Biosensors and Bioelectronics ■ (■■■■) ■■■–■■■

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

journal homepage: www.elsevier.com/locate/bios

Detection of norovirus virus-like particles using a surface plasmon resonance-assisted fluoroimmunosensor optimized for quantum dot fluorescent labels

Hiroki Ashiba [a,*], Yuki Sugiyama [b], Xiaomin Wang [c,1], Haruko Shirato [d], Kyoko Higo-Moriguchi [e], Koki Taniguchi [e], Yoshimichi Ohki [b], Makoto Fujimaki [a]

[a] Electronics and Photonics Research Institute, National Institute of Advanced Industrial Science and Technology (AIST), Tsukuba, Japan
[b] Department of Electrical Engineering and Bioscience, Waseda University, Shinjuku, Japan
[c] Nanoelectronics Research Institute, National Institute of Advanced Industrial Science and Technology (AIST), Tsukuba, Japan
[d] Department of Virology II, National Institute of Infectious Diseases, Musashi-Murayama, Japan
[e] Department of Virology and Parasitology, Fujita Health University School of Medicine, Toyoake, Japan

ARTICLE INFO

Article history:
Received 21 June 2016
Received in revised form
26 August 2016
Accepted 28 August 2016

Keywords:
Norovirus detection
Sensor design
Sensitivity enhancement
V-trench biosensor
Surface plasmon resonance
Quantum dot

ABSTRACT

A highly sensitive biosensor to detect norovirus in environment is desired to prevent the spread of infection. In this study, we investigated a design of surface plasmon resonance (SPR)-assisted fluoroimmunosensor to increase its sensitivity and performed detection of norovirus virus-like particles (VLPs). A quantum dot fluorescent dye was employed because of its large Stokes shift. The sensor design was optimized for the CdSe-ZnS-based quantum dots. The optimal design was applied to a simple SPR-assisted fluoroimmunosensor that uses a sensor chip equipped with a V-shaped trench. Excitation efficiency of the quantum dots, degree of electric field enhancement by SPR, and intensity of autofluorescence of a substrate of the sensor chip were theoretically and experimentally evaluated to maximize the signal-to-noise ratio. As the result, an excitation wavelength of 390 nm was selected to excite SPR on an Al film of the sensor chip. The sandwich assay of norovirus VLPs was performed using the designed sensor. Minimum detectable concentration of 0.01 ng/mL, which corresponds to 100 virus-like particles included in the detection region of the V-trench, was demonstrated.

1. Introduction

Norovirus is an extremely contagious gastroenteritis virus that can infect with less than 20 virus particles (Morillo and Timenetsky, 2011). To prevent the spread of infection, complete cleaning and sterilizing is needed at a location where a patient of norovirus infection appeared. A highly sensitive method of on-site norovirus detection is desirable for confirming the sterilization. Highly sensitive biosensors should be developed for the inspection device against norovirus in the environment.

Norovirus consists of large numbers of genotypes. At least 33 genotypes have been known for human norovirus (Kageyama et al., 2004). The inspection device should deal with the various genotypes. An immunoassay possesses high specificity against an antigen owing to the use of a corresponding antibody; on the contrary, in general, recognition of various genotypes of norovirus using an antibody is difficult. In a recent work, cross-reactive antibodies for various genotypes of norovirus has been isolated (Higo-Moriguchi et al., 2014). These antibodies exhibit a relatively low affinity in return for the cross-reactivity. Thus, sensitivity enhancement is required to a sensing instrument for developing the cross-reactive biosensor.

Surface plasmon resonance-assisted fluoroimmunoassay (SPRF), or surface plasmon field-enhanced fluorescence spectroscopy (SPFS), is known as a method of highly sensitive biosensing (Attridge et al., 1991; Liebermann and Knoll; 2000; Roy et al., 2002; Toma et al., 2013). SPRF utilizes enhanced electric field induced by surface plasmon resonance (SPR) excitation for an immunoassay using a fluorescent label. Luminescence from a fluorescent label is enhanced by the SPR and the sensor achieves increased sensitivity. We have developed a simple and compact sensing instrument for SPRF: a V-trench biosensor (Nomura et al., 2013; Ashiba et al., 2016). The sensor have demonstrated virus detection, e.g., influenza virus at a concentration of 10^4 pfu/mL (pfu: plaque forming unit) was detected (Ashiba et al., 2016). A V-trench biosensor, which is compact and sensitive, suits for the on-site detection of norovirus.

* Corresponding author.
E-mail address: h.ashiba@aist.go.jp (H. Ashiba).
[1] Present address: Research and Development Division, Optoquest Co., Ltd, Ageo, Japan.

http://dx.doi.org/10.1016/j.bios.2016.08.099
0956-5663/

Although SPRF is sensitive, the sensitivity needs further increase since the targeted sensitivity of norovirus in the environment is extremely high. A predominant noise source of a fluorescent sensor is a leaked or scattered excitation light, which should be cut by a detector filter. Protein fluorescent labels exhibit small Stokes shift in general, i.e., their excitation and emission wavelengths are close. Effective separation of closed wavelengths is not easy, requires high quality optical filters, and in some case generates a noise signal. In addition, the cut of an excitation light results in the cut of luminescence from a fluorescent label because of an overlap of spectra. As the result, the signal-to-noise ratio decreases. Therefore, the use of a fluorescent label with a large Stokes shift is considered to be effective.

In this study, we focused on the use of a quantum dot fluorescent dye for increasing the sensitivity of SPRF. A V-trench biosensor for the SPRF using a quantum dot fluorescent label was developed. Electric field simulation for optimal design of the sensor was conducted. The developed sensor was examined using quantum dot fluorescent labels and demonstrated detection of norovirus virus-like particles (VLPs).

2. System design

A schematic diagram of a V-trench biosensor is shown in Fig. 1. A fluidic channel with a V-shaped cross-section ("V-trench") on a sensor chip works as a sample holder, a sensing surface, and a finely-designed prism that satisfies an SPR excitation condition. An optical system is simplified owing to the functionalized sensor chip.

A quantum dot fluorescent dye is an inorganic fluorescent substance exhibiting Stokes shift as large as hundreds of nanometers (Medintz et al., 2005). Moreover, the quantum dot fluorescent dye possesses properties useful for the practical use such as strong luminescence intensity and no photobleaching. In this study, we employed commercially available quantum dot fluorescent dyes: Qdot nanocrystals (Life Technologies, Thermo Fisher Scientific). Qdots have core-shell structure, typically consisted of CdSe core and ZnS shell, and emit fluorescence at various wavelengths depending on its size. Qdots are excited by a light of broad wavelength range, from visible to ultraviolet, and the efficiency of excitation is higher when the shorter excitation wavelength is used (https://tools.thermofisher.com/content/sfs/manuals/mp19000.pdf). Thus, the excitation wavelength of the V-trench biosensor should be short, specifically 500 nm or less. Au is commonly-used material of SPR excitation; however, Au is undesirable in this wavelength range because of low SPR excitation efficiency. Herein we employed Al, which is suit for SPR excitation in short wavelengths, as the SPR excitation material. The design of V-trench biosensor based on the SPR on Al and Qdot fluorescent labels is described below.

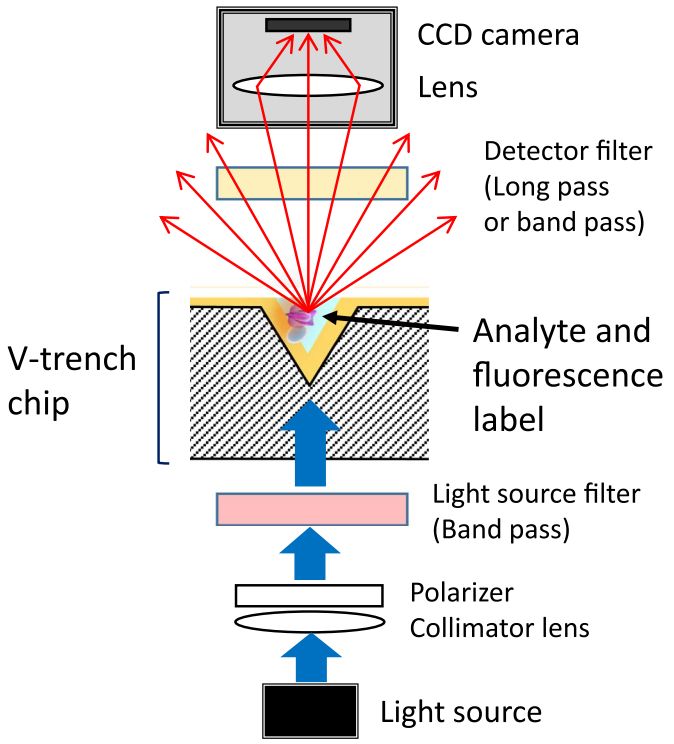

Fig. 1. Schematic diagram of an optical system of a V-trench biosensor.

Firstly, an excitation wavelength for exciting SPR and Qdots was investigated. Then, the optimal dimensions of V-trenches for SPR excitation, specifically vertex angle and thickness of Al, were decided. For deciding the optimal excitation wavelength, three factors were considered: (i) excitation efficiency of Qdots, K_1, (ii) degree of electric field enhancement by SPR, K_2, and (iii) autofluorescence intensity of a substrate of sensor chips, K_3. K_1 and K_2 relate with luminescence intensity of Qdots. Detection signal becomes larger when K_1 and K_2 are larger. K_3 relates with background noise and should be small. Herein, a value of K_1K_2/K_3 was employed as an index for the excitation wavelength selection. On the basis of K_1K_2/K_3 values for each wavelength, taking into account the practical points such as availability of a light source and cost of a system, the excitation wavelength was decided.

For K_1, properties of Qdots are provided from the supplier. Several Qdots with visible fluorescence were considered as candidates of the fluorescent label: Qdot 565, Qdot 605, Qdot 625, and Qdot 705. Excitation spectra of Qdots were obtained from a webpage of supplier (Fluorescence SpectraViewer, Thermo Fisher Scientific). Relative intensities of the spectra were calibrated by a molar extinction coefficient at 405 nm (https://tools.thermofisher.com/content/sfs/manuals/mp19000.pdf), and the calibrated values were used as K_1 (the values are shown in Supplementary Fig. S1).

For K_2, electric field enhancement factors of SPR, $|E/E_0|^2$, were numerically calculated by electric field simulation based on the transfer matrix method (Born and Wolf, 1980). The vicinity of the surface of V-trenches was modeled with a multiplexed layers to calculate the optimal condition for SPR excitation and $|E/E_0|^2$. A schematic diagram of the calculation model is shown in Fig. 2(A). An excitation light at an incident angle, which corresponds to a vertex angle of V-trenches, enters from the bottom. As a material of the bottom substrate layer, we employed polystyrene that had been used in the previous study (Nomura et al., 2013). An Al layer is placed on the polystyrene substrate with a native oxide on its surface. A protein layer of 20-nm thick is placed on the oxide to represent surface modification on the sensor chip, including immobilized antibodies and antigens. The top layer is water. Optimal vertex angle of V-trenches and thickness of Al layer that maximize $|E/E_0|^2$ at the boundary between the protein and water layers were calculated for each excitation wavelength. In this calculation, the aluminum oxide was assumed to be Al_2O_3. The complex refractive indices of polystyrene (http://refractiveindex.info), Al_2O_3 (Lichtenstein, 1979), and water (Querry et al., 1998) were referred from the literature. The complex refractive indices of Al and thickness of the native oxide were evaluated using an ellipsometer (VASE, J.A. Woollam, Lincoln, NE, USA) with an Al film prepared using a sputtering system (CFS-4EP-LL, Shibaura Mechatronics, Yokohama, Japan). The evaluated thickness of native oxide was 5 nm and complex refractive indices of Al were values shown in Supplementary Fig. S2. These values were used for the calculation. The complex refractive indices of the protein layer were set to be 1.45.

Please cite this article as: Ashiba, H., et al., Biosensors and Bioelectronics (2016), http://dx.doi.org/10.1016/j.bios.2016.08.099

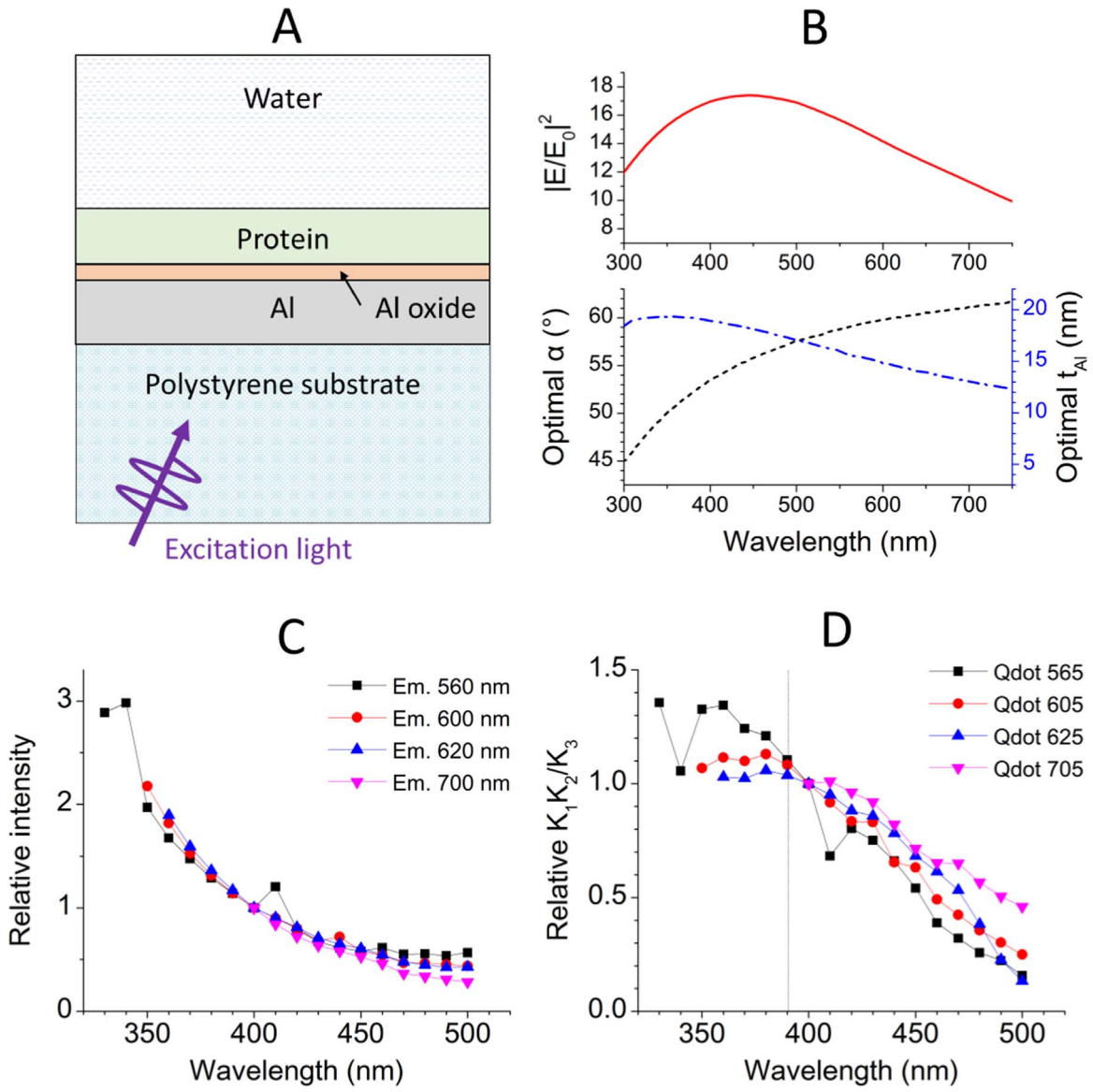

Fig. 2. Design of the V-trench biosensor for quantum dot fluorescent labels. (A) Multi-layer model used in electric field simulation. (B) Calculated values of vertex angle, α, and Al thickness, t_{Al}, of a V-trench chip optimized to maximize electric field enhancement factor, $|E/E_0|^2$, and $|E/E_0|^2$ at the optimal condition against excitation wavelength. In the lower panel, a broken line (black) shows the values of optimal α and a dash dot line (blue) shows the values of optimal t_{Al}. (C) Measured values of relative intensity of autofluorescence of polystyrene substrate against excitation wavelength. The symbols shows different emission wavelengths (Em.). (D) Evaluated relative values of K_1K_2/K_3 against excitation wavelength. The plotted values expresses an index of signal-to-noise ration of the sensor. A broken line shows an excitation wavelength of 390 nm.

The calculation result including the optimal vertex angle, optimal Al thickness, and $|E/E_0|^2$ against an excitation wavelength is shown in Fig. 2(B). Electric field enhancement induced by SPR excitation is present in a broad range of excitation wavelength from ultraviolet to near infrared. The maximum value of $|E/E_0|^2$ is 17.4 at an excitation wavelength of 450 nm, vertex angle of 55.9°, and Al thickness of 18 nm. Although the maximum is seen at 450 nm, more than 90% of the maximum $|E/E_0|^2$ is obtained in a wavelength range of 360–550 nm, which is considered to be applicable. $|E/E_0|^2$ shown in Fig. 2(B) were used as K_2.

For K_3, autofluorescence of a substrate of a sensor chip was measured using a spectrofluorometer (FluoroMx-4, Horiba, Kyoto, Japan). An excitation light with a wavelength ranged from 300 to 500 nm was irradiated to a plate of polystyrene (CR-3500, DIC, Tokyo, Japan) with a thickness of 1.5 mm. Spectra of an emitted light were obtained for each excitation wavelength. Fig. 2(C) shows the spectra of autofluorescence for an emission wavelength of 560, 600, 620, and 700 nm, corresponding to Qdot 565, Qdot 605, Qdot 625, and Qdot 705, respectively. Each spectrum is normalized by an intensity at an excitation wavelength of 400 nm. Data points near the second harmonic of the excitation light are excluded from the spectra. A similar trend was observed for each emission wavelength. The autofluorescence increased with the decrease in excitation wavelength. In a practical measurement, the emitted autofluorescence is attenuated by an Al film and then observed by a detector. The autofluorescence enters into the Al film at any incident angle; however, only the autofluorescence with an incident angle near that of the excitation light is observed by the detector. Thus, for simplification, we evaluated the attenuation effect of the Al film by calculating absorption of a light entered at the incident angle of excitation light. An intensity of transmitted light, I_{out}, is expressed as $I_{out}=I_{in}\ \exp((-4\pi k/\lambda)(t_{Al}/\sin(\alpha/2)))$, where I_{in} is an intensity of incident light, k is an extinction coefficient of Al, λ is a wavelength of incident light, t_{al} is a thickness of Al film, and α is a vertex angle of the V-trench. I_{out} was calculated substituting the measured intensity of autofluorescence to I_{in}. Also, the optimal

Please cite this article as: Ashiba, H., et al., Biosensors and Bioelectronics (2016), http://dx.doi.org/10.1016/j.bios.2016.08.099

thickness and vertex angle calculated by the electric field simulation (Fig. 2(B)) were used as t_{Al} and α. The values of I_{out} for each excitation wavelength were used as K_3 and shown in Supplementary Fig. S3.

Using obtained K_1, K_2, and K_3, relative values of K_1K_2/K_3 against excitation wavelength were calculated as shown in Fig. 2(D). Each dataset is normalized by a value at an excitation wavelength of 400 nm. An excitation wavelength with large K_1K_2/K_3 results in high signal-to-noise ratio. For all Qdots, K_1K_2/K_3 increases from 500 nm to shorter wavelengths and reaches a plateau in a wavelength region of 360–390 nm. Thus, an excitation wavelength at 390 nm or less is desirable. On the other hand, use of ultraviolet light with a shorter wavelength has disadvantages including a cost of light source and deterioration of the resin part of the instrument. Therefore, in this study, we employed 390 nm as the excitation wavelength. At this wavelength, the optimal vertex angle and Al thickness are derived as 52.9° and 19 nm, respectively, and $|E/E_0|^2 = 16.7$ from the electric field simulation (Fig. 2(B)). The Qdots were evaluated using the designed system; the one exhibited the highest signal-to-noise-ratio was selected for detection of norovirus VLPs.

3. Materials and methods

3.1. Materials

Phosphate-buffered saline (PBS) was purchased from Wako Pure Chemical Industries (Osaka, Japan). 10-Carboxydecylphosphonic acid (10-CDPA), the Amine Coupling Kit [including *N*-hydroxysuccinimide (NHS) and 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC)], and the Biotin Labeling Kit - NH_2 were purchased from Dojindo Laboratories (Kamimashiki, Japan). Bovine serum albumin (BSA) was purchased from Sigma-Aldrich (St. Louis, MO, USA). Ethanolamine hydrochloride was purchased from Alfa Aesar, Thermo Fisher Scientific (Waltham, MA, USA). Streptavidin conjugate of Qdot 565, Qdot 605, Qdot 625, Qdot 705, and Alexa Fluor 700 were purchased from Life Technologies, Thermo Fisher Scientific (Waltham, MA, USA). Protein G Sepharose was purchased from GE Healthcare (UK).

3.2. Preparation of VLPs and antibodies

Since a cell culture system for human norovirus have not been established, the human norovirus VLPs have been developed and used in virological studies. The VLPs are morphologically and antigenically similar to native norovirus particles (Jiang et al., 1992; Green et al., 1993; Prasad et al., 1994). Strain Narita 104 VLPs (r104: genogoup II, genotype 4 [GII.4]) were prepared by infecting subconfluent Tn5 insect cells with the recombinant baculoviruses as described previously (Hansman et al., 2006). Anti-norovirus monoclonal antibody, 12A11, was used as capture antibody against norovirus VLPs, which showed intra-GII cross-reactivity. Using r104 as antigen, 12A11 was isolated from a pharge-displayed antibody library (single chain variable fragment [scFv] form) prepared from human B lymphocyte-rich tissues of a few dozen people (Higo-Moriguchi et al., 2014). Anti-norovirus polyclonal antibodies were used as detection antibody against norovirus VLPs. Hyperimmune sera to the 13 genotypes of VLPs, including GI.1, GI.2, GI.3, GI.4, GI.6, GII.1, GII.3, GII.4, GII.5, GII.6, GII.7, GII.12, and GII.13, were prepared in rabbits. The 13 of antisera were mixed in equal volume to express cross-reactivity of the detection antibody. Protein G Sepharose were used to purify the IgG antibody molecules from the mixed antisera.

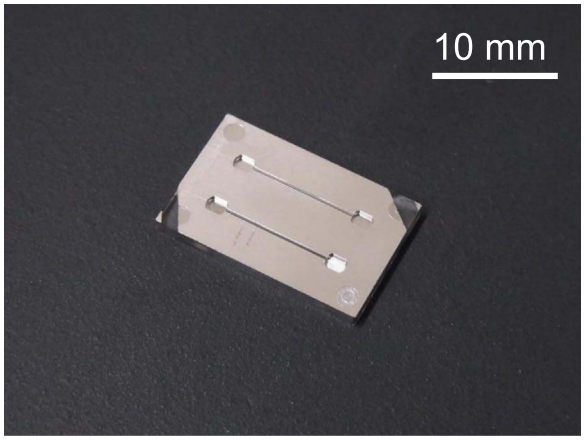

Fig. 3. Photograph of V-trench sensor chip for Qdot fluorescent dyes. Two trenches are formed on the chip.

3.3. Fabrication of V-trench sensor chips

A substrate of the designed V-trench sensor chip was fabricated by injection molding of polystyrene (Cluster Technology, Higashi-Osaka, Japan). The molding material was CR-3500. A vertex angle, length, and opening width of a V-trench were set to be 52.9°, 10 mm, and 0.3 mm, respectively. Fluid reservoirs were equipped at the both ends of the V-trench. An Al film as the SPR excitation layer was formed by sputtering (CFS-4EP-LL). The polystyrene substrates were placed in a chamber under 0.5 Pa of Ar atmosphere, and Al was sputtered with an RF power of 200 W. The thickness of sputtered Al film was set considering incline of the surface of V-trench and formation of native oxide on the surface. For example, under assumption that Al of 2.5-nm thick from its surface is oxidized and forms Al oxide of 5-nm thick, the sputtered thickness of 51 nm results in Al thickness of 19 nm on a V-trench when its vertex angle is 52.9°. Thus, sputtered thickness of 51 nm was employed, which is considered to meet the optimal condition for SPR excitation of the designed chip. A photograph of the fabricated V-trench chip is show in Fig. 3.

3.4. Experimental apparatus

Fluorescence measurements of the V-trench chips were conducted using a custom-made fluorescence imager with an excitation wavelength of 390 nm (Shibasaki, Chichibu, Japan). A photograph of the instrument is shown in Supplementary Fig. S4. A light source, V-trench chip, and a camera were placed in a line such as shown in Fig. 1. An outer dimensions of the optical system was $260 \times 180 \times 160\ mm^3$. A collimated excitation light from a light-emitting diode (LED) with a wavelength of 390 nm passed through a polarizer and a bandpass filter (MX0390, Asahi Spectra, Tokyo, Japan) and then irradiated the V-trench chip from the backside. The excitation light was a circle with a diameter of 5 mm and irradiated at the center of V-trench. The excitation light was P-polarized against the surface of V-trenches to excite SPR. When a fluorescent substance exists in the vicinity of the surface, fluorescence is excited by the enhanced electric field of SPR. A fluorescence image was obtained by a 16-bit monochrome charge-coupled device (CCD) camera with a Peltier cooling unit (BU-51LN, Bitran, Gyoda, Japan). A bandpass filter was placed in front of the camera as a detector filter and its center wavelength was selected depending on the emission wavelength of fluorescent dyes: a center wavelength of 560 nm for Qdot 565, 600 nm for Qdot 605, 620 nm for Qdot 625 (FB560-10, FB600-10, and FB620-10, Thorlabs, Newton, NJ, USA), and 700 nm for Qdot 705 (MX0700, Asahi Spectra). These filters effectively cut the excitation light; e.g., an

Please cite this article as: Ashiba, H., et al., Biosensors and Bioelectronics (2016), http://dx.doi.org/10.1016/j.bios.2016.08.099

optical density (OD) of FB560-10 at a wavelength of 390 nm is approximately 7. A luminescent intensity was evaluated from the obtained fluorescence image by an averaged value of brightness of the region of V-trench where the excitation light was irradiated. Cooling temperature of the camera was −16 °C and exposure time to obtain the fluorescence images was 60 s. Dark current signal of the camera was subtracted from the luminescent intensity.

3.5. Evaluation of fluorescence of quantum dots

Fluorescence measurements were conducted to evaluate luminescence of Qdots using the fabricated V-trench sensor chips. The V-trench chips were treated using a plasma cleaner (PDC-32G, Harrick Plasma, Ithaca, NY, USA) at an RF power of 18 W for 20 s to increase hydrophilicity of its surface. No further surface modifications were performed in this experiment. The V-trench were filled with deionized water by putting a 15 μL of droplet on it. The water flowed and filled in the V-trench by capillary force. The fluorescence image of the water, used as the reference blank, was obtained. After removing the water using an N_2 gun, 15 μL of a solution of the 1 nM Qdot streptavidin conjugate in deionized water was applied to the V-trench and its fluorescence image was obtained. Qdot 565, 605, 625, and 705 were evaluated with changing the detector filter. A power density of the excitation light irradiated to the sensor chip was 1.5 mW/cm^2. Luminescent intensities of the water and Qdot were evaluated from the fluorescence images.

3.6. Detection of norovirus VLPs

Detection of norovirus VLPs were examined using the developed system. A schematic diagram of the surface of the V-trenches constructed for norovirus VLP detection is shown in Fig. 4. The VLPs were detected by a sandwich assay. A self-assembled monolayer (SAM) of a phosphonic acid derivative was employed to immobilize proteins on the surface of aluminum oxide. The phosphonic acid derivatives form dense and stable SAMs on the surface of metal oxides compared to a silane coupling agent (Silverman et al., 2005; Klauk et al., 2007). In the following protocol, all reactions were carried out at room temperature and the V-trenches were washed with PBS between the reactions unless otherwise specified. The washing was conducted using a pipette and 0.4 mL of PBS was flowed onto tilted V-trenches five times. Reagents applied to the V-trenches were put on the fluid reservoirs equipped at the both ends of the trench and flowed by capillary force. The V-trench chips were treated with a plasma cleaner at an RF power of 18 W for 20 s and immersed overnight in a 1 mM solution of 10-CDPA in ethanol. The chips were rinsed with ethanol, dried using an N_2 gun, and placed on a hot plate at 60 °C for 1 h to stabilize the SAM. As the reference blank, 15 μL of PBS was applied to the V-trench and fluorescence image was obtained. After removing the PBS, 10 μL of an equal-volume mixture of a 100 mM solution of NHS and a 100 mM solution of EDC was applied to the V-trench for 30 min to activate carboxyl groups of the SAM. A capture antibody was immobilized on the SAM by applying 10 μL of 30 μg/mL solution of the monoclonal anti-norovirus antibody in PBS for 30 min. A 1 M solution of ethanolamine hydrochloride at pH 8.5 in deionized water and a 1 wt% solution of BSA in PBS were serially reacted for blocking (10 μL for 30 min each). The norovirus VLPs were diluted with PBS containing 1 wt% BSA to a concentration ranged from 0.01 to 100 ng/mL, and 10 μL of each solution was reacted with the capture antibody for 20 min. PBS containing 1 wt% BSA without VLPs was used as the negative control (0 ng/mL). A detection antibody was reacted with the VLPs by applying 10 μL of 30 μg/mL solution of the biotinylated polyclonal anti-norovirus antibodies (prepared with the Biotin Labeling Kit) in PBS containing 1 wt% of BSA for 20 min. A 5 nM solution of Qdot streptavidin conjugate in PBS containing 1 wt% BSA was reacted to label VLPs with Qdots (10 μL for 10 min). Fluorescence images of the V-trench chip labeled with Qdots were obtained applying 15 μL of PBS. A power density of the excitation light irradiated to the sensor chip was 1.5 mW/cm^2. Luminescent intensities of the reference blank and VLPs with Qdots were evaluated from the fluorescence images.

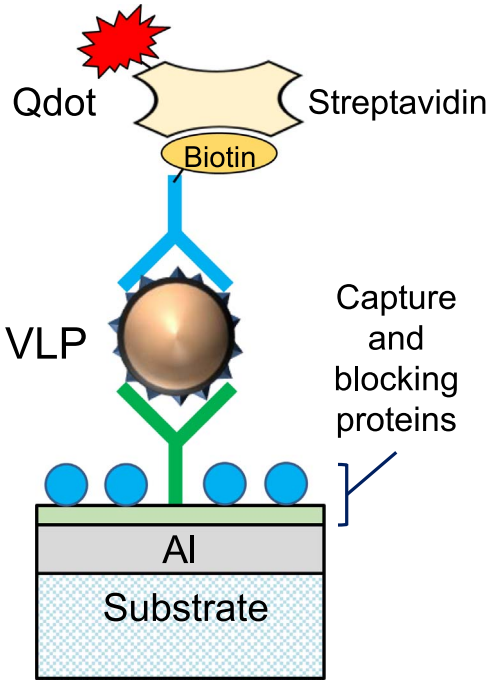

Fig. 4. Schematic diagram of the surface of the V-trenches constructed for norovirus virus-like particle (VLP) detection.

4. Results

Luminescent intensities of Qdot solutions and deionized water (reference blank) directly applied to the V-trenches are shown in Fig. 5. The plotted values shows the average of luminescent intensities evaluated from three fluorescence images and the error bars are standard deviations. The luminescent intensities of Qdots increased with the increase in an emission wavelength of Qdots, whereas that of the reference blank decreased.

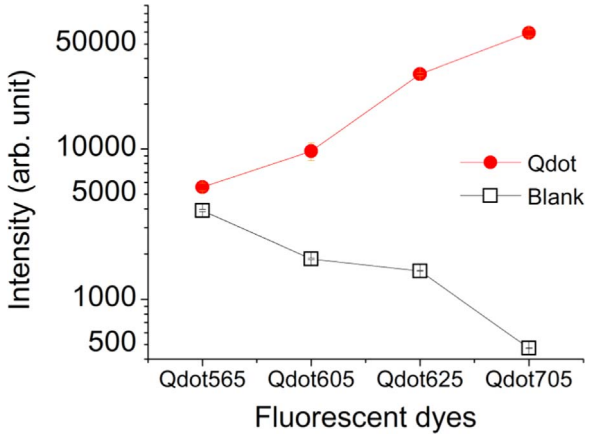

Fig. 5. Luminescent intensities of solutions of Qdot fluorescent dyes ("Qdot") and deionized water ("Blank") measured using the developed V-trench biosensor.

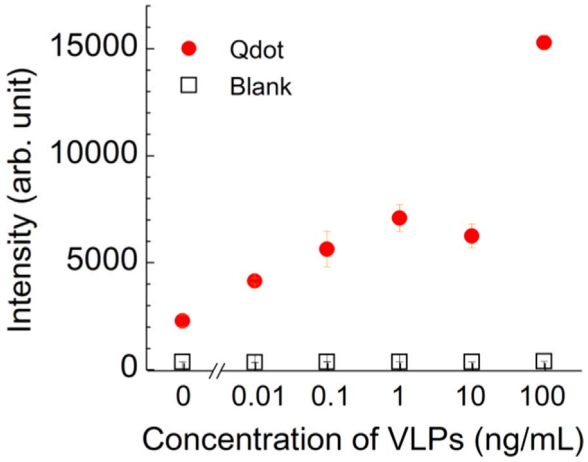

Fig. 6. Luminescent intensities measured with a sandwich assay of norovirus virus-like particles (VLPs) using the developed V-trench biosensor.

Luminescent intensities against the concentration of norovirus VLPs are shown in Fig. 6. The luminescent intensities of VLPs with Qdots and the reference blank are shown. Qdot 705 was used as the fluorescent label in this experiment. The plotted values shows the average of luminescent intensities evaluated from three fluorescence images and the error bars are standard deviations. The luminescent intensities of VLPs with Qdots were mostly correlated with the concentration of VLPs. The minimum detectable concentration of VLPs was 0.01 ng/mL for this measurement.

5. Discussion

5.1. Comparison of quantum dots

As shown in Fig. 5, Qdot 705 exhibited the highest luminescent intensities of Qdot and the lowest luminescent intensities of reference blank. Thus, we used Qdot 705 that possesses the highest signal-to-noise ratio for the detection of norovirus VLPs. Since a part of pixels in the evaluated region showed the saturated brightness for the fluorescence images of Qdot 705 (data not shown), the potential signal-to-noise ratio should be even higher. The blank luminescence potentially originates from the leaked and scattered excitation light and the autofluorescence of substrate. In this experiment, the excitation light was negligible because of the high OD of the detector filter (approximately OD 7 at 390 nm); thus, the decrease in the blank luminescence at long-wavelength side originated from the decrease in autofluorescence of the substrate of V-trench chips. For the increase in the Qdot luminescence at long-wavelength side, we deduce it is due to the difference in luminescence properties of the Qdots; for example, Qdot 625 and 705 possesses higher molar extinction coefficients than Qdot 565 and 525 (Fig. S1). The combined effects resulted in the highest signal-to-noise ratio of Qdot 705.

5.2. Detection of norovirus VLPs

The demonstrated minimum detectable concentration of VLPs, 0.01 ng/mL, is much smaller than that of commercially-available immunochromatography kits, 0.25–12.5 ng/mL (ImmunoCatch-Noro, Eiken Chemical, Tokyo, Japan; GE test Noro Nissui, Nissui Pharmaceutical, Tokyo, Japan; Quick Navi-Noro 2, Denka Seiken, Tokyo, Japan). The number of VLPs detected in this measurement is estimated as follows. The VLP used in this study is assumed to be a spherical particle structured with 180 of capsid proteins with a molecular weight of 58000 (Shirato, 2011), and thus a molecular weight of the VLP is 1.4×10^7. Therefore, 0.01 ng/mL corresponds to 4.3×10^5 particles/mL. The applied volume of the VLP solution was 10 μL; however, most of the volume remained in the reservoirs since the volume of the V-trench was 0.5 μL. Furthermore, the excitation light (5 mm in diameter) was irradiated to a half of the V-trench (10 mm in length). Therefore, by multiplexing the concentration and the volume, the number of VLPs contained in the measured region of the V-trench is estimated to be approximately 100.

Although the developed system successfully detected the small number of norovirus VLPs, its sensitivity can be further increased by means such as optimizing the surface modification conditions or decreasing aluminum oxide layer to increase $|E/E_0|^2$. For example, in Fig. 6, the luminescent intensity of the negative control (0 ng/mL) was higher than that of its reference blank. Nonspecific adsorption of the detection antibodies or Qdots would cause this luminescence. By optimizing a method of blocking and washing, the nonspecific adsorption can be suppressed resulting in the decreased luminescence of the negative control.

The protocol of VLP detection employed in this study should be simplified to be used for on-site inspection since it requires multiplexed processes and takes time before the measurement. The simplification and shortening of time is achievable by introducing a method such as pre-mixing of a sample and reagents before applying. In addition, installation of flow system to the sensor will be effective for a quick and easy-to-use method of detection. These methods contribute to realize a practical instrument for the on-site inspection of norovirus.

6. Conclusion

Aiming at on-site detection of norovirus, a simple, compact, and sensitive instrument for SPRF, V-trench biosensor, optimized for quantum dot fluorescent labels was developed. The sensor was designed to excite Qdot fluorescent dyes by enhanced electric field induced by SPR on an Al film, and the excitation wavelength of 390 nm was selected. The developed sensor chips and measurement system were evaluated using Qdot solutions. Qdot 705 exhibited the highest signal-to-noise ratio compared to Qdot 525, Qdot 605, and Qdot 625. Detection of norovirus VLPs were performed using the developed system by the sandwich assay using a phosphonic acid SAM and the cross-reactive antibodies. Minimum detectable concentration of norovirus VLPs was 0.01 ng/mL.

In the present study, detection of one genotype of norovirus VLPs (GII.4) have been demonstrated. However, the developed system is potentially applicable to various genotypes of VLPs; the capture antibody was widely reactive to the genogroup II and the detection antibodies are reactive to the 13 genotypes in genogoup I and II. A capture antibody with intra-GI cross-reactivity is also available (Higo-Moriguchi et al., 2014). Detection of various genotypes of norovirus VLPs is scheduled in the future work to validate the cross-reactivity and high sensitivity of the developed system.

Acknowledgement

This work was supported by The Japan Society for the Promotion of Science (JSPS) KAKENHI Grant number 26246008.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.bios.2016.08.099.

References

Ashiba, H., Fujimaki, M., Wang, X., Awazu, K., Tamura, T., Shimizu, Y., 2016. Jpn. J. Appl. Phys. 55, 067001.

Attridge, J.W., Daniels, P.B., Deacon, J.K., Robinson, G.A., Davidson, G.P., 1991. Biosens. Bioelectron. 6, 201–214.

Born, M., Wolf, E., 1980. Principles of optics: Electromagnetic Theory of Propagation, Interference and Diffraction of Light, 6th ed. Pergamon, Oxford.

Green, K.Y., Lew, J.F., Jiang, X., Kapikian, A.Z., Estes, M.K., 1993. J. Clin. Microbiol. 31, 2185–2191.

Hansman, G.S., Natori, K., Shirato-Horikoshi, H., Ogawa, S., Oka, T., Katayama, K., Tanaka, T., Miyoshi, T., Sakae, K., Kobayashi, S., Shinohara, M., Uchida, K., Sakurai, N., Shinozaki, K., Okada, M., Seto, Y., Kamata, K., Nagata, N., Tanaka, K., Miyamura, T., Takeda, N., 2006. J. Gen. Virol. 87, 909–919.

Higo-Moriguchi, K., Shirato, H., Someya, Y., Kurosawa, Y., Takeda, N., Taniguchi, K., 2014. J. Med. Virol. 86, 558–567.

Jiang, X., Wang, M., Graham, D.Y., Estes, M.K., 1992. J. Virol. 66, 6527–6532.

Kageyama, T., Shinohara, M., Uchida, K., Fukushi, S., Hoshino, F.B., Kojima, S., Takai, R., Oka, T., Takeda, N., Katayama, K., 2004. J. Clin. Microbiol. 42, 2988–2995.

Klauk, H., Zschieschang, U., Pflaum, J., Halik, M., 2007. Nature 445, 745–748.

Lichtenstein, T., 1979. Handbook of Thin Film Materials. College of Engineering and Applied Science, University of Rochester, Rochester.

Liebermann, T., Knoll, W., 2000. Colloids Surf. A 171, 115–130.

Medintz, I.L., Uyeda, H.T., Goldman, E.R., Mattoussi, H., 2005. Nat. Mater. 4, 435–446.

Morillo, S.G., Timenetsky, M.D.C.S.T., 2011. Rev. Assoc. Med. Bras. 57, 453–458.

Nomura, K., Gopinath, S.C.B., Lakshmipriya, T., Fukuda, N., Wang, X., Fujimaki, M., 2013. Nat. Commun. 4, 2855.

Prasad, B.V.V., Rothnagel, R., Jiang, X., Estes, M.K., 1994. J. Virol. 68, 5117–5125.

Querry, M.R., Wieliczka, D.M., Segelstein, D.J., 1998. In: Parik, E.D. (Ed.), Handbook of Optical Constants of Solids II, Academic Press, San Diego, p. 1059.

Roy, S., Kim, J.-H., Kellis, J.T., Poulose, A.J., Robertson, C.R., Gast, A.P., 2002. Langmuir 18, 6319–6323.

Shirato, H., 2011. Jpn. J. Infect. Dis. 64, 95–103.

Silverman, B.M., Wieghaus, K.A., Schwartz, J., 2005. Langmuir 21, 225–228.

Toma, K., Vala, M., Adam, P., Homola, J., Knoll, W., Dostálek, J., 2013. Opt. Express 21, 10121–10132.

Please cite this article as: Ashiba, H., et al., Biosensors and Bioelectronics (2016), http://dx.doi.org/10.1016/j.bios.2016.08.099