Label-free detection of DNA molecules moving in micro-fluidic channels by infrared absorption spectroscopy

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Abstract

Infrared absorption spectroscopy (IRAS) in a multiple internal reflection (MIR) geometry is integrated with silicon-based micro-fluidic channels to allow detection and separation of DNA molecules in the micro-channels. The micro-channels were fabricated on a Si MIR-prism using the conventional silicon lithographic techniques. In the present method we can probe ~~both solution-phase and surface-bound~~ biomolecular species through IR spectral profiles of those biomolecules, allowing us to detect and separate biomolecules without fluorescence labeling. The applicability of this approach is demonstrated with a representative test case: transport transformation of single-stranded DNA molecules (oligonucleotides) in the micro-fluidic channels.

Keywords: Micro-fluidic channel, DNA, Infrared spectroscopy

1. Introduction

Development of ~~novel noble~~ techniques of separating biological molecules is required for ‘high-speed’ and ‘high-resolution’ analysis of genetic information [1-5]. Micro-structures and channels fabricated by the use of the matured semiconductor nanotechnology are widely used in separation techniques such as ‘micro-chip-electrophoresis’ [6-8]. In the conventional method, biomolecules are separated in micro-channels by their size and quantity of their electric charges, and the separation is monitored via fluorescence signals from tags attached to the molecules [9,10]. However, fluorescent tags might have undesirable influences on biomolecular interactions, and moreover, labeling of molecules with fluorescent tags is time-consuming. To overcome these problems, we propose a novel label-free detection scheme using infrared absorption spectroscopy (IRAS). In this scheme, we can detect and distinguish biomolecules through infrared absorption spectral profiles of the biomolecules, instead of fluorescent signals from labeled molecules. IRAS is in many ways an ideal detection technique, since virtually all molecules of interest absorb IR radiation. In contrast to UV/vis absorption, IRAS is a fingerprinting method. Characteristic functional groups are apparent by quick inspection of IRAS spectral profiles, and the unique IR signature of many molecules allows identification of ~~an~~ unknown chemical species via comparison with spectral libraries [11-13]. Previously, we have shown that DNA molecules can be *in-situ* detected in aqueous solution using IRAS in the multiple internal reflection geometry (MIR-IRAS) [14-16]. In the MIR method [17], a focused infrared light beam goes through a Si prism, internally reflecting many times, and biomolecular species in aqueous solution can be monitored by measuring IRAS spectra of the biomolecules in the vicinity of the prism surface where the evanescent field is present.

In this study, we apply MIR-IRAS to the detection of DNA molecules during electro-osmosis or electrophoresis in micro-fluidic channels. In Fig.1 is schematically

shown the principle of our detection scheme. Micro-fluidic channels are formed on a Si prism (crystal) through which a focused infrared light beam penetrates, internally reflecting many times. When the infrared light is totally reflected at the surface, the evanescent field is generated in the vicinity of the prism surface. A solution containing DNA molecules flows through the micro-fluidic channels. When the molecules cross the evanescent field of infrared light, they produce infrared absorption signals which are fingerprints of the molecules of interest. One can therefore detect and distinguish DNA molecules by in-situ collecting infrared absorption spectra. The advantage of our method is that the MIR-IRAS method is very surface sensitive. The evanescent field is confined within sub-microns from the surface, and the exponential decay of the evanescent IR wave causes surface-associated species to be weighted more heavily in sampling than their solution-phase counterparts.

In the present study, we fabricate micro-fluidic channels on a Si prism surface using the conventional lithographic method, and demonstrate that transport of single-stranded DNA in the micro-channels can be monitored through infrared spectral profiles.

2. Experiment

2.1. Fabrication of micro-fluidic channels

Our micro-fluidic channels ~~consist~~ ~~are consisted~~ of a Si substrate (Si MIR-prism) with sizes of 35 x 20 x 0.5 mm³ and a quartz substrate with 0.13 mm in thickness. We formed micro-fluidic channels on the Si substrate. The procedure that we employed for the formation of micro-fluidic channels (micro-capillaries) is schematically shown on the left side of Fig. 2. First, we prepared a Si MIR prism in which a focused infrared light beam penetrates, internally reflecting many times. The prism has two beveled edges through which an infrared light beam enters and exits the prism. Second, we formed a silicon dioxide layer (SiO₂) with a thickness of approximately 1.5-μm on the prism surface. After that, we used the conventional

lithographic method to form micro-fluidic channels on the SiO₂ layer. Each channel was 1.5 μm in height and 200 μm in width. The Si prism surface in the micro-fluidic channels may contact with chemically-active solution and should be inert against chemical attack by the solution. We therefore formed a passivation layer of SiO₂ on the prism surface. The detailed process for the fabrication of the SiO₂-based micro-fluidic channels is illustrated in Fig. 3. Firstly, we formed a thin layer of SiO₂ with a thickness of 1.5 μm on the prism surface by wet oxidation. Then, we utilized the conventional photolithographic method to form grooves on the SiO₂ layer which function as the micro-fluidic channels. Finally, a passivation layer of SiO₂ with 0.1 μm thickness was formed on the bottom of the channels by dry oxidation. Dry oxidation has been carried out at an oxidation temperature of 1100 °C.

On the other hand, we opened up two holes in the quartz substrate that were used for the inlet and the outlet of sample solution. Finally, we utilized the conventional wafer bonding technique [18,19] to bond the quartz substrate with the Si substrate to form micro-fluidic channels on the Si MIR-prism. Figures 4(a) and 4(b) shows typical scanning electron microscope (SEM) images of the micro-fluidic channels that were formed on the Si MIR prism. Solutions of DNA in heavy water (D₂O) were introduced into the micro-fluidic channels due to capillary action. Figure 4(c) shows a photograph of the micro-fluidic channels we fabricated. Figure 5 shows a photograph of the DNA solution moving through the channels. We can see that the solution flowed uniformly in the channels; that is, the flow in each channel moved at almost the same speed. This indicates that the micro-fluidic channels we fabricated were rather uniform in size and shape.

2.2. DNA samples

30-mer oligonucleotides of adenine and cytosine were purchased and used without further purification. The single-stranded oligonucleotides were solved in D₂O. The reason

why we used D_2O instead of H_2O as the solvent is that H_2O has the strong scissoring peak around 1640cm^{-1} where the bases of DNA exhibit specific vibration modes (C=O, C-N stretching and also NH_2 scissoring modes). On the other hand, D_2O has no significant vibration modes at these wavelength regions; it has scissoring mode at 1230cm^{-1} . The concentration of oligonucleotides in ~~D_2O -solution~~ was typically $200\text{ }\mu\text{M}$. Solutions of 30-based oligonucleotides (dA_{30} , dC_{30}) were put into a small sample reservoir, while the micro-fluidic channels and a waste reservoir were filled with pure ~~D_2O -solution~~. After that, a DC voltage was applied between the two ends of the micro-fluidic channels, that is, between the sample and waste reservoirs. Behavior of oligonucleotides moving through the micro-fluidic channels was monitored by MIR-IRAS to investigate the electrokinetic properties of DNA.

2.3. MIR-IRAS measurement

An infrared light beam from an interferometer (BOMEM MB-100) was focused at normal incidence onto one of the two bevels of the Si MIR-prism, and penetrated through the Si prism, internally reflecting about 60 times. The light that exited the Si prism through the other bevel on the opposite side was focused onto a liquid-nitrogen cooled mercury-cadmium-telluride (MCT) detector. The resolution of the interferometer was set at 4 cm^{-1} . Since the refractive index of Si, about 3.4, is larger than that of SiO_2 , about 1.5, internal reflection of infrared lights occurs at SiO_2/Si interfaces. Strictly speaking, reflection at $\text{SiO}_2/\text{solution}$ interface may occur since the refractive index of SiO_2 is larger than that of water, approximately 1.3. However, if a SiO_2 layer on Si is much smaller than the penetration depth of the evanescent field, approximately $0.5\text{ }\mu\text{m}$, we can regard the SiO_2 layer as an absorbing medium layer. This is the case in the present experimental condition, since the thickness of the SiO_2 layer was $0.1\text{ }\mu\text{m}$. Indeed, we observed an intense, broad absorption

band around 1850 cm^{-1} for the MIR prism with the micro-fluidic channels. The broad absorption band may be attributed to the absorption of the SiO_2 layers. However, this absorption was not too strong to obscure the absorption by the solution over the SiO_2 layer. In addition, note that this absorption band was not overlapped with the absorption bands specific peculiar to DNA bases that should appear between 1500 and 1700 cm^{-1} . When the oxide layer were thicker, exceeding the penetration depth, we would not be able to monitor infrared absorption of the solution in microfluidic channels due to strong absorption of infrared light by the SiO_2 layer. We confirmed that for a SiO_2 passivation layer thickness of $0.1\text{ }\mu\text{m}$, we can monitor infrared absorption of the solution over the SiO_2 layer. Therefore, we can clearly distinguish the specific absorption bands of oligonucleotides moving in the micro-fluidic channels, as will be demonstrated in the next section.

3. Results

3.1. IR spectra of DNA in the micro-fluidic channels

Figure 6 shows typical IRAS spectra collected for solutions of 30-mer adenine (dA_{30}) and cytosine (dC_{30}) in D_2O that were introduced into the micro-fluidic channels. For dA_{30} , we can clearly identify two intense peaks at 1627 and 1560 cm^{-1} . We have previously observed an intense peak at 1628 cm^{-1} on the spectrum collected for oligonucleotides of adenine floating in D_2O [14-16]. We assigned the peak at 1627 cm^{-1} to the ring deformation mode of the base of adenine. The peak at 1560 cm^{-1} has not been observed in our previous works. Therefore, it could be due to impurities that had been contained in the sample solutions, most probably due to triethylammonium acetate (TEAA) which is widely used as a binding molecule in HPLC of DNA. For dC_{30} , some peaks can be identified in the wavenumber region of $1500 - 1700\text{ cm}^{-1}$. These peaks can be attributed to vibration modes of the base of cytosine, except for the peak at 1560 cm^{-1} . Thus, we confirmed that DNA molecules present

in micro-channels can be detected with high sensitivity by the MIR-IRAS technique. Here, it should be pointed out that the penetration depth of the infrared evanescent field is approximately 1 μm for an infrared wavelength of 1000 cm^{-1} , which is close to the height of our micro-fluidic channels, approximately 1.5 μm , as is shown in Fig. 4(a). This means that we can detect most of the DNA molecules moving through the channels. Furthermore, it should be noted that we can readily distinguish DNA molecules from undesired impurities through a quick inspection of infrared spectral profiles, as is shown in Fig. 6. This is a great advantage of our method over other separation methods such as the conventional fluorescent techniques.

3.2. Real-time monitoring of DNA moving through the micro-fluidic channels

We have examined the drift of DNA solutions in the micro-fluidic channels; which force dominates in drifting DNA molecules in the channels: the force of electro-osmosis or that of electrophoresis. ~~In the electro-osmotic flow, anions in the vicinity of the inner walls of micro-fluidic channels move towards the cathode electrode, and samples drift in the same direction due to the viscosity of the electrolyte. On the other hand, electrophoresis is the movement of an electrically charged substance under the influence of an electric field. This movement is due to the Coulomb force that acts on the charged substance. In order to examine the movement of DNA molecules,~~ We applied a potential of 50 V between two Pt electrodes located at the ends of each micro fluidic channel. Since oligonucleotides moved toward the cathode, we determined that electro-osmosis dominated in the present experimental condition. Therefore, we have in-situ collected MIR-IRAS spectra for 30-mer oligonucleotides, dA₃₀ and dC₃₀, drifting through the micro-fluidic channels due to the electro-osmotic force. ~~In the present study, as mentioned above, we used D₂O as the solvent. When a dilute solution of NaCl was used as the solvent, the flow of DNA solution in the micro-fluidic channels~~

~~drastically varied depending on the NaCl concentration; electrophoresis dominated for a high concentration of NaCl. Our MIR-IRAS technique enabled us to clearly observe the transition of the flow of DNA solution from electro-osmosis to electrophoresis that was caused by varying the NaCl concentration. The results will be reported elsewhere.~~

In order to monitor the flow of DNA solutions in the micro-fluidic channels, we formed the so-called “plugs” of DNA solution by applying a DC voltage in a manner as depicted in Fig. 7. First, a solution containing DNA molecules was put into the sample reservoir and then was injected into the channels by electro-osmosis flow. After a short-term (approximately 1 min.) injection, the sample solution in the reservoir was replaced with D_2O to produce sample plugs in the channels. After that, the sample plugs moved towards the waste reservoir through the micro-fluidic channels under the influence of an electric field.

Figure 8 shows a series of IRAS spectra obtained for dA_{30} and dC_{30} molecules moving through the micro-fluidic channels. These spectra have been collected at intervals of 13 seconds. For dA_{30} , the peak at 1627 cm^{-1} , which is due to the ring vibration of the adenine base, initially showed up and then disappeared, as is shown in Fig. 8(a). This clearly indicates that sample plugs containing DNA (dA_{30}) crossed the path of internally-reflecting infrared light. As can be seen in Fig. 8(b), similar results have been obtained for oligonucleotide of cytosine, dC_{30} . The peak at 1650 cm^{-1} is due to the C=O stretching vibration mode of the cytosine base. Thus, we can conclude that movement of DNA plugs in the micro-fluidic channels can be monitored using the MIR-IRAS technique. Furthermore, it should be noted that we can readily distinguish DNA molecules from undesired impurities through a quick inspection of infrared spectral profiles. As mentioned above, a broad peak around 1550 cm^{-1} is most probably due to TEAA which is widely used in the purification of DNA. Figure 8 shows that this impurity can be distinguished from DNA molecules moving through the micro-channels. This is a great advantage of our method over other separation methods such

as the conventional fluorescent techniques.

4. Conclusions

Infrared absorption spectroscopy (IRAS) in a multiple internal reflection (MIR) geometry was integrated with silicon-based micro-fluidic channels to allow detection and separation of DNA molecules in the micro-channels. The micro-fluidic channels were fabricated on a Si MIR-prism using the conventional silicon lithographic techniques. In the present method we can probe both solution-phase and surface-bound biomolecular species through IR spectral profiles of those biomolecules, allowing us to detect and separate biomolecules without fluorescence labeling. The applicability of this approach was demonstrated with a representative test case: transport transformation of single-stranded DNA molecules (oligonucleotides) in the micro-channels. The present method can be also applied to investigation on the behavior of other biomolecules in micro-fluidic channels.

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Figure captions:

Fig.1: DNA detection system using micro-channel electrophoresis conjugated with MIR-IRAS.

Fig. 2: Procedure for the fabrication of micro-fluidic channels.

Fig. 3: Procedure for the formation of patterned SiO₂ layers for micro-fluidic channels.

Fig. 4: (a) Cross sectional SEM image and (b) optical microscope image of the micro-channels fabricated on the Si prism surface. (c) Photograph of a DNA detection system with micro-channels.

Fig. 5: Photograph of the DNA solution moving through the micro-fluidic channels with 200 μm width.

Fig. 6: Infrared absorption spectra of (a) dA₃₀ and (b) dC₃₀ stored in the micro-fluidic channels.

Fig. 7: Procedure of control of applied voltage for introduction of sample plugs.

Fig. 8: Time evolution of infrared absorption spectra of (a) dA₃₀ and (b) dC₃₀ moving through the micro-fluidic channels.

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