

Sensitive detection of Interleukin-6 (IL-6) on the plasmonic chip by Grating Coupled-Surface Plasmon-field enhanced Fluorescence Imaging

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1. Introduction

As for a sensitive immunosensor, we focused on the Surface Plasmon-field enhanced Fluorescence Spectroscopy (SPFS) based on the Surface Plasmon Resonance (SPR) in some powerful techniques. Recently, we have sensitively detected analytes with Grating Coupled-Surface Plasmon-field enhanced Fluorescence Imaging (GC-SPFI). In this study, Interleukin-6 (IL-6), which acts as both pro-inflammatory and anti-inflammatory cytokine, was detected on the plasmonic chip by the GC-SPFI instrument. IL-6 was quantitatively evaluated at low concentration due to the effect of fluorescence enhancement.

2. Experiments

Measurement

The GC-SPFI instrument we fabricated was composed of a He-Ne laser ($\lambda = 632.8$ nm) for irradiation light, an Electron Multiplying Charge Coupled Device (EM-CCD) camera (Andor, Ruca R) for detection, and a sample stage for horizontally setting a plasmonic chip. Laser light irradiated to the rear panel with scanning the incident angle by rotational mirror. The CCD camera was fixed at “the reverse coupling” angle; the fluorescence is re-coupled with surface plasmon polariton and re-emitted at the specific detection angle. Sample solutions were injected into silicon well on the plasmonic chip. The fluorescence intensities were analyzed from the fluorescence images taken every 1° of incident angle.

Preparation of the plasmonic chip

The replica with 500 nm pitches was fabricated by UV-nanoimprint lithography, and the chips were coated with a silver, titanium as an adhesive layer, and silica films by RF-sputtering. Sandwich immunoassay was applied on the plasmonic chip with the following procedures.

Sandwich assay for IL-6

The silica surface was aminated and anti-IL-6 capture antibody was immobilized with PEG cross-linker. After blocking with Bovine Serum Albumin (BSA), the IL-6 was dropped and detected using Alexa-labeled anti IL-6 detection antibody. IL-6 was prepared at concentrations of 0, 2, 5, 10, 20, 50 pg/mL.

3. Results and Discussion

Fig.1 shows the reflectivity and the fluorescence intensity plotted against the incident angle. The reflectivity

(broken line) was reduced at SPR angle. The fluorescence intensities (solid line) evaluated against the incident angle from 2° to 8° showed the fluorescence peak.

The fluorescence image of Alexa combining with IL-6 at the edge of grating was shown in Fig.2. Solid circle and broken square correspond to the laser spot and the grating area, respectively. In Fig.2, the fluorescence is obviously enhanced on the grating compared with that out of grating.

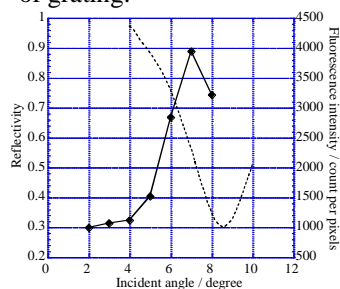


Fig.1 The reflectivity (broken line) and fluorescence intensity (solid line)

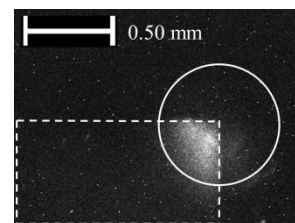


Fig.2 The fluorescence image of Alexa at the edge of grating

The fluorescence peak values were plotted for each concentration of IL-6 as shown in Fig.3. The fluorescence intensity at 0 pg/mL is due to non-specific adsorption. Against the IL-6 concentration of 50 to 2 pg/mL, the fluorescence intensity was quantitatively evaluated.

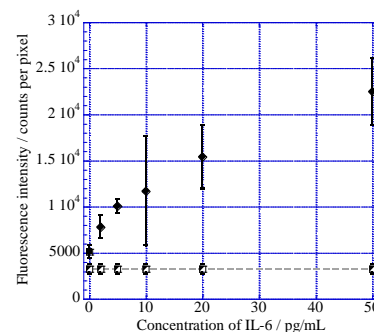


Fig.3 The calibration curve for the detection of IL-6

4. Conclusion

IL-6 was quantitatively detected against the IL-6 concentration of 50 to 2 pg/mL with the plasmonic chip by GC-SPFI instrument. Thus, GC-SPFI with the plasmonic chip was found to be a valid measurement tool for an immunosensor.

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