

A NANOBIOSENSOR FABRICATED BY NANOIMPRINTING TECHNOLOGY

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Abstract: A new biosensor with a high sensitivity and a low process cost is presented in this paper. Localized surface plasmon resonance generated inside the nanogrooves was verified to have a high sensitivity and this structure could be produced using a nanoimprinting technology which realizes a high reproducibility and a mass-production. The present sensor had five times as high sensitivity as the conventional colloidal localized surface plasmon resonance sensor. And the detection of BSA antigen using the present sensor was attained in this research.

Keywords: Biosensor, Surface Plasmon Resonance, Nanoimprinting

1. INTRODUCTION

Biosensors detecting the proteins' interactions are expected to gain importance as devices for the rapid diagnosis of the incipient disease, and to realize the preventive medical care. Among the various detection techniques available (e.g., fluorescence), localized surface plasmon resonance (LSPR) has received great attention since it does not require any labeling of the analytes and enables real-time sensing [1]. LSPR is a physical phenomenon which is caused by the interaction between the free electrons in metal and the incident light. When the frequency and wave number of the incident light corresponds to the resonance eigenvalues of the metal surface, a strong coupling occurs and the energy of the incident light is transferred to the oscillation of the free electrons. Since these eigenvalues of the metal surface depend on the refractive index of the ambient materials, the protein interactions on its surface can be detected as a change of the surrounding refractive index. The eigenvalues are also decided by the shape and sizes of the metal nano-structure. To fabricate the metal nano-structures, a number of techniques have been proposed, such as a fixation of metal colloids onto substrates and the nanosphere lithography [2, 3]. Despite these advances in LSPR technology, the sensitivity and process cost for the sensor chips are not satisfactory yet, especially for the commercial products. In this study, we realized a high sensitivity and a low process cost of the sensor chip by incorporating the nanoimprinting

technology [4] with the fabrication of the LSPR sensor chips.

2. MATERIALS AND METHODS

2.1 Fabrication of sensor chip

Fig. 1 shows the process diagram of the sensor chips. Periodic nano grooves were prepared on the master substrate by semiconductor procedures (Fig. 2). Then a metal mold was fabricated from the master substrate using a nickel electroforming process (SA2000, Digital Matrix Co.). This mold was used to produce replicas, subsequently. An acrylic based polymer resin was dropped onto the glass substrate (S1224, Matsunami Glass Ind., Ltd) and this mold was pressed on it with UV dose. Many replicas can be produced from the same one metal mold and the process cost and the

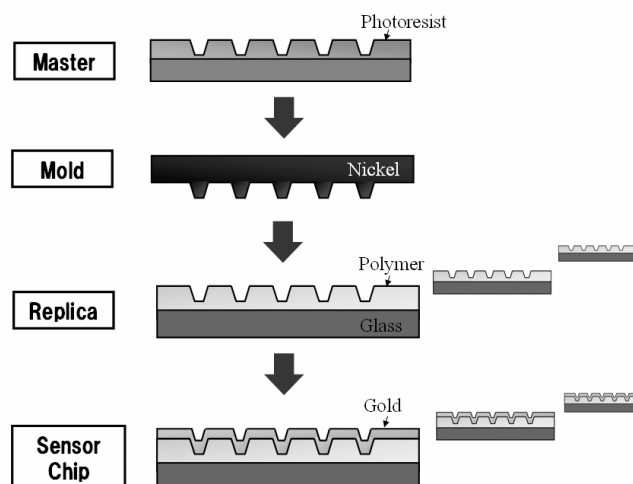


Fig. 1 Fabrication process diagram

reproducibility can be, therefore, much improved by this method. Finally a gold layer was sputtered onto the nano-patterned polymer surface for the generation of LSPR. Fig. 3 shows a picture of the fabricated sensor chip. The nano-patterned area had a different color from the surrounding area, which was caused by the absorption by LSPR.

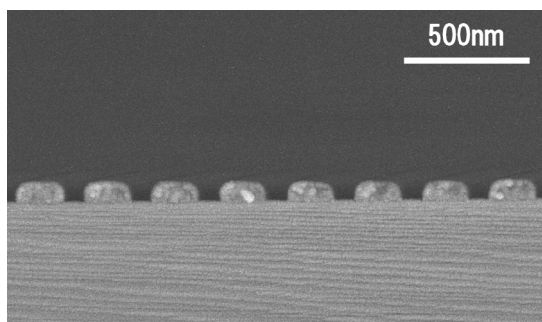


Fig.2 SEM image of the cross-sectional nanopatterns.

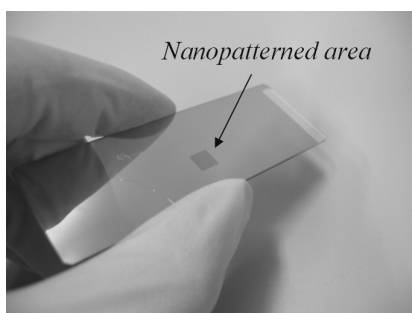


Fig.3 A picture of the LSPR sensor chip

2.2 Preparation for a biosensor surface

For the detection of the specific target proteins, a modification of the sensor surface with the probe proteins which binds to the complementary proteins is necessary. In the LSPR sensor system, the sensing region from the surface is under several tens nanometers. And the closer to the sensor surface the association occurs, the more sensitive the sensor can be. Therefore, the thickness of the probe layer should be as thin as possible. To realize a thin probe protein layer, we adopted a commercialized linker layer (Orla 18, Orla Protein Technologies Ltd.) in which the protein A and self assembled monolayer protein are fused. The antibodies for the detection of the specific target proteins were immobilized onto this linker layer.

2.3 Experimental setup

Fig. 4 shows the experimental setup to observe the LSPR characteristics. Linearly polarized white light was collimated and irradiated to the nanopatterned sensor surface. And the reflection light was collected to the spectrometer (MK-300, Bunkoukeiki Co.) equipped with a cooled CCD camera (DU420-OE, Andor Technology). Home-made flow cell was attached to the sensor chip to change the sample materials.

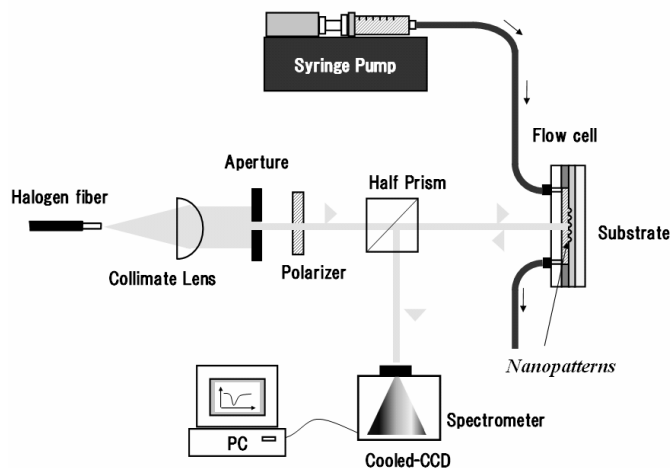


Fig.4 Experimental setup

3. SIMULATION

3.1 Calculation of reflection spectra

The simulation model depicted in Fig. 5 was figured out from the AFM and SEM measurement results of the fabricated sensor chip. This model is periodically aligned along x-direction. Fig.6 shows the reflection spectra from the patterned surface of this substrate calculated by RCWA (Rigorous Coupled Wave Analysis) method. The incident light was linearly polarized along the x-direction and its incident angle was normal to the sensor surface. The refractive index (n) of the sample was changed from 1.00 to 1.33 and 1.36. The reflection dips were at 648.8, 859.4 and 875.1nm, respectively. The decrease of the reflection under the wavelength of 550 nm corresponds to the interband transition of gold. The sensitivity calculated from the peak shift between $n=1.33$ and 1.36 was 522.3 nm/RIU, while the sensitivity of the conventional gold colloidal LSPR sensor is around 100 nm/RIU.

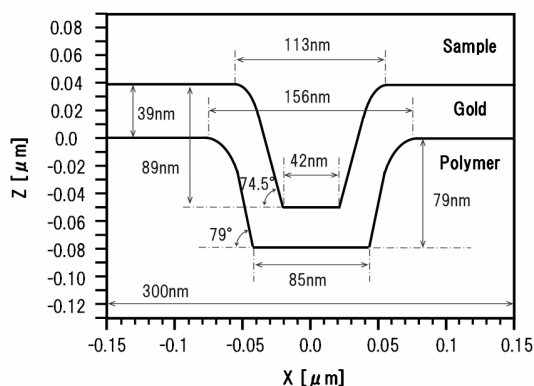


Fig.5 Model for RCWA simulation

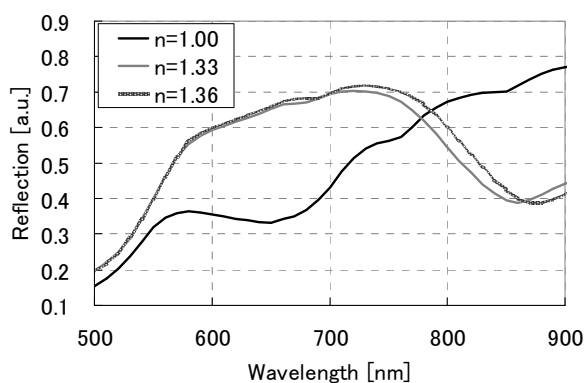


Fig.6 Reflection spectra calculated by RCWA

3.2 Calculation of the electric field distribution

When the coupling between the incident light and the free electrons in the metal occurs, strong enhancement of the electric field is generated in the very vicinity of the metal surface. This enhanced area corresponds to the sensing region in which the resonant condition is affected by the change of the refractive index. One main characteristic of the LSPR is that this sensing region is confined under the light diffraction limit, generally several tens nanometer, from the sensor surface which means that it can detect the protein interactions in nanometer scale with high exclusiveness. In this study, FDTD (Finite Difference Time Domain) method is adopted to observe the electric field distribution at the condition of the LSPR occurrence ($n=1.33$, wavelength=859.4nm). The amplitude of the incident electric field was 0.05 and a simplified model in which the radii of the pattern edges were omitted was used for the limitation of the FDTD computational memory. It was observed that the resonant electric field was sharply localized and

enhanced inside the nanogroove as shown in Fig. 7. The localization of the electric field was under 100 nm and the intensity of enhancement was more than 20 times. It is expected that this strongly enhanced resonance inside the nanogroove can contribute the higher sensitivity than the conventional LSPR which occurs outside the metal particles.

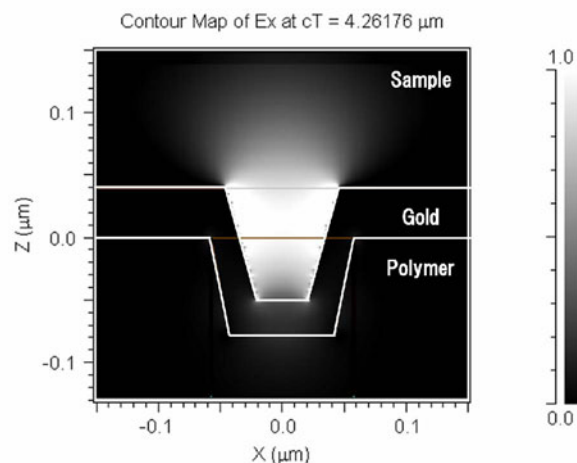


Fig.7 Electric field distribution calculated by FDTD method

4. RESULTS AND DISCUSSION

4.1 Reflection spectra and sensitivity

The experimental data of the reflection spectra was measured by the experimental setup shown in Fig. 4. The nanopatterned structure was same as depicted in Fig. 5. Fig. 8 shows the experimental reflection spectra of this substrate. The sample was changed from $n=1.00$ (air) to $n=1.33$ (water) and $n=1.36$ (ethanol). The corresponding reflection dips were observed at the wavelength of 666.8, 828.0 and 844.2 nm, respectively. The

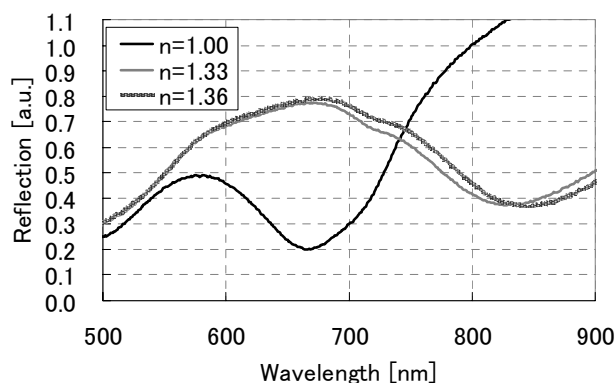


Fig.8 Experimental reflection spectra

sensitivity calculated by the aforementioned way was 541.5 nm/RIU. It was proved that the results had good agreement between the simulation and the experiment and that the nanogroove LSPR sensor had a much higher sensitivity than the conventional LSPR sensors.

4.2 Verification as a biosensor

As a next step, a detection of the specific protein was conducted to verify that this sensor can be used as a biosensor. Fig. 9 shows a schematic illustration of the surface configuration. The sample solution containing the anti-BSA (Bovine Serum Albumin) antibodies (0.03 mg/ml) was let flow onto the sensor surface after the preparation of the ORLA 18 layer. The sample volume was 60 μ l and the flow rate was 6.0 μ l/min. Fig. 10 shows the time sequence data of the LSPR peak wavelength shift. The peak shift of 1.4 nm was observed as a result of the anti-BSA antibody's association with the ORLA 18. Subsequently, BSA (0.1 mg/ml) antigen was let flow over the sensor surface. The peak shift of 0.5

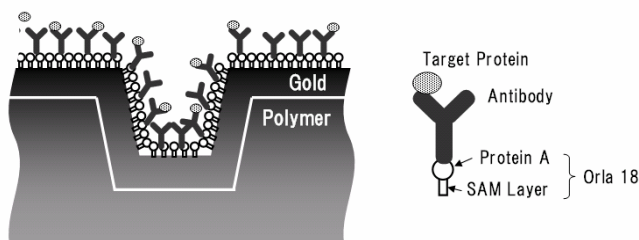


Fig.9 Schematic illustration of the surface configuration

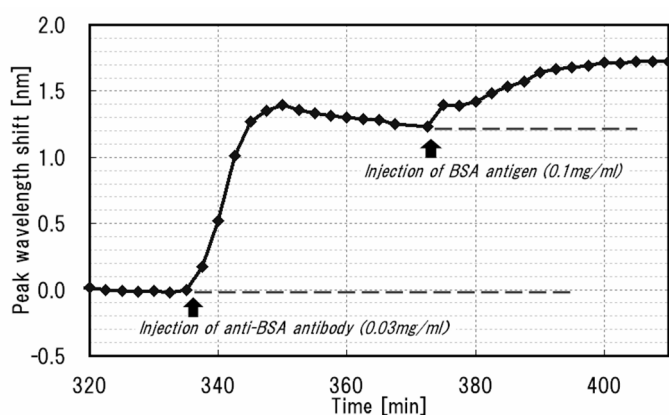


Fig.10 Time sequence data of the LSPR peak

nm was observed. As the noise level of this system was under 0.05 nm, the S/N to detect the BSA antigen was more than 10. By changing the kinds of the antibodies, we can apply this system to the different kinds of proteins.

5. SUMMARY

We have proposed a new biosensor incorporating LSPR and the nanoimprinting technology. This provides a low cost sensor chip with a high reproducibility. Furthermore, the LSPR in the nanogroove structure was proved to achieve a high sensitivity. It was also verified to work as a biosensor for detecting the specific target protein. We expect this sensor can be contribute to realize the protein chip and POC (point-of-care) chip for the medical usages.

6. ACKNOWLEDGEMENT

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