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Surface plasmon field-enhanced fluorescence spectroscopy apparatus with a convergent optical system for point-of-care testing

Mitsuaki Toda [a,1], Yusuke Arima [a], Hiromi Takiguchi [b], Hiroo Iwata [a,*]

[a] Institute for Frontier Medical Sciences, Kyoto University, Sakyo-ku, Kyoto 606-8507, Japan
[b] Graduate School of Engineering, University of Tokyo, Bunkyo-ku, Tokyo 113-8656, Japan

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ABSTRACT

Surface plasmon field-enhanced fluorescence spectroscopy (SPFS) is a promising methodology for point-of-care (POC) testing. The SPFS devices that have been reported are equipped with an angle rotating stage to adjust the surface plasmon resonance (SPR) angle. In a clinical setting, however, the SPR angle determination is a tedious and time-consuming process. In this study, we employed an SPFS instrument with a convergent optical system that allows the omission of this procedure. We demonstrated that this instrumentation allowed the sensitive determination of low concentrations of α-fetoprotein in serum and reduced the variation effect caused by the protein concentrations in samples. The SPFS with a convergent optical system is suitable for POC testing.

Point-of-care (POC)[2] testing allows medical personnel to carry out clinical tests at the bedside or near patients [1]. The results are immediately available, thereby permitting the rapid application of clinical management decisions. Various methods such as paper chromatography [2], electrochemical analysis [3], surface plasmon resonance (SPR) [4–7], and the like [8,9] have been examined to develop point-of-care testing (POCT) devices. Surface plasmon field-enhanced fluorescence spectroscopy (SPFS) [10] (Scheme 1) is a promising method for the development of a highly sensitive POC device. SPFS uses a field-enhanced optical field of a surface plasmon mode for the excitation of fluorophores placed near the metal–dielectric interface. The evanescent field of a surface plasmon is enhanced by a factor of approximately 15 compared with the incident field for a gold–water interface at the specific resonance angle, and then the signal decays exponentially into the dielectric medium, approximately Lz = 190 nm [11]. Thus, an SPFS-based sensor allows the sensitive determination of low concentrations of target molecules without the removal of unbounded complexes or B/F separation steps. Yu and coworkers reported an SPFS-based sensing device and achieved highly sensitive detection of free prostate-specific antigen (f-PSA) [12]. Our group also developed an SPFS-based device and demonstrated quantitative detection of α-fetoprotein (AFP), a marker of hepatoma, at levels below the cutoff concentration for the clinical test [13]. Some problems, however, remain for the SPFS-based sensing devices used for POCT. Protein concentration in patients' blood varies within the range of 30 to 100 mg/ml [14,15], resulting in large variation of its refractive indexes. Conventional SPFS devices monitor a change in fluorescence intensity at a fixed incident angle near the SPR angle, which is influenced by the refractive index near the metal–dielectric interface. Therefore, the incident angle must be carefully adjusted to be close to the SPR angle for each patient to maximize the field intensity of the surface plasmon and, thus, the fluorescence intensity.

The SPFS-based devices that have been previously reported are equipped with an angle rotating stage to adjust the SPR angle [12,13]. These devices should be small and consist of simple optical units for their application to POCT. In this study, we developed an SPFS-based device with a convergent optical system. Parallel incident light was focused through the lenses and a prism onto the back side of sensor chip surface, giving a fixed range of incident light angles. We examined the SPFS-based device with a

* Corresponding author. Fax: +81 75 751 4119.
E-mail address: iwata@frontier.kyoto-u.ac.jp (H. Iwata).

[1] Current address: Office of Society–Academia Collaboration for Innovation, Kyoto University, Sakyo-ku, Kyoto 606-8501, Japan.

[2] *Abbreviations used:* POC, point-of-care; SPR, surface plasmon resonance; POCT, point-of-care testing; SPFS, surface plasmon field-enhanced fluorescence spectroscopy; AFP, α-fetoprotein; BSA, bovine serum albumin; EDC, 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride; NHS, *N*-hydroxysuccinimide; D-PBS, Dulbecco's phosphate-buffered saline powder; FBS, fetal bovine serum; LED, light-emitting diode; PMMA, poly(methyl methacrylate); CCD, charge-coupled device; ND, neutral density; SAM, self-assembled monolayer; ahAFP–A647, anti-human AFP 6D2 antibody labeled with AlexaFluor 647; a.u., arbitrary units; ELISA, enzyme-linked immunosorbent assay; CLIA, chemiluminescence immune assay; RIA, radioimmunoassay.

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convergent optical system for detecting AFP included in different concentrations of bovine serum albumin (BSA).

Materials and methods

Materials, reagents, and antibodies

11-Mercaptoundecanoic acid (Sigma–Aldrich, St. Louis, MO, USA), 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC; Dojindo, Kumamoto, Japan), ethanol and *N*-hydroxysuccinimide (NHS; Nacalai Tesque, Kyoto, Japan), and polyoxyethylene sorbitan monolaurate (Tween 20 equivalent, Wako Pure Chemical Industry, Osaka, Japan) were of reagent grade and used as obtained. Dulbecco's phosphate-buffered saline powder (D-PBS; Nissui Pharmaceuticals, Tokyo, Japan), BSA (Sigma–Aldrich), and AFP (HyTest, Turku, Finland) were used as obtained. Fetal bovine serum (FBS; Biowest SAS, Nuaille, France) was heated at 56 °C to inactivate the complement system before use. Water was purified with a MilliQ system (Millipore). Monoclonal mouse anti-human AFP antibody (clones 1D5 and 6D2) was obtained from Mikuri Immunological Laboratories (Osaka, Japan), and its solution was prepared and stored in accordance with supplier instructions. Anti-human AFP (clone 6D2) was labeled by fluorescence dye (AlexaFluor 647) following the supplier's instruction for the labeling kit (Invitrogen, Life Technologies, Carlsbad, CA, USA). The concentration of AlexaFluor 647-labeled anti-human AFP (clone 6D2) in the stock solution was determined by the method presented in the instruction from the absorbance at 280 and 650 nm and molar absorptivity (ε_{Ab} = 203,000) of antibodies.

Preparation of SPFS apparatus with convergent optical system

The SPFS apparatus with a convergent optical system (referred to as SPFS–convergent) prepared in this study is schematically shown in Fig. 2A (see Results). A 650-nm light source of pointed light-emitting diode (LED; BL15-1212, Kodenshi, Kyoto, Japan) was collimated using an aspheric optical lens (65989, Edmond Optics Japan, Tokyo, Japan). The light was passed through an iris (IH-08R, Sigma Koki, Tokyo, Japan) to cut the light of which the directivity was larger than 5° and then linearly *p*-polarized with an optical filter (NT47-215, Edmond Optics Japan). An S-LAL10 glass plate coated with a chromium underlayer (1 nm) and a thin gold layer (49 nm) was optically coupled to a hemi-cylinder prism via immersion oil (reference index 1.720; Cargille Laboratories, Cedar Grove, NJ, USA). A flow cell was assembled on the glass plate by fixing a poly(methyl methacrylate) (PMMA) plate through a spacer made of silicone rubber (thickness: 1 mm), and silicone tubes (inner diameter: 0.5 mm; outer diameter: 1 mm) were connected to the space on the glass plate through the PMMA plate [13]. The sample solution or D-PBS was delivered to the glass plate through the flow cell with a peristaltic pump (SMP-21, Tokyo Rikakikai, Tokyo, Japan). The *p*-polarized and collimated light was focused on the gold layer by an aspheric optical lens (48164, Edmond Optics Japan).

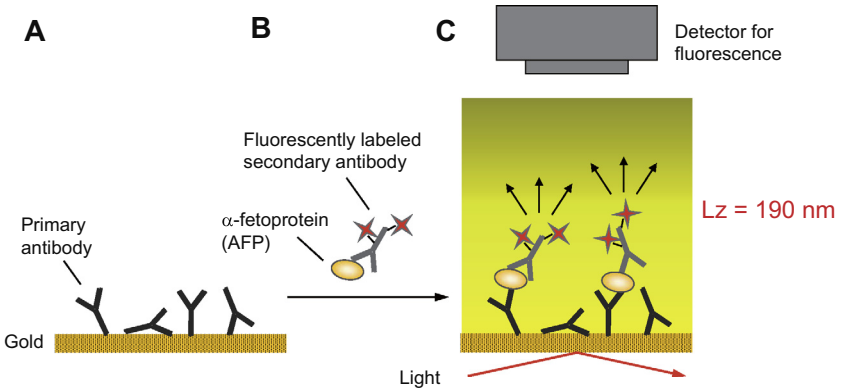
The reflected light was captured by a charge-coupled device (CCD; ICX086AK, image sensor for NTSC color video cameras, Sony, Japan) camera module (without cooling, outputting NTSC signals), and its image was recorded by a personal computer with recording software (VirtualDubMod version 1.4.13.1jp2) and analyzed by ImageJ (version 1.4.3). The fluorescence image on the surface of the glass plate was collected through an objective lens (SLWD Plan20×, Nikon, Tokyo, Japan) and 670-nm interference filter (Optical Coatings Japan, Tokyo, Japan) with a high-sensitivity EM–CCD camera equipped with a charge multiplier (MC681-SPD, Texas Instruments, Dallas, TX, USA) cooled by a built-in peltier cooler. Acquired images were captured with an image capture board (MT-PCI2, Micro-Technica, Tokyo, Japan) and analyzed with homemade intensity scanning software. The fluorescence light intensities in selected areas (100 × 100 pixels, corresponding to 210 × 210 μm) were determined.

Preparation of SPFS apparatus with angle rotation stage

The SPFS instrument of Kretschmann configuration was fabricated in-house (referred to as SPFS–1spot) [13] following the setup described by Liebermann and Knoll [10] with minor modifications. Briefly, a glass plate with a thin gold layer was coupled to a triangular prism via immersion oil (Cargille). A flow cell was prepared as mentioned above. A laser diode (Coherent, Santa Clara, CA, USA) was used as a source of incident light (λ = 635 nm, 0.95 mW). The laser intensity was reduced to 10% with a neutral density (ND) filter. The laser was linearly *p*-polarized and then irradiated through a triangular prism at the back side of the glass plate. The incident angle was kept constant during fluorescence detection. The reflected light intensity was monitored with a photodiode detector (S2281-04, Hamamatsu Photonics, Hamamatsu, Japan). The fluorescence image on the surface of the glass plate was collected by a CCD camera (MC681-SPD) and analyzed as mentioned above.

Immobilization of antibodies on sensor chip

Glass plates (S-LAL10, refractive index: 1.720; 25 × 25 × 1 mm) were purchased from Sigma Koki (Tokyo, Japan). They were coated with a gold layer as reported previously [13,15]. Briefly, S-LAL10



Scheme 1. Schematic illustration of SPFS-based immunoassay for the detection of AFP. (A) The primary antibody was immobilized on the sensor surface. (B) AFP–antibody complex was flowed over the sensor surface. (C) The primary antibodies captured the AFP complexes, and a fluorescence detector (CCD camera) captured the fluorescent image used by the AFP complexes bound on the sensor surface.