Detection of biomolecules on porous Si by infrared spectroscopy: Applications to biosensing

Michio Niwano
Laboratory for Nanoelectronics and Spintronics
Research Institute of Electrical Communication,
Tohoku University
2-1-1 Katahira, Sendai, Japan

In the field of genomics, the focus is now shifting to the gene expression and to the interplay between DNA and proteins. To elucidate these phenomena, it is quite important to analyze and to understand structural and functional relationships of biomolecules in various clinical states. In general, a biosensor is an electronic device capable of detecting biological phenomena by the conversion from biomolecular signals to physical signals. The so-called DNA chip is a typical example of such biosensors. In DNA chips, complementary DNA sequences are detected through DNA hybridization and fluorescence of added chromophores. Various types of biosensors have so far been developed for the detection of DNA hybridization. For those biosensors, however, labeling DNA with chromophores and radioisotopes is needed in order to distinguish between reacted and non-reacted species.

We propose a label-free method of in-vitro analyzing the chemical bonding state of DNA in aqueous solutions by infrared absorption spectroscopy in the multiple internal reflection geometry (MIR-IRAS). The advantages of this method are threefold: First, labeling of DNA with fluorescent tags or radioisotopes is not necessary. Second, MIR-IRAS is intrinsically sensitive to surface (or interface) vibrations and consequently is suitable for biosensor detection in which only surface or interface phenomena are involved. Third, in our method, a Si prism, through which infrared light penetrates, internally reflecting at the prism surfaces, also serves as an electrochemical electrode. By applying a positive or negative potential to the Si electrode (prism), we may separate and concentrate DNA in aqueous solutions (electrophoresis).

In the present work, we have tried to improve the sensitivity of DNA detection by forming thin porous Si layers on the prism surface. When biomolecules such as DNA and proteins stick on a nanostructured porous Si surface, the surface density of biomolecules will be quite high as compared to the case of flat Si surfaces, leading to an improvement in the sensitivity of biomolecule detection.

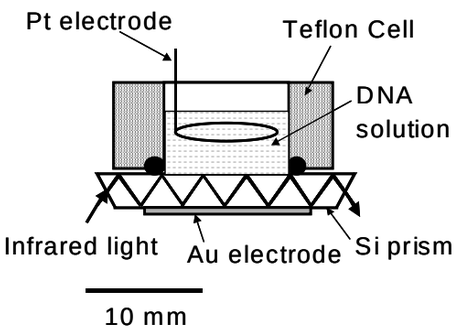

Fig. 1 MIR-IRAS biosensor in conjugation with electrophoresis.

Our biosensor (bio-cell) in conjugation with electrophoresis is schematically shown in Fig. 1. Si prisms used for MIR-IRAS measurements were 0.5 x 10 x 30 mm^3 with 45^o bevels on each of the short edges. The infrared radiation that exited an interferometer was focused at normal incidence onto one of the two bevels of the sample, and penetrated through the wafer, internally reflecting about 60 times. We deposited gold metallic layers with about 10 nm in thickness onto the backside of the Si electrode to apply a potential to the electrode (Si prism).

We show in Fig. 2 IRAS spectra measured for 10-based single-stranded (ss) DNA molecules immobilized on a nanostructured porous Si surface and a flat Si surface. Absorption peaks in the range of 1500 - 1750 cm^{-1} are due to vibration modes of DNA and linker molecules. It is obvious that the absorbance is significantly enhanced on the porous Si surface.

Figure 3 shows a series of IRAS spectra of the 10-based ss-DNA of guanine (dG_{10}), collected while a positive potential of 6 V was applied to the Si electrode. The peak intensity increases with time, which clearly indicates that negatively-charged DNAs were attracted by the Si electrode and were gradually concentrated on the prism surface.

Accordingly, we can conclude that porous Si-based MIR-IRAS biosensors in conjunction with electrophoresis enable us to detect DNA with quite high sensitivity and to manipulate DNA in aqueous solution.

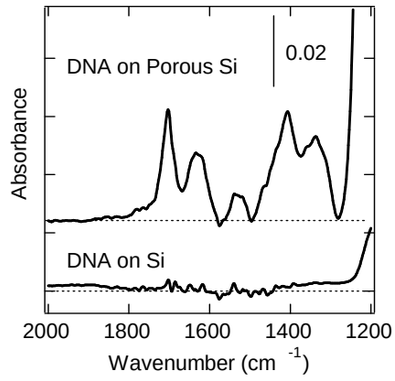

Fig. 2 IRAS spectra of 10-based DNA molecules immobilized on Si surfaces.

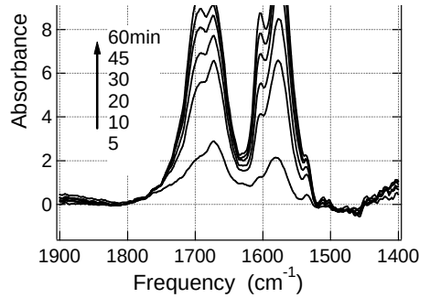

Fig. 3 IRAS spectra of 10-based ss-DNA of guanine under a potential of 6 V applied to the Si electrode.