Plasmonic Biosensing and Imaging with Metal Nanoslits

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Abstract
This study has developed sensitive gold nanoslit biosensing and imaging with surface plasmon (SP) effect for real-time analysis of biomolecular interactions. The main advantages of the proposed system include: 1) a minimum disturbance from buffer solution; 2) requires only a compact detection system; and 3) provides a practical kinetic study of biomolecular interactions.

Introduction
The surface plasmons (SPs) on the substrate-metal interface could be excited with the help of periodic nanoslits, which transmit SPs into the metal-buffer sensing interface via the metal nanoslit coupling. Therefore, to design a high sensitivity nanoslit SPR biosensor to match the requirements mentioned previously (to avoid buffer solution disturbances and for only a simple metrology system), the rigorous coupled wave analysis (RCWA) and finite-difference time-domain (FDTD) method are adopted to design a suitable period and width of the nanoslit array. The designed nanoslit array will select a proper resonant wavelength band from normally incident light for which its reflection spectrum can provide a better depth-to-width ratio detection dip to improve the detection limit. We also found that a thickness of the metal layer below 100 nm can provide a better depth-to-width ratio dip in the reflection spectrum. Therefore, the design mechanism is totally different from the nanoslit SPR biosensor based on EOT. Certainly, the fabrication of the nanoslit structure can utilize the e-beam or focused ion beam lithography process to develop accurate samples, which is unlike the fabrication of the phase difference grating. Therefore, the proposed SPR biosensing and imaging via the metal nanoslit coupling has advantages including high sensitivity, low disturbance from buffer solution, and compact measurement system.

The fabrication of the proposed nanoslit SPR device was commenced by depositing a gold film onto a BK7 glass substrate of thickness 1 mm. The radio frequency sputtering process was used to deposit a 60 nm thick gold film. The nanoslit structure measured 50 nm wide, with a 480 nm period. A uni-dimensional periodic nanoslit with an area of 100×100 μm^2 was then fabricated using a dual-beam focused ion beam system (DB-FIB, FEI Nova-200). The focused ion beam column is equipped with a Ga^+ source and operated at 30 keV with 30 pA beam current. After the ion beam milling process, an experimental prototype with the metal nanoslit array is ready for sensing.

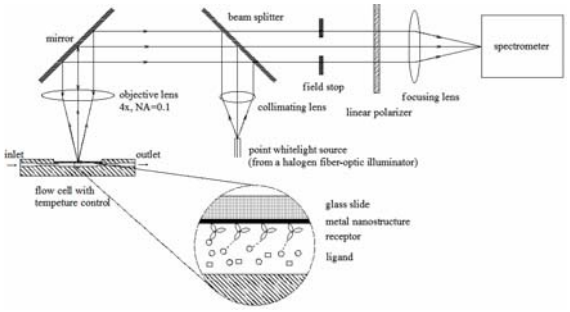

Fig. 1. Schematic illustration of a normally incident spectroscope.

The following reagents were all used in the current experiments: carboxyl-terminated 16-mercaptohexadecanoic acid (MHDA, Aldrich), *N*-ethyl-*N*-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC hydrochloride, $C_8H_{17}N_3 \cdot HCl$, MW = 191.70 g/mol, Fluka), *N*-hydroxysuccinimide (NHS, $C_4H_5NO_3$, MW = 115.09 g/mol, Fluka), 0.5 μM protein G (from streptococcus sp., MW = 22,600 g/mol, Sigma) dissolved into a 10 mM Tris-HCl buffer solution (pH 7.4, and 150 mM NaCl), and 1.0 μM anti-albumin antibody (from goat, Sigma). The biosensor surface was rinsed in deionized water, cleaned with ethanol, and dried in pure nitrogen gas. The biosensor was then soaked in a 1 mM MHDA ethanol solution for 12 h. After being soaked, the biochip was once again rinsed, cleaned and dried using deionized water, ethanol, and nitrogen, respectively, and then soaked in a 2 mM EDC 5 mM NHS solution for 12 h.

Fig. 1 shows the optical setup for the biosensor metrology system. In this system, a white light emitted from a halogen fiber-optic illuminator is collimated into a microscope objective lens (4×, NA= 0.1, Nikon) and focused on the sensing region with a spot diameter of less than 100μm. The reflected light follows the reverse path to be collected by the same microscope objective, passes through the field stop and linear polarizer, and then enters a detection spectrometer. With the help of the nanoslits, this simple optical measurement system not only avoids the requirement of the coupling prism and matching-index oil used in the Kretschmann configuration, but also ignores the disturbances from the buffer solution. Therefore, it is superior to other SPR metrology systems based on prism or nanostructure coupling. During the current molecular interaction detection processes, the specimens were pumped at a constant flow rate of 100 μl/min into a copper reaction cell maintained at a temperature of 30±0.1°C. As a result

of the dielectric constant of the gold film's sensitivity to temperature, temperature control in the SPR metrology system will be a key factor. The present system setup placed a heater on the top of the reaction cell with a thermal coupler near the sensing region to maintain the temperature change within 0.1℃. This method reduces instability of the optical system in long-term measurements to detect small vibrations in order to improve the signal-to-noise ratio. The CCD integration time of the spectrometer was 2s while the measurement period of the reflection resonance spectrum was 10s.

Before analyzing the biomolecular interactions on the sensing surface of the nanoslit SPR sensor, the reflection resonance spectra under injected deionized water into the reaction cell were measured to verify the long-term stability over a few hours. The SPR dip is preliminarily determined by detecting the minimum reflection intensity point near the designed resonance wavelength. Usually, 451 raw data points around the minimum intensity point are used to precisely estimate the resonance wavelength by fitting a high-order polynomial curve to filter out the measurement noise. The 451 data points cover over 18 nm spectral width. The long-term stability of the nanoslit SPR sensor with the simple spectroscopy system is found to be better than 0.02 nm root mean square. The system provides a dynamic sensing capability to realize the resonance wavelength shift by switching different refractive index liquid solutions (deionized water = 1.33, and alcohol = 1.36). In this dynamic testing, deionized water was first injected into the reaction cell and a steady-state resonance wavelength was reached after few minutes. At about the 19th minute, alcohol injected into the reaction cell was mixing with deionized water to induce a resonance wavelength shift with a one to two minute transition state; then, a final wavelength shift of 11 nm occurred. After a cycle, deionized water and alcohol were injected in turn again. The wavelength shift by the different liquid solutions is equivalent to the simulation value based on the RCWA. Therefore, the sensitivity of the proposed nanoslit SPR sensor is achieved.

To study the kinetics of biomolecular interaction, the sensing surface of the nanoslit SPR sensor was modified to form a self-assembled monolayer first. A 10 mM Tris-HCl buffer solution was initially injected to test the system stability. After about 10 min (shown in Fig. 2), the resonance wavelength shift was changed little when the Tris-HCl buffer had an unbalanced interaction with the MHDA surface with EDC and NHS. Once the measurement signal had almost stabilized (after 50 minutes, as in Fig. 2), the 0.5 μM protein G was injected into the reaction cell. At this moment, protein G starts to bind on the modified sensor surface resulting in the resonance wavelength being slightly changed. Finally, the protein adsorption on the surface was detected by a resonance wavelength shift of 0.04 nm after the Tris-HCl buffer wash over. At the 130th minute, 1 μM antibody solution was injected into the reaction cell, the wavelength shift exhibited obvious variation. After

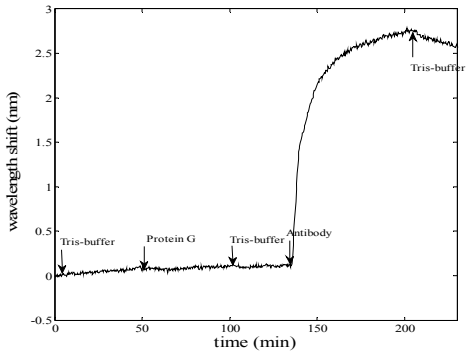

Fig. 2. Dynamic response of nanoslit SPR biosensor during antibody interaction with protein on sensing region.

nearly reaching dynamic equilibrium with the wavelength shift of 2.7 nm, the 10 mM Tris-HCl buffer solution was injected to the reaction cell to wash out non-binding antibodies. The wavelength shift was approximately 2.5 nm after the antibody interaction with the protein on the sensing surface. The wavelength shift of biomolecular interaction is difficult to estimate by simulation, because the effective binding ability is unpredictable due to various factors such as biomolecular activity, environmental conditions, functional binding sites, etc. However, the dynamic biomolecular interaction experiment reveals kinetic information for the study of biomolecular binding behavior. Therefore, the experimental results demonstrate that the proposed biosensor not only retains the sensitivity, but also provides long-term stability for biomolecular kinetic study.

Conclusions

The proposed SPR biosensing and imaging via metal nanoslit coupling has been developed to dynamically detect biomolecular interactions at great sensitivity under a simple reflection spectrum metrology system. This simple system not only avoids the requirement for the coupling prism and matching-index oil used in the Kretschmann configuration, but also is unaffected by the disturbances of the buffer solution. The proposed SPR system not only retains the same sensitivity level as that of a conventional SPR biosensor based on grating coupling but also achieves the optimal measurement resolution with a simple spectrometer. Moreover, the proposed SPR biosensor provides long-term kinetic study for real-time biomolecular interaction.

References

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