Label-free DNA sequence detection using oligonucleotide functionalized optical fiber

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Label-free DNA sequence detection using oligonucleotide functionalized optical fiber

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The authors present a label-free method for direct detection of deoxyribonucleic acid (DNA) sequences. The capture DNA is immobilized onto the surface of a silica optical fiber tip by means of the layer-by-layer electrostatic self-assembly technique. Hybridization of target DNA with complementary capture DNA increases the optical thickness of the fiber tip. This phenomenon can be detected by demodulation of the spectrum of a Fabry-Pérot cavity fabricated in the optical fiber. Experimental results demonstrate sequence specificity and sensitivity to nanogram quantities of target DNA sequences with short (~5 min) hybridization time. © *2006 American Institute of Physics*. [DOI: 10.1063/1.2364459]

The detection of the base sequences of deoxyribonucleic acid (DNA) is of great importance in many fields such as genetics, pathology, pharmacogenetics, food safety, criminology, and civil defense. One example is the immediate detection of biological weapons in airports, subways, and similar settings. Disease diagnosis is another important application for DNA sensors. For instance, *Mycobacterium tuberculosis* (TB) has a worldwide mortality rate of 3×10^6 annually. Linked with human immunodeficiency virus, it is highly infectious and transmissible. Current methodologies such as the TB rapid cultivation detection technique[1] require a few days to obtain definitive results; in contrast, the direct detection of DNA could be completed in minutes. Rapid diagnostics for infectious diseases have been shown to be highly valuable in facilitating major improvements in disease management.[2]

However, traditional DNA detection methods involve some form of labeling, such as fluorescence, to signal a binding event, which makes the process very expensive and cumbersome. In addition, fluorescent dyes are easy to photobleach. Furthermore, the readout of those arrays involves highly precise and expensive instrumentation and requires sophisticated numerical algorithms to interpret the data, which makes the analysis time consuming.[3]

In contrast, the method for direct DNA sequence detection reported here requires no labels as indicators. On the surface of silica optical fiber tip with a 125 μm diameter, single stranded capture DNA is immobilized by layer-by-layer electrostatic self-assembly (L-b-L ESA).[4] When the target (complementary) DNA sequence is present in a tested sample, the optical thickness of the fiber probe increases. This is mainly due to the change in density of the DNA monolayer.[5] In this study, the change in optical thickness is measured by a multicavity Fabry-Pérot (FP) interferometer formed by a short piece of hollow fiber sandwiched between two pieces of optical fiber,[6] as shown in Fig. 1. Interference of light reflected from the three fiber endfaces results in periodic oscillations in the reflection spectrum. Demodulation of the output spectrum provides the change in optical cavity length due to any immobilization or hybridization event. Experiments have shown that the sensor features sequence specificity, speed, and ease of use. The successful immobilization and hybridization of DNA on fused silica materials is very important for DNA analysis probes. With different FP cavity designs, this method can be extrapolated into a range of optical sensors for DNA detection.

The sensor design was tested using the oligonucleotides listed in Table I, which were purchased from Genosys and used without further purification. The process of identifying the existence of a specific sequence involves immobilization of the single stranded capture DNA onto the fiber tip, which then becomes the "probe." Then, the probe is immersed into the sample. If the target sequence is contained in the sample,

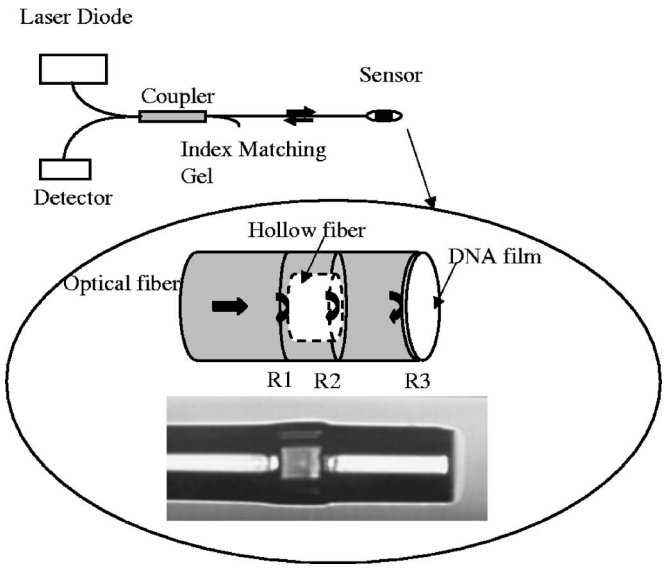

FIG. 1. Multicavity Fabry-Pérot (FP) interferometer is formed by a short piece of hollow fiber sandwiched between two pieces of optical fiber. Demodulation of the output spectrum created by reflections from the three fiber/air interfaces indicates film growth due to either immobilization or hybridization.

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TABLE I. Oligonucleotide sequences used in immobilization and hybridization experiments.

Name	Purpose	Sequence (5′–3′)
ssDNA-A	Probe	TCCAGACATGATAAGATACATTGATG
ssDNA-B	Target	CATCAATGTATCTTATCATGTCTGGA
ssDNA-C	Negative control	CTCACGTTAATGCATTTTGGTC

hybridization occurs. Otherwise, any noncomplementary oligonucleotides can be easily rinsed away.

The immobilization of capture DNA onto the fiber tip takes advantage of the L-b-L ESA technique shown in Fig. 2. This method consists of film deposition a single monolayer at a time by alternating the terminal charge of each assembled monolayer. This implies that in principle the selection of polyelectrolytes is not restricted to specific molecular structures. Due to the negative charge of its sugar phosphate backbone, DNA is considered as a polyanion. The thickness of each layer can be adjusted very precisely by changing the ionic strength and *p*H of the solution. In addition, the adsorption processes are independent of the substrate size and topology.[7]

To improve self-assembly growth characteristics, ameliorate denaturation, and allow sufficient configurational freedom for hybridization, five bilayers of polyelectrolyte precursor film were deposited by alternately immersing the sensor in polyallylamine hydrochloride (PAH) and polysodium 4-styrenesulfonate solutions (2 mg/ml, *p*H of 5.0) for 5 min, with PAH, the positively charged polymer, as the outermost surface. The sensor was then rinsed with ultrapure water (Barnstead Nanopure UV/UF, 18 MΩ) and dried.

Then the sensor was immersed in a solution of ssDNA-A (*p*H of 5.5, 19.26 nmol/ml, 0.02*M* NaCl) for 5 min. Representative results are shown in Fig. 3, indicating cavity thickness increases of 3.4 and 3.6 nm and thus successful immobilization onto the tip of silica fiber. This is larger than the unperturbed radius of gyration of the ssDNA chain of about 2 nm, and smaller than the end-to-end distance of about 5 nm, if a Gaussian coil of a Kuhn step length model is used for the unstretched conformation of the immobilized ssDNA.[5] This may be caused by the salt concentration. At low NaCl concentrations, the negative charges on the DNA chain tend to repel each other, stretching the DNA strands.

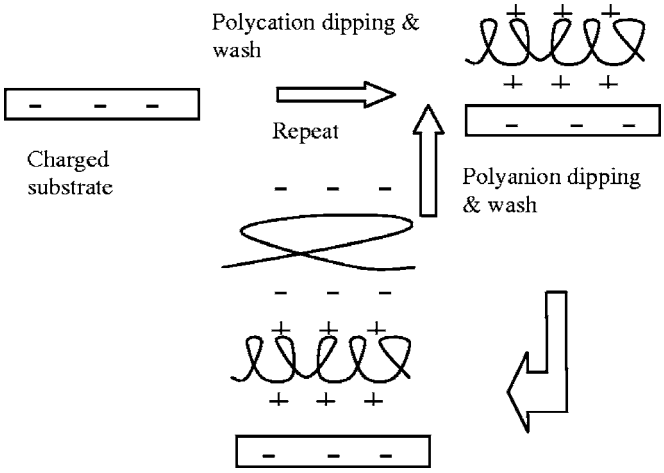

FIG. 2. L-b-L ESA is obtained by alternating adsorption of anionic and cationic polyelectrolytes onto the substrate.

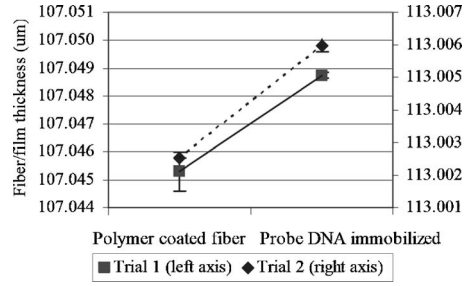

FIG. 3. DNA was successfully immobilized onto the fiber tip with 3.4 and 3.6 nm thicknesses, respectively in two trials. Data to the left and right indicate film thickness before and after DNA immobilization, respectively.

When the concentration of NaCl increases, the Na^+ alleviate the repulsion, and the DNA strands may coil up. A further increase in NaCl may shield some of the phosphate groups on the DNA strands and make the DNA extend from the surface to form a brushlike structure. Thus, the effective thickness of 3–4 nm is reasonable when the tilt angle is also considered. Slight differences in thickness may be caused by different ionic strengths of the DNA solution during these two experiments.[8] This step completes the fabrication of the DNA detection sensor.

Hybridization experiments were performed by immersing sensors in a solution containing complementary DNA, denoted as ssDNA-B (*p*H of 5.5, 76.4 nmol/ml, 0.02*M* NaCl). After 25 min, they were washed in ultrapure water to remove unhybridized DNA and dried. Representative thickness increases due to hybridizations of 4.3 and 4.0 nm are shown in Fig. 4, indicating that the sensor can successfully detect the complementary DNA sequence. Since the dsDNA is very stiff and may be modeled as a wormlike chain using merely linear elasticity of a thin, uniform rod, the 26 base pair dsDNA can be regarded as a rod with a contour length of about 9 nm.[5] If the average effect of ssDNA and dsDNA is considered, this film thickness change is reasonable. 123.8 nmol/ml solutions of noncomplementary ssDNA-C were used as the negative control. Sensors were immersed for 40–60 min. The concentration of noncomplementary DNA was much higher than the complementary ones, and the immersion time was much longer. However,

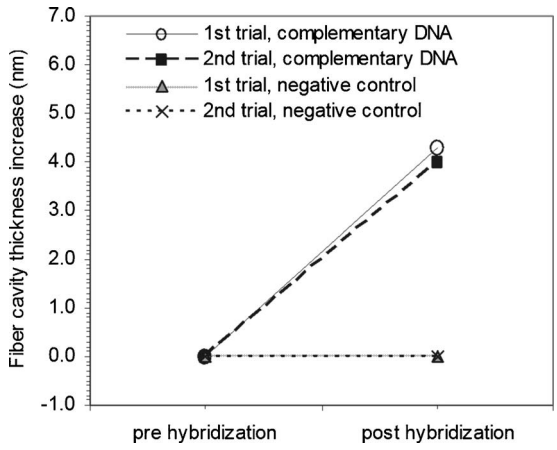

FIG. 4. Detection of specific hybridization of 26-mer target to immobilized probe; probe: ssDNA-A immobilized on sensor tip; target: ssDNA-B, 76 nmol/ml, *p*H of 5.5, 0.02*M* NaCl, immersion time of 30 min; negative control: 22-mer ssDNA-C, 123.8 nmol/ml, immersion time of 40–60 min. The DNA can successfully detect complementary DNA, whereas no noncomplementary DNA attachment is observed.

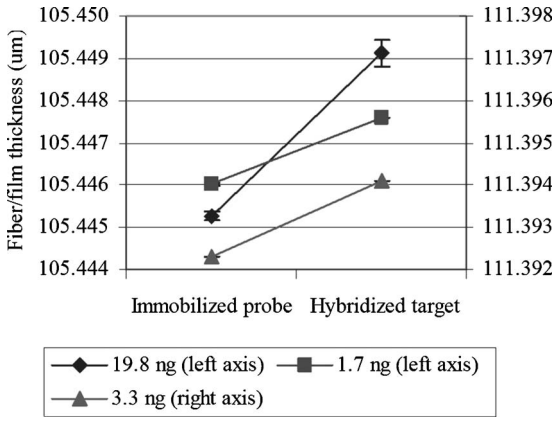

FIG. 5. Hybridization of small quantities (1.7–19.8 ng) of ssDNA-B sequence to ssDNA-A probes immobilized on the sensor interface. Data to the left and right indicate film thickness before and after hybridization, respectively.

the thickness of the fiber tip did not change after washing the probe with de-ionized water (Fig. 4), indicating no attachment of the noncomplementary DNA to the probe, and demonstrating that the DNA sensor is capable of detecting a specific DNA sequence.

To evaluate the sensitivity of this label-free DNA sequence detection method to small quantities of target DNA, hybridization was observed in successively smaller volumes. 19.1 nmol/ml ssDNA-B solutions (0.02*M* NaCl, *p*H of 5.5) were diluted with stock of 103.8 nmol/ml ssDNA-C. The fiber sensor was inserted into the solution volume generated from a needle tip. Sensor fiber cavity thickness changes induced by hybridization (Fig. 5) indicate that the sensor can detect DNA quantities as small as 1.7 ng following short (~5 min) hybridization times. The effect of single base mismatches is currently being investigated and will be reported at a later date.

In summary, we have demonstrated a label-free DNA sequence detection method using the L-b-L ESA method to immobilize the capture DNA directly onto the optical fiber surface, forming part of an interferometric cavity. When complementary DNA samples are hybridized, the thickness of the cavity increases. Otherwise, a noncomplementary DNA sample cannot be bound to the surface and are easily washed away. Detection of the thickness change indicates the successful hybridization. Experiments show that the method is simple, sequence specific, and sensitive to nanogram quantities of the target DNA sequence. With different Fabry-Pérot cavity designs, this method can be extrapolated into a range of optical sensors for DNA detection featuring miniature size, cost efficiency, speed, and ease of use. After optimization, the sensor probe size can be further minimized to the order of microns or nanometers. In addition, the successful immobilization and hybridization of DNA on the fused silica material is very important for DNA analysis probes. Based on this principle, a variety of DNA sensors can be developed, including those based on a FP cavity, which can be intrinsic or extrinsic. This will be discussed in future papers.

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