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In-Situ Detection and Classification of DNA by Porous Alumina Filter in Conjugation with Infrared Absorption Spectroscopy

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1. Introduction

Recently, we have developed a label-free detection scheme that uses infrared absorption spectroscopy (IRAS) to detect hybridization of two complementary oligonucleotides.[1,2] The advantage of our method is that labeling of DNA with fluorescent tags or radioisotopes is not necessary. We can determine DNA hybridization through infrared spectral profiles; the hybridization process is accompanied by a spectral change in the vibration regions of the bases of DNA, providing a means of detection.

In this paper, a method for *in-situ* detecting and separating DNA using porous alumina with the ordered, nano-scaled honeycomb-structure and infrared absorption spectroscopy (IRAS) in the multiple internal reflection (MIR) geometry is described.

Porous alumina layer is formed on a Si wafer that serves as the prism in which infrared light penetrates internally reflecting many times, as is shown in Fig. 1. When a positive potential is applied to the Si prism, DNA molecules move to the vicinity of the prism surface through straight nano-pores in the porous alumina layer, producing infrared absorption signals. In our detection system, labeling oligonucleotides with fluorophore is not necessary. In addition, DNAs can be separated from various neutral contaminants in the solution and also can be classified depending on the size and length of DNA; small DNAs can penetrate through the pores, producing IR signals, while large DNA cannot reach the vicinity of the prism surface.

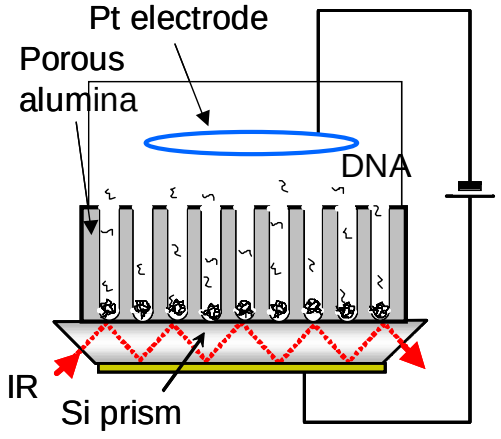

Fig. 1. Porous alumina-based DNA sensor. When a positive potential is applied to the Si prism, DNA molecules move to the vicinity of the prism surface through straight nano-pores in the porous alumina layer.

2. Experimental procedures

Si samples used for MIR-IRAS measurements were prepared from a p-type Si(111) wafer. The samples were 17×16×0.5 mm^3 in size with 45° bevels on each of the short edges. A metallic Al film of 2 μm in thickness was evaporated on the Si prism surface. Gold (Au) was evaporated on the backside of the Si prism to make an ohmic contact to the Si prism. In order to form porous alumina on the Si prism, we used an oxalic acid solution as the electrolyte and applied an anodic potential of 40 V to the Si prism (electrode) during Al anodization.

Figure 2 shows a typical scanning electron microscope (SEM) image of a porous alumina layer formed on a Si surface. The diameter of the pores was approximately 80 nm.

All the MIR-IRAS spectra were measured in D_2O. The reason why we used D_2O instead of H_2O was as follows: H_2O has strong scissoring modes around 1650 cm^{-1}, where the bases of DNA have specific vibration modes that are quite sensitive to base-pairing. On the other hand, D_2O has no significant vibration modes at this frequency region.

3. Results

Figure 3 shows a series of IRAS spectra of a

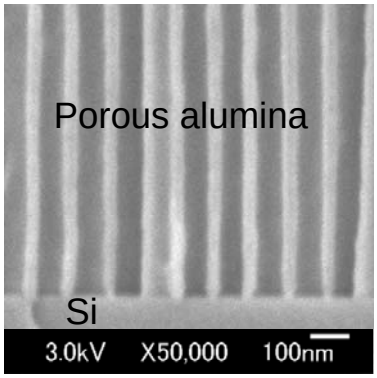

Fig. 2. Typical scanning electron microscope (SEM) image of the porous alumina film (filter) on the Si prism surface. The diameter of the pores was approximately 80 nm.

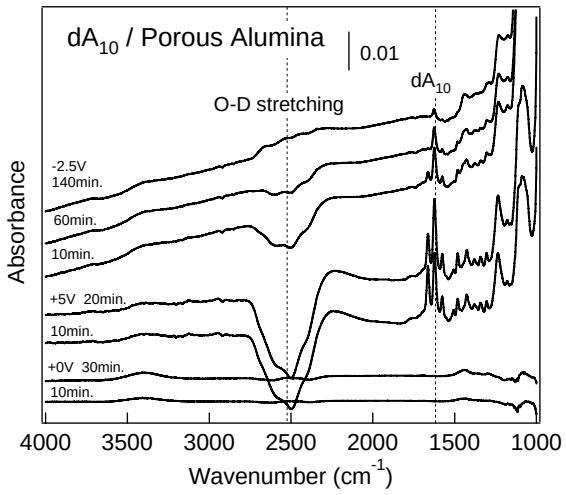

Fig. 3. IRAS spectra of the 10-based oligonucleotides (dA10) under a positive (+5V) or negative (-2.5V) potential applied to the Si electrode.

10-based oligonucleotide of adenine (dA_{10}) that have been collected while a positive or negative potential was applied to the Si electrode. Peaks around 1600 cm^{-1} are due to the vibrational modes of the base of adenine.[3] We can see that the peak intensity increased with time when a positive potential was applied to the Si prism. This clearly indicates that DNA moved to the Si prism through straight pores and was gradually condensed in the vicinity of the prism surface where the evanescent field of IR is present. On the other hand, a broad peak due to heavy water (D_2O), which appeared around 2500 cm-1, decreased its intensity, indicating that water molecules moved away from the prism surface. We suppose that the hydronium ion (D_3O^+) was removed from the prism surface.

When a negative potential was subsequently applied to the prism, the DNA peaks decreased their intensities, indicating that DNA was expelled from the Si prism surface. Furthermore, it can be seen that the peak due to D_2O recovered its intensity, indicating that water returned to the prism surface.

4. Conclusion

We have investigated a method for *in-situ* detecting and separate DNA using porous alumina combined with infrared absorption spectroscopy (IRAS) in the multiple internal reflection (MIR) geometry. IR data demonstrated that by combining the MIR-IRAS method with a porous alumina film (filter), we can not only detect DNA with quite high sensitivity, but also separate DNA molecules in aqueous solution.

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