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

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A B S T R A C T

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.

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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.

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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.

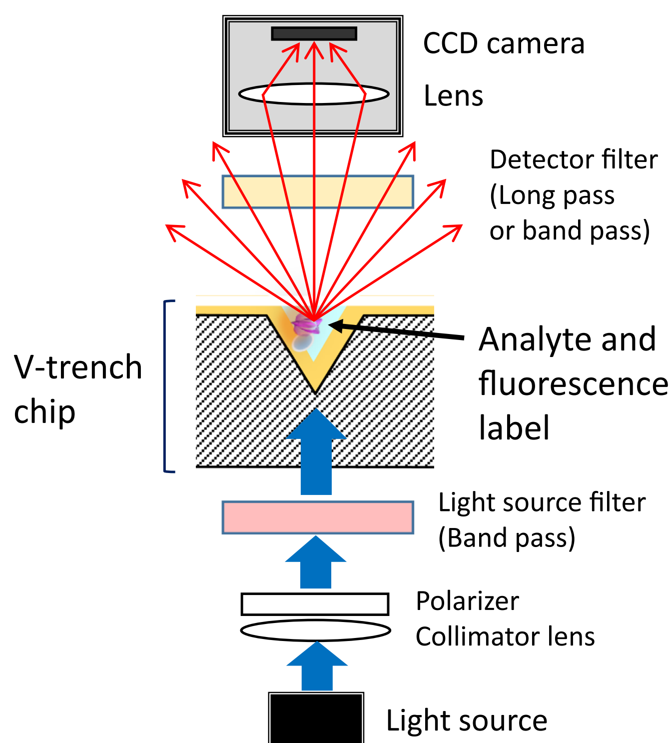

Fig. 1. Schematic diagram of an optical system of a V-trench biosensor.

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.

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