Optimized Sandwiched Surface Plasmon Resonance Enhanced Biosensor for Multiplex Biomarker Detection

Tianxun Gong[1], U.S. Dinish[2], Shuwen Zeng[1], Malini Olivo*[2,3], Ken-Tye Yong*[1]
[1]School of Electrical and Electronic Engineering, Nanyang Technological University, Singapore, 639798
[2]Bio-Optical Imaging Group, Singapore Bioimaging Consortium (SBIC), Agency for Science Technology and Research (A*STAR), 11 Biopolis Way, Singapore, 138667,
[3]Bio-photonics group, School of Physics, National University of Ireland, Galway, Ireland
Email: *malini_olivo@sbic.a-star.edu.sg
: *ktyong@ntu.edu.sg

Abstract— **In this study, we performed finite element method (FEM) simulations to optimize the configuration of gold nanorods (GNR) enhanced surface plasmon resonance (SPR) sensor and discovered its application for multiplex antigens detection. Our work analyzed the near-field coupling between the sensing film and GNR. By systematically study the effect of gold film thickness, GNR-to-film distance and GNR dimensions on SPR, it was found that for GNR width smaller than 40nm, length change in GNR brought about significant SPR wavelength shift on the sensor, while the sensor is insensitive for GNR-to-film distance. As an application, we adopted GNRs of width 20 nm and aspect ratios from 2 to 4 and demonstrated the concept of conjugating gold film and GNRs with anti-Immunoglobulin G (anti-IgG) antibodies for multiplex detection of various IgG proteins with more than 100nm separation on their SPR wavelengths.**

Keywords- Surface plasmon resonance; biosensor; gold nanorods; multiplex detection; IgG protein

I. INTRODUCTION

Surface plasmon resonance based sensors have been widely used to study biomolecule interaction during recent years. Generally, as light beam totally reflected from the interface between waveguide and outer medium, an evanescent filed will propagate along the surface of interface. As described by widely used Kretschmann configuration [1], when a metal (typically gold for its chemical stability) film is coated on the surface of glass prism, SPR will be stimulated when the frequency of photons matches the natural frequency of surface electrons oscillating against the restoring force of positive nuclei, which can be detected by measuring the absorption of reflected light. SPR is highly sensitive to the external medium, a small refractive index change at the metal film/analyte surface will result a significant shift of wavelength or light incident angle for SPR [2]. SPR biosensors use this phenomenon to measure the amount of bound analyte and subsequently the concentration of analyte in a sample. Thus the changes in the refractive index due to analyte binding to the specific receptor, which is immobilized on gold film coated sensing surface can be monitored by SPR biosensor [3].

There is an increasing demand of developing biosensor devices which are capable of detecting biomarker proteins for human diseases. These applications require biosensors technologies to be sensitive, rapid and cost-effective. SPR biosensor technology is a well-established technique and has advantages such as real-time monitoring of molecular interactions [4], specific for a variety of different analytes [5] and comparably low cost and high sensitivity [6]. Many types of detection formats have been developed for SPR sensors based on type of targeting analytes, binding characteristics and concentration of samples. The most commonly adopted formats as described by J. Homola [4] are direct binding format, sandwiched binding format, competitive binding format and inhibition binding format. Considering small amount of analytes usually cannot generate a sufficient refractive change, sandwiched binding format receives more attention due to its improvement on sensitivity.

Various modifications and extensions of exploiting metallic nanoparticles in SPR biosensors have been developed because their surfaces are rich of free electrons thus bring high sensitivity to the surrounding environment. When electromagnetic wave directed to metallic nanoparticles which have much smaller size than the incident wavelength, it will introduce a plasmon that oscillates locally around the nanoparticles [7]. Similar to SPR, this phenomenon is known as localized surface plasmon resonance (LSPR), which is sensitive to the local dielectric environment changes. When combining metallic nanoparticles with SPR biosensors as a sandwiched structure, the metallic nanoparticles not only induce the increase in binding mass and refractive index, but also introduce the combination of LSPR with SPR which perturb the evanescent field thus led to enhanced sensitivity. Natan's group demonstrated the use of gold nanoparticles (GNP) in SPR sensors to increase the sensitivity through the binding of a secondary antibody in the sandwich detection format in 1990s [8, 9]. Then in next decade, several groups published their research on GNPs enhanced SPR biosensors. Hutter's

and Misawa's group described DNA Hybridization detection by using SPR interaction between GNPs and gold substrate film [10, 11]. SPR based GNPs labeled sensitive kinetic assay for carbamate–acetylcholinesterase interaction has been developed by Zhang's group [12]. Recently, a sensitivity improved SPR biosensor for biomarker detection has been described by Prasad's group [13]. Instead of using GNPs, they adopted GNRs for their unique behavior on tunable longitudinal plasmonic peak which enables an effective plasmonic coupling between sensing film and nanoparticle. By applying this platform they are able to detect tumor necrosis factor alpha (TNF-R) antigen with extreme high sensitivity estimated to be 0.03 pM, which is 1-2 orders of magnitude higher than the traditional immunoassay method. Inspired by this method, in this work, we systematically studied the effect of GNR dimensions, GNR-to-film distance and gold film thickness on SPR sensor. We then proposed an optimized sandwiched SPR biosensor for multiplex biomarker detection by using gold sensing film coupling with GNRs with different aspect ratios.

II. METHOLOGY

A. Model Setup

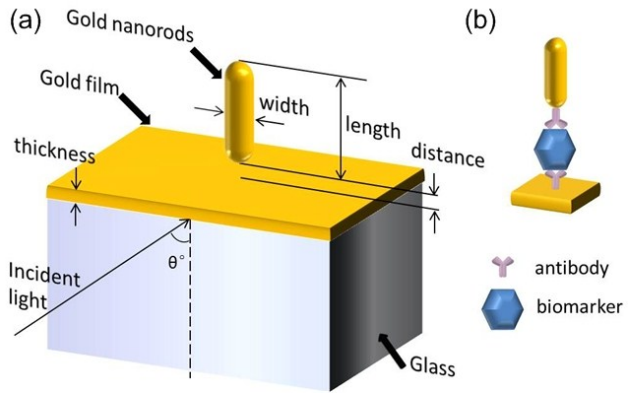

Figure 1. (a) Schematic of sanwitched SPR enhanced biosensor model.(b) Sandwichted structure formed by GNR, biomarker and gold sensing film.

As shown in Figure 1(a), the glass block is evenly coated with a thin gold film as a sensing film. In order to introduce the GNRs forming the sandwiched structure for biomarker detection, the sensing film surface is first immobilized with capture antibodies. Then the sample solution containing biomarkers which are highly specific to the capture antibodies be applied to the surface. After biomarkers captured by antibodies on the surface, GNRs solution which immobilized with capture antibodies previously will be applied to the film surface. They will bind to the captured biomarkers on the sensing film surface thus to form the second layer for the sandwiched structure, as shown in Figure 1(b). According to Prasad's group's experimental result, the GNRs are able to vertically bind to the film surface[13]. When optical light is projected to the interface between the glass block and gold film with a certain incident angle, the SPR and LSPR will occurs thus the reflected light will have an absorption peak at specific wavelength, the biomarkers therefore can be detected. Since SPR and LSPR are highly sensitive to environment changes, we need carefully study the effect of GNR dimensions, GNR-to-film distance and gold film thickness on SPR and optimize the system for multiplex biomarker detection.

B. Simulation Setup

The studies are performed by using FEM simulation software COMSOL Multiphysics. The model is solved in 2D RF module, where TM in-plane waves are adopted in order to excite the SPR. TM planar wave is generated at a port boundary condition from the bottom of the glass propagates along y direction which is defined as

$$k1x = k_0\_rfwh * n1 * \cos(\alpha) \quad (1)$$

$$k1y = k_0\_rfwh * n1 * \sin(\alpha) \quad (2)$$

$$H_{0z} = \exp(-i * k1y * x) \quad (3)$$

where α is the propagation angle, k_0_rfwh is a built-in parameter for free space wave number. n1 refers the real part of the refractive index of glass and k1x is the propagation constant. Since tilt incident wave is introduced, we use Floquet periodic boundary condition for the left and right boundary in y direction. For first medium (glass, n=1.51108) which light propagates in, the Floquet boundary condition is Eq. (1) and Eq. (2). While for second (gold film) and third layer (water, n=1.33), n1 in these two equations should be replaced by their refractive index. Since light will be refracted when propagates through each interface, which can be calculated through Snell's law, α in these two equations should also be replaced by the refraction angle in each medium respectively. The boundary condition ensures that a wave, when reaching the bottom boundary transmits to the top boundary with the appropriate phase shift. By this, no artificial effects will be introduced to modeling region. We use perfect match layer (PML) to ensure no reflection from top boundary for calculation accuracy. The simulation is performed by using wavelength modulation through wavelength scan and the absorption power of Ey (y component of electrical field) will be calculated out.

III. REULTS AND DISCUSSION

To study various parameters and their effect to SPR, the angle of incident light should be determined first. According to Homola's group's study [2, 14, 15], when the incidence angle of the light source is set to a small value ($\leqslant 65^o$), the SPR occurs with longer resonance wavelength and a high sensitivity. However, the SPR peak will become broader for smaller incident angle. Therefore, we fix the incident angle at 67^o to compromise both the sensitivity and peak width.

A. Gold Film Thickness

The thickness of gold film is a very important factor for SPR enhancement. In this study, the distance between GNR and gold film is fixed at 10nm. To simplify the simulation,

we treat GNR as a cube, thus the width and length are equal. In order to find the optimal thickness which gives the maximum SPR enhancement, for each cube size, the SPR peak power for different gold film thickness is calculated by wavelength scan. As shown in Figure 2, when GNR size increases from 5nm to 50nm, the gold film thickness which brings maximum SPR decreases from 42nm to 31nm. This result enables us to find an optimum thickness range according to the GNR size we expect to use for the biosensor. Furthermore, it confirms that the coupling of GNR on SPR sensing film will bring a perturbation to the evanescent field thus causing SPR intensity change. Since for most synthesizes and applications, the width of GNRs range from 10nm to 30nm [16], we choose the gold film thickness to be 40nm for best SPR enhancement.

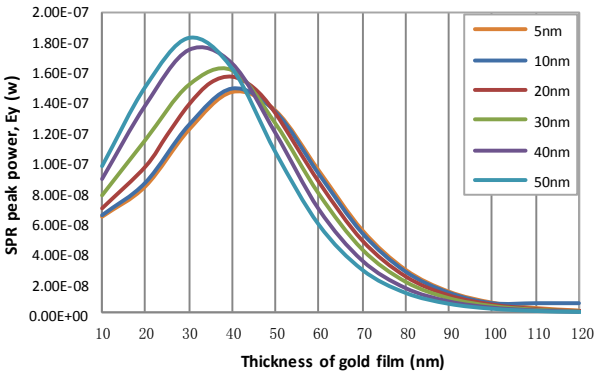

Figure 2. The SPR peak power for gold film thickness varies from 10nm to 120nm for different GNR size.

B. GNR-to-film Distance

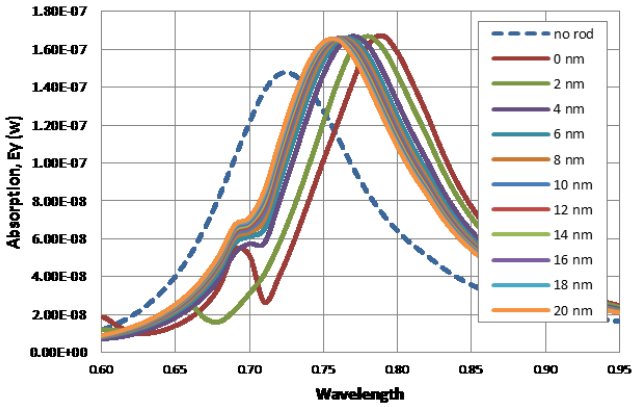

Figure 3. The SPR absorption spectra without GNR and with GNR by varing GNR-to-film distance.

The distance between GNR and film depends on the linker, antibody and protein size. In this study, the GNR size is set to be 20nm (cube size for simplify calculation) with a 40nm thickness gold film. Since we are going to demonstrate biosensor for IgG proteins detection, consider the compound length of IgG protein, anti-IgG antibody and linkers is between 10nm to 20nm, we therefore focus on the SPR effect with distance varies from 0nm to 20nm. Results in Figure 3 indicate the distance changes have slightly effect on SPR, less than 10nm for distance change from 10nm to 20nm. This interesting result indicate that the biosensor is less sensitive to distance change, thus the dimension changes for different IgG proteins and antibodies will have negligible effect for the sensing accuracy. It should be noted that, compare with SPR without GNR, existence of even a such small GNR shows a significant perturbation to the SPR field, introduces not only SPR wavelength shift but SPR enhancement.

C. GNR's Length and Width

As already observed from above results, the existence of GNR has strong effect to SPR field, both wavelength and intensity. It is necessary for us to study the SPR by changing width and length of GNR. The distance between GNR to film is fixed at 10nm. Figure 4 shows increase either length or width results red-shift on SPR peak wavelength. However, the result indicates SPR is much more sensitive to GNR length changes. Especially for GNR has a length between 30nm to 90nm and a width between 10nm to 40nm, SPR shifts ~50nm for each 10nm increase on GNR length, only less than 20nm shift for same increase on GNR width, on the contrary. This may due to the electrical field has most components on y direction which are perpendicular to the gold film and therefore excite the longitudinal SPR mode on GNRs. It should be noted that most type of GNRs have dimensions in this region [17].

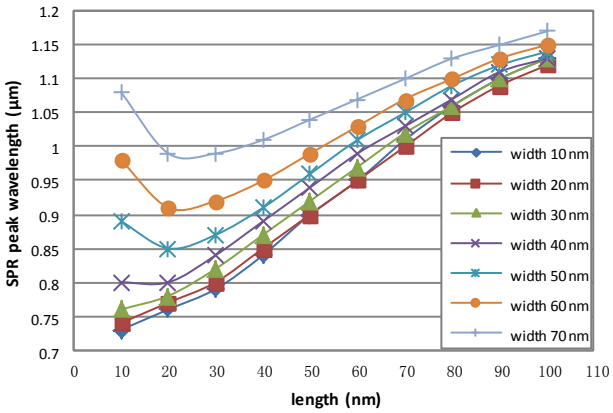

Figure 4. SPR peak wavelength for different GNR width and length on gold film.

D. Biosensor Concept for Multiplex IgG Proteins Detection

Based on the characteristics we found, we optimized this sensing system and proposed the concept of using GNRs with different aspect ratio for multiplex IgG proteins detection. Consider this SPR sensor is more sensitive to GNR length changes, we select three types of GNRs for multiplex detection. These three types of GNRs have 20nm in width and 40nm, 60nm, 80nm in length, respectively. According to the results in Figure 4, these three types of GNRs should result different SPR shift significantly. Mouse-IgG, human-IgG and rabbit-IgG proteins were chosen to demonstrate the concept. Since their size different is small

thus their size different effect to SPR can be neglected, as we shown in Figure 3. Therefore, we set the GNR-to-film distance to 20nm with a 40nm thick gold film. In order to perform multiplex detection, the gold film is immobilized with three types of antibodies (anti-Mouse-IgG, anti-human-IgG and anti-rabbit-IgG) first to specifically capture the IgG proteins. While three types of GNRs are immobilized with these three types of antibodies correspondingly. The anti-IgG antibodies immobilization processes are well established [18, 19]. Then sample solution contains certain type of IgG protein is applied onto the sensing film. At the last step, solution of GNRs with immobilized antibodies is applied onto the film. Since only the type of GNRs with corresponding immobilized antibody to the IgG protein in the sample solution will be captured to the film, therefore, the specific SPR peak which corresponding to the certain GNR will exist. As shown in Figure 5, according to the SPR absorption peak, we are able to tell the type of GNR and its corresponded IgG protein in the sample solution with SPR wavelength separation more than100nm.

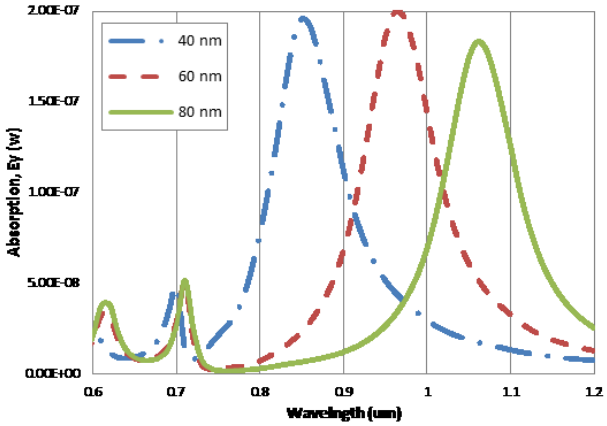

Figure 5. SPR absorption spectra for GNRs with 20nm width and aspectratio from 2 to 4 on 40nm gold film with 20nm distance.

IV. CONCLUSION

In this work we have demonstrated GNR combine with gold film for a SPR-based biosensor. The effects of GNR dimensions, GNR-to-film distance and gold film thickness on SPR were studied through FEM simulation. We found (i) the optimized thickness for the sensor is ~40nm for maximum SPR absorption, (ii) SPR sensor is insensitive to the GNR-to-film thickness within 10nm-20nm range, (iii) Increase in GNR length results significant SPR red-shift while the sensor shows much less sensitive to GNR width change. Based on these results, we demonstrated adopting three types of GNRs with 40nm, 60nm and 80nm in length and 20nm in width for an optimized sandwiched SPR enhanced biosensor for Multiplex IgG proteins detection. According to our concept and simulation result, the biosensor is able to detect these proteins by SPR absorption peak corresponding to different types of GNRs. We believe this biosensor can achieve rapid, cost effective multiplex biomarker detection not only for IgG proteins but wide range of biomolecular interactions.

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