Color Surface Plasmon Resonance Imaging of Protein Microdot Arrays

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A novel method for readout of protein microdot arrays was presented by using a laboratory-built color SPR imaging system which used multiwavelength light as an excitation source and color CCD as an image detector. True color images were directly recorded from IgG microdot arrays. At the incident angles of 44–47°, the colors complementary to the resonance absorption were recorded, depending on the concentration of IgG between 0.05 and 1.00 mg/mL, which indicates that the color signals contain both qualitative and quantitative information.

As a highly sensitive surface analytical method, surface plasmon resonance (SPR) imaging is becoming an absorbing analytical platform applicable to the characterization of thin films and research of various biological processes.[1–5]

Although any light with wavelength longer than 520 nm, whether mono- or multiwavelength, can be employed to excite the resonance in SPR with gold sensing films,[6] SPR imaging was mainly conducted using monochromatic light as the excitation source.[7–9] Monochromatic SPR images were hence resulted. However, there were a few reports concerning with the exploration of SPR imaging with white or multiple wavelength light as the excitation sources. Knobloch et al.[10] were the first to characterize patterned silver films using white light. Johansen et al.[11] presented a type of SPR measurement based on multiple wavelengths to visualize varying bulk refractive indices in microwells. Otsuki et al.[12,13] developed a wavelength-scanning SPR imaging method to describe the structure of thin layer in nanometer scale. Recently, Yuk et al.[14,15] presented a spectral SPR imaging method, which introduced that resonance wavelength of protein spots was assigned to an appropriate color. However, there is no attempt to directly record a true color image from biomolecular microdot arrays and get some quantitative information from the true color image using the SPR imaging technique.

This paper aimed thus at exploring an approach to read directly the true color image of protein microdot arrays by multiple wavelength SPR imaging technique or color SPR imaging (CSPRI). Furthermore, qualitative and quantitative information could be obtained from the color signals.

A CSPRI device was designed and built in our laboratory which is shown schematically in Figure 1. The incident light emitted from an 8-W halogen lamp was collimated and polarized through a simple lens system. The whole body of prism/gold film/flow cell was mounted on a motorized rotational stage to regulate and optimize accurately the incident angle. The reflected light was imaged through an achromatic lens onto a color CCD detector (Watec Co., Ltd., Japan) working on the visible light spectrum, and the image data were acquired by a computer via a frame grabber card (Join Hope Image Technology Ltd., China).

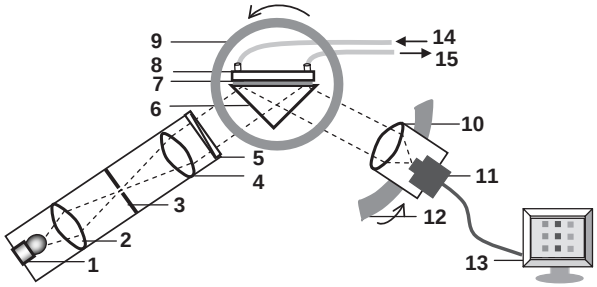

Figure 1. Schematic diagram of CSPRI system. 1, halogen lamp; 2, achromatic doublet; 3, diaphragm; 4, achromatic doublet; 5, polarizer; 6, prism; 7, gold sensor; 8, flow cell; 9, motorized rotational stage; 10, achromatic doublet; 11, CCD; 12, rotational arm; 13, computer; 14, inlet; 15, outlet.

Sheep immunoglobulin G (IgG) was chosen as testing protein and was immobilized on gold film in this study. The processes of immobilization were carried out at room temperature. A cleaned gold film (50 nm in thickness) was first modified chemically with carboxylic acid-terminated alkanethiol mercaptoundecanoic acid (MUA). The MUA-assembled gold film was further immersed into an aqueous solution of *N*-hydroxysuccinimide (EDC) and 1-ethyl-3-[3-(dimethylamino)propyl]carbodiimide hydrochloride (NHS) to form NHS ester monolayer. Onto the modified surface was spotted IgG dissolved in 10 mM sodium phosphate buffer (pH 7.4) at 0.05, 0.50, and 1.00 mg/mL, respectively. After reaction for about 5 h, the surface was rinsed with sodium phosphate buffer and then immersed in ethanolamine solution to block the remaining reactive sites.[16,17] The excessive ethanolamine was removed by rinsing with exhaustive water, and the surface was flushed with a stream of nitrogen gas.

Figure 2 shows the typical color images of IgG microdot arrays taken against air. The measurements were carried out at room temperature. Because the monomolecular layer of IgG with thickness $\approx$11 nm[18] matches the resonance conditions at our selected incident angles, both of the IgG microdots and their surroundings will produce incident angle-dependent color images (about 1 degree for eye recognition). The difference in thickness between IgG microdots and their surroundings can be read and detected by monitoring the color of their images. Interestingly, the color of protein dots varies with the concentration of IgG at the same incident angle, which implies that quantitative information should be included in the color signals. In a word, not only the type or thickness of sample but also its content can be discriminated from color images. As a color high-throughput readout technique, the CSPRI system can be very useful to identify and quantify protein microdot arrays, which is significant to proteomics study and new drug discovery

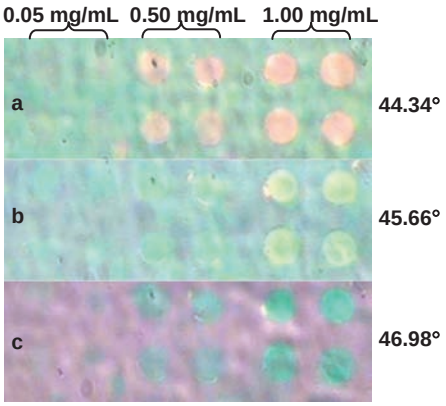

Figure 2. SPR images taken from IgG microdot arrays chemically immobilized at different concentrations obtained at the incident angles of 44.34, 45.66, and 46.98°.

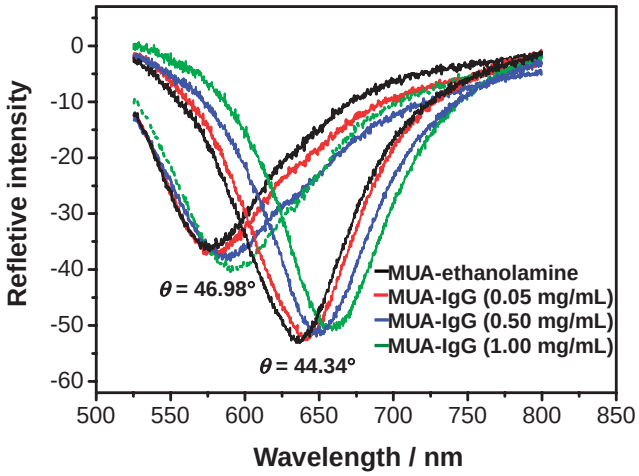

Figure 3. Spectra of IgG chemically immobilized on MUA-modified gold surface at various concentrations, measured at the incident angles of 44.34 and 46.98°, respectively. The absorbance curves indicate the complementary color which should be observed on their corresponding images.

and development.

With the aid of SPR method,[19] the spectra of IgG at various concentrations were measured and shown in Figure 3, which indicated the color of protein dots was complementary to that of the maximum SPR absorption as has been expected in theory.[10] For instance, at the incident angle of 44.34°, the MUA-ethanolamine-modified gold film had the maximum resonance absorption at 635 nm and the complementary color (the background) was bluish green (Figure 2a); while for IgG dots, because their maximum resonance absorptions shifted to 640, 649, and 660 nm at the concentrations of 0.05, 0.50, and 1.00 mg/mL, respectively, they developed their colors around buff (Figure 2a). When the incident angle increased to 46.98°, the background changed its color to violet (Figure 2c) because of the shift of the adsorption from 635 to 576 nm, but the protein dots changed their colors to greenish blues (Figure 2c) as the resonance absorption shifted to 579, 586, and 594 nm, respectively.

It should be noted that the resonance absorption wavelengths of IgG remained very close as its concentration varied. The samples were hence not distinguished from each other by color but by saturation.

In conclusion, CSPRI of protein microdot arrays have been demonstrated with successfulness using IgG as a testing sample. The measured color complementary to the maximum absorption of SPR was a type of analytical signals applicable to the determination of biological solutes at different concentrations. CSPRI can thus be expected as an intuitive imager for label-free biochips.

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