### LABEL-FREE OPTICAL BIOSENSOR USING NANOPERIODIC STRUCTURE FOR MONITORING OF BIOMOLECULAR INTERACTION

Tatsuro Endo, Yasutaka Morita, Yuzuru Takamura, Eiichi Tamiya School of Materials Science, Japan Advanced Institute of Science and Technology

1-1 Asahidai, Tatsunokuchi, Ishikawa, 923-1292, Japan

# 1. Introduction

In recent years, label-free biosensors not requiring external modifications have been receiving intense attention. A label-free optical biosensor, which retains many of the desirable features of conventional surface plasmon resonance (SPR) reflectometry, namely, the ability to monitor the kinetics of biomolecular interactions in real time without a label has been developed with several important advantages: the sensor device is easy to fabricate, and simple to implement, requiring only an UV-VIS spectrophotometer or flatbed scanner. Importantly, the biosensor can be easily multiplexed to enable highthroughput screeing of biomolecular interactions in an array-based format [1-3].

In this research, the development of a novel label-free optical biosensor based on nanoperiodic structures is aimed. This optical method promises to offer a massively parallel detection capability in a highly miniaturized package.

### 2. Experiments

2.1 Formation of the nanoperiodic structure substrate

4,4'-dithiodibutyric acid (DDA) was added to the gold substrate surface produced by the thermal evaporation, and a self-assembled monolayer (SAM) was formed. Silica nanoparticles (100 nm i. d.) modified with 3aminopropyltriethoxysilane ( $\gamma$ -APTES), were added to the SAM-functionalized gold substrate surface using 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC).

## 2.2 Evaluation of the surface characteristics

The surface characteristics evaluation was carried out by the UV-VIS spectroscopy and atomic force microscopy (AFM). All absorbance spectra were taken from 400 to 800 nm on the UV–VIS spectrometer at room temperature. To analyze the surface of nanoperiodic structure substrate quality in terms of nanoparticle density, periodicity, and monolayer formation, surface analysis of nanoperiodic structure substrate using AFM was carried out in tapping mode using silicon tips on cantilevers with a nominal spring constant of 18 N/m for scanning in air.

#### 2.3 Real-time monitoring of the avidin adsorption

Different concentrations of avidin (1 ng/ml ~ 100  $\mu$ g/ml) were introduced to the nanoperiodic structure substrate surface, and the real-time change in the absorption spectrum was observed.

## 3. Results and discussion

3.1 Surface analysis of the nanoperiodic structure suface.

The nanoperiodic structure substrates formed by surface modified silica nanoparticles, absorbance peak was observed at 552 nm. It is known that nanoparticles such as gold, silver, and copper possess strong absorption in the visible region, often coined as surface plasmon absorption.

The AFM image clearly illustrated that the surface modified silica nanoparticles were one particle in depth, forming a nanoperiodic assembly (Fig. 1).



Fig.1 AFM image of the nanoperiodic structure substrate.

## 3.2 Real-time monitoring of the avidin adsorption.

The detection of the different concentration of avidin adsorption was performed by using the nanoperiodic structure substrate. As a result of fixing wavelength to 550 nm, and performing real-time monitoring of the avidin adsorption, the spectrum change accompanying different concentration of avidin adsorption was observed (Fig. 2).



Fig.2 Real-time monitoring of the avidin adsorption

### 4. Conclusions

In this research, surface modification of silica nanoparticles and SAM substrates was carried out to realize a two-dimensional arrangement of silica nanoparticles. A unique nanoperiodic structure of surface modified silica nanoparticles was attached to the DDA modified surface of gold substrate.

#### References

- X. Liu, D. Song, Q. Zhang, Y. Tian and Hanqi Zhang, *Talanta*, **62** (2004) 773.
- [2] P. I. Nikitin, M. V. Valeiko and B. G. Gorshkov, Sens. Actuators B: Chem., 90 (2003) 46.
- [3] B. Lin, J. Qiu, J. Gerstenmeier, P. Li, H. Pien, J. Pepper and B. Cunningham, *Biosens. Bioelectron.*, 17 (2002) 827