

# Characteristics of a Bimetal-Layer Chip of a Surface Plasmon Resonance Sensor in the Intensity Interrogation for Tumor Marker Detection

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## Abstract

The characteristics of a bimetallic surface plasmon resonance (SPR) chip were investigated to detect a tumor biomarker, carcinoembryonic antigen (CEA). The linewidth and the tangential slope of the reflectance curve of the bimetallic SPR chip was compared with those of the reflectance curve of a conventional gold (Au) SPR chip. The changes in reflectance in response to the variation in CEA in the critical concentration range were analyzed at an angle where the tangential slope of the reflectance curve was maximum. From linear regression analysis, the sensitivity of the bimetallic SPR chip with respect to the CEA in critical concentration was obtained.

**Keywords:** Bimetal, Surface Plasmon Resonance, Reflectance, Tumor, Carcinoembryonic Antigen

## 1. INTRODUCTION

Over the last two decades, a surface plasmon resonance (SPR) sensor has gained research interests in life-science technologies including drug discovery, medical diagnostics, food safety, and environmental monitoring owing to its extremely high sensitivity, rapidity, and label-less detection [1-3]. The SPR sensor is basically an optical sensor which is very sensitive to the refractive index change at or near the noble metal (e.g., Gold (Au), silver (Ag)) surface [1-3]. Even though the SPR sensors are based on optics, it works well in turbid samples. The common configuration (Kretschmann configuration) of the SPR sensor has the noble metal film sandwiched between the prism and the sample medium. The p-polarized light illuminates the metal film through the prism, and the reflectance from the metal film is monitored by a photodetector. As the incident angle increases, the reflectance dips and attains a minimum at an angle, and gradually rises from the minimum. The angle at which the reflectance is minimum is known as the SPR angle. The curve of the reflectance

versus the incident angle is called the reflectance curve. When binding events such as binding of the analyte to the receptor immobilized on the metal surface occur, the reflectance curve and the SPR angle shift due to the refractive index change. There are four interrogations for the SPR sensor under the Kretschmann configuration, namely, angle, intensity, wavelength, and phase interrogations [4]. Among these, the intensity interrogation has attracted interests owing to the choice of open window as the material. [5-7]. In the intensity interrogation, the incident light is stationary at the angle with the steepest tangential slope of the reflectance curve, and the reflectance is monitored. To enhance sensitivity in the intensity interrogation, a narrower linewidth of the reflectance curve enables a steeper tangential slope. Optical properties of Ag make the linewidth narrower [6,8]. Thus, the addition of Ag to the conventional Au SPR chip to enhance the sensitivity or signal-to-noise ratio has been one of the main subjects of research [4-8].

Directional immobilization of the receptor is an efficient way to improve sensitivity of biosensors. Protein G has been known as an antibody binding protein, and three immunoglobulins G (IgG) binding domains were at the COOH-terminal part of the protein G, which mainly interacts with fragment crystallisable (Fc) region of IgG [9-11]. If protein G is formed on the sensor surface, it seizes Fc region of IgG. Thus, antigen binding site, fragment antigen-binding (Fab) fragment of the antibody can be exposed to outer side, enabling antigen to bind with the antibody more effectively.

Carcinoembryonic antigen (CEA) is known as a serum cancer biomarker associated with colon cancer, lung cancer, breast cancer, and other cancer in clinical tests [12,13]. Normal CEA

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level for healthy people lies between 3 ng/ml and 5 ng/ml, and the CEA level can increase up to 10 ng/ml due to other benign diseases. If the CEA level is over 20 ng/ml, then the cancer patients are considered to be in metastatic state [12,13].

In this study, a bimetallic SPR chip composed of Au and Ag was prepared to detect minute concentration of the tumor marker, CEA. Significant factors of the bimetallic SPR chip in the intensity interrogation were investigated and compared to those of the conventional Au SPR chip. Anti-CEA was immobilized using protein G, and the responses of the bimetallic SPR chip with respect to various CEA concentrations were analyzed. Linear regression is used to calculate the sensitivity obtained from the plot of SPR response versus CEA concentration.

## 2. EXPERIMENTAL

P-polarized incident light from a light emitting diode (LED) (770 nm) excites surface plasmons. The wedge shaped beam with an angle range of  $7.14^\circ$  passes through the hemisphere-shaped prism (BK-7) and is shone on the metal film, which is in direct contact with the prism. The reflected beam from the metal film is acquired by the 2D-CMOS image sensor. The bimetallic chip was prepared on the BK-7 glass substrate using e-beam evaporator. The optimized thicknesses of Ag and Au were 40 nm and 10 nm, respectively. The optimization of the SPR chip was conducted by considering linewidth, the maximum slope and reflectance (R) variation responding to refractive index (n) change obtained from the calculated reflectance curves [14]. Total thickness of Ag and Au layers was fixed at 50 nm, and five combinations of Ag/Au ratios, which were 40 nm/10 nm, 35 nm/15 nm, 30 nm/20 nm, 25 nm/25 nm, and 30 nm/20 nm were compared. Among the five combinations, the Ag/Au (40 nm/10 nm) chip showed the narrowest linewidth ( $0.82^\circ$ ), the largest maximum slope ( $170\%/^\circ$ ), and the greatest reflectance variation responding to refractive index changes ( $R_{n=1.337} - R_{n=1.335} = 30.73\%$ ). The system has three sample channels, out of which one channel is used as a reference channel to eliminate environmental interference. All SPR responses due to biomolecule interactions were obtained by deducting the reference channel signal from the sample channel signal. For the immobilization of antibodies, at first, protein G was formed on the SPR chip. Then, the antibody of CEA (anti-CEA) was injected to be immobilized on the protein G-formed SPR chip. Next, bovine serum albumin (BSA) was flowed over the SPR chip surface to prevent non-specific binding events. Various concentrations of CEA were finally injected into the SPR sensor

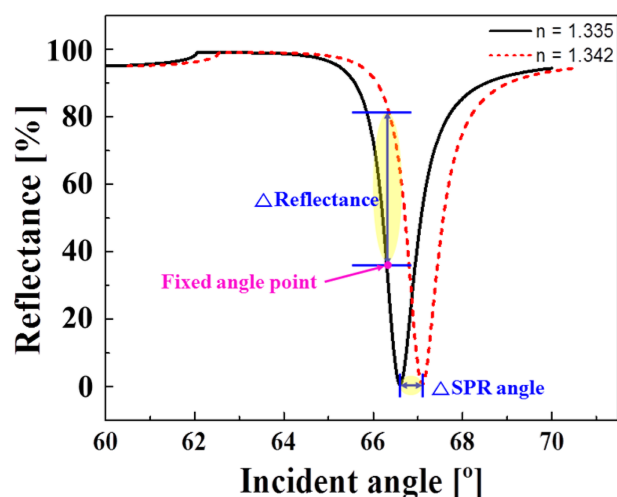


Fig. 1. Shift of the reflectance curve due to refractive index change in sample medium

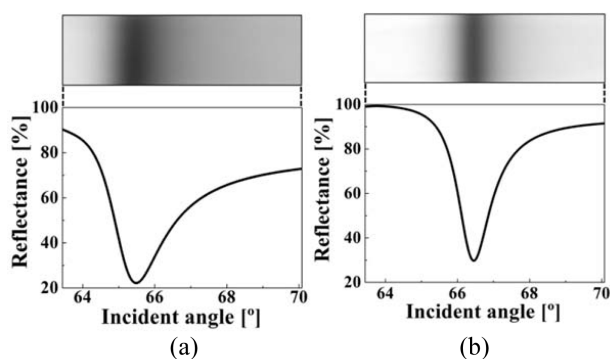
system, and the SPR responses were analyzed. Three identical experiments were carried out to get an average SPR response. Flow rate of all samples were kept at  $10 \mu\text{l}/\text{min}$ .

Phosphate buffered saline (PBS), protein G recombinant and BSA were purchased from Sigma Aldrich (MO, USA), and CEA and anti-CEA were purchased from abcam (Cambridge, UK). All proteins were dissolved in the PBS solution.

The theoretically calculated reflectance curves are shown in Fig. 1 [14]. Fig. 1 shows the shift of the reflectance curve due to the change in refractive index in the sample medium. The refractive index change can be confirmed by the change of the critical angle shown in Fig. 1. Detection principles of the angular and intensity interrogations can be explained using the graphs presented in Fig. 1. As the reflectance curve shifts the SPR angle also shifts, and in the angular interrogation mode this SPR angle variation provides the refractive index change at or near the SPR chip surface. On the other hand, at a fixed incident angle the reflectance is changed by the shift of the reflectance curve due to the refractive index change in the sample. In our interrogation mode, we monitored the reflectance associated with a fixed incident angle with the steepest tangential slope in the reflectance curve, so that the largest reflectance variation can be achieved.

## 3. RESULTS AND DISCUSSIONS

Reflection images and corresponding reflectance curves for the conventional Au chip and the bimetallic chip are shown Fig. 2(a) and Fig. 2(b), respectively. The dark portions in the reflection

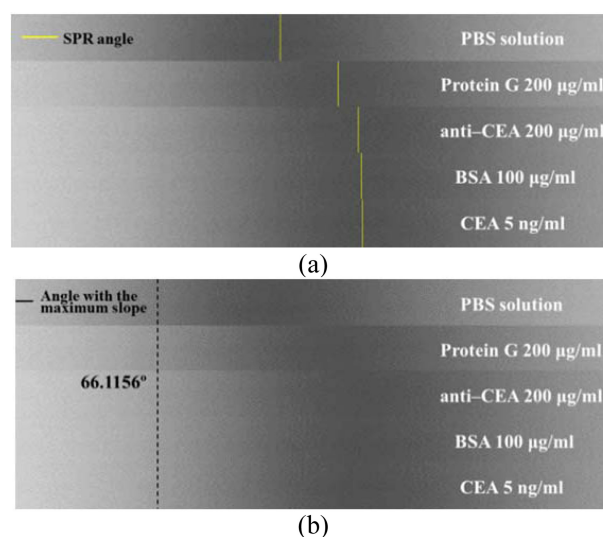


**Fig. 2.** Reflectance images acquired by the CMOS image sensor (upper image) and the corresponding reflectance curves (lower image) with responding to the PBS solution. (a) Conventional Au SPR chip, (b) Bimetallic (Ag/Au) SPR chip

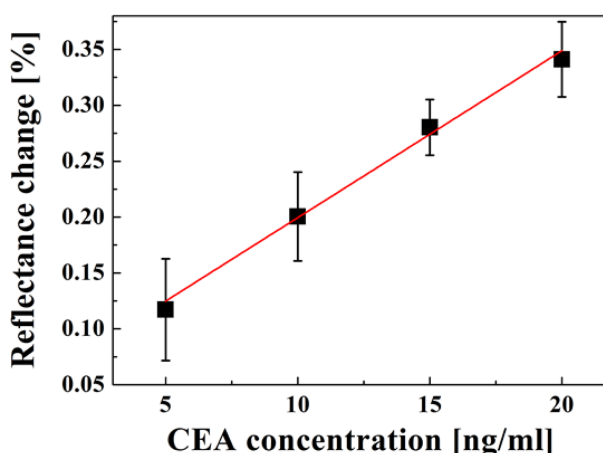
images signify that the incident light was absorbed by the metal films. The SPR angles of the Au (48 nm) chip and the bimetallic chip for a PBS solution were  $65.4856^\circ$  and  $66.4504^\circ$ , respectively. As shown in Fig. 2, the linewidth (full width at half maximum: FWHM) of the Au chip ( $1.871^\circ$ ) was broader than that of the bimetallic chip ( $1.105^\circ$ ). The maximum slope of the bimetallic reflectance curves ( $94.6774\%/^\circ$ ) was steeper than that of the Au reflectance curve ( $67.4052\%/^\circ$ ) as expected. Thus, in the intensity interrogation mode it would be more profitable to use the bimetallic chip to get the larger output signal.

For the detection of CEA, anti-CEA has to be immobilized on the surface of the SPR sensor chip. As shown in Fig. 3(a), the SPR angle shifts to the right from the SPR angle for the PBS solution as protein G, anti-CEA, BSA, and CEA were sequentially injected into the sensor chip. Adsorption of proteins causes the change in refractive index at the SPR sensor chip surface, resulting in the shift of the SPR angle. In the reflectance curve for PBS, the incident angle with the maximum tangential slope in the reflectance curve was  $66.1156^\circ$ . The black vertical dash line in Fig. 3(b) indicates the incident angle where the tangential slope of the reflectance curve for PBS is maximum. The reflectance was monitored at this angle. At this fixed angle ( $66.1156^\circ$ ), the average reflectance changes responding to 5 ng/ml, 10 ng/ml, 15 ng/ml, and 20 ng/ml of CEA were 0.1172%, 0.2005%, 0.2803%, and 0.3411%, respectively. The standard deviation of the reflectance of the PBS solution at this fixed angle without any proteins (the blank) was 0.00547%, and thrice this value is equal to 0.0164%. The reflectance changes corresponding to the changes in the CEA concentrations in the range from 5 ng/ml to 20 ng/ml were considered to be meaningful, since they were all more than three times the standard deviation of the blank value.

Fig. 4 represents the calibration curve obtained for CEA



**Fig. 3.** SPR reflectance images of the bimetallic SPR chip responding to PBS, protein G, anti-CEA, BSA, and CEA of 5 ng/ml. (a) Yellow vertical lines indicate the corresponding SPR angles. (b) Black vertical dash line indicates the incident angle where the tangential slope of the reflectance curve for PBS is maximum.



**Fig. 4.** Calibration curve for the bimetallic SPR chip responding to CEA concentrations in the range from 5 ng/ml to 20 ng/ml. Error bars represent the standard deviation calculated from three identical experiments.

detection using the bimetallic SPR chip. Reflectance change has a linear relationship with CEA concentration over the range of 5–20 ng/ml. The sensitivity, that is linear slope of the calibration curve in Fig. 4 was  $0.01493\%/(\text{ng/ml})$ . Error bars in Fig. 4 represent the standard deviation calculated from three identical experiments. From the obtained values, the calculated detection limit was 1.1 ng/ml which is below the commonly used CEA concentrations for diagnosis. The correlation coefficients obtained in Fig. 4 was 0.9962.

#### 4. CONCLUSIONS

In this study, the characteristics of the bimetallic SPR chip were investigated. The linewidth and the tangential slope of the bimetallic SPR reflectance curve were 0.59 times narrower and 1.41 times steeper than those of the conventional Au SPR reflectance curve, which had relatively higher sensitivity in the intensity interrogation. Responses of the bimetallic SPR chip with respect to various concentrations of the tumor biomarker, CEA were analyzed. The reflectance change was linearly related to the CEA concentrations ranging from 5 ng/ml to 20 ng/ml. Thus, the proposed bimetallic chip and the SPR detection scheme, intensity interrogation would aid to diagnose cancers in clinics.

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