

Micro-capillary-based evanescent field biosensor for sensitive, label-free DNA detection

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Abstract: We proposed and demonstrated a micro-capillary-based, high-sensitivity evanescent field biosensor for the cost-effective, rapid, and sensitive analysis and detection of specific DNA sequences. By functionalizing the surface of the tubing wall with ssDNA probe sequences, label-free DNA detection is achieved. The wavelength shift response of the surface-functionalized biosensors to DNA hybridization is monitored in real time. Our experiments show that the biosensor can operate at room temperature and is capable of performing label-free hybridization detection, analyte concentration measurement and nucleotide mismatch detection through a single sensing device. The sensor has many advantages, such as a simple manufacturing process, standardized production control, reliable quality, low cost and an economic demodulator. The compact nature and miniature size of the biosensing detection system makes it a good candidate for the rapid and highly sensitive detection of low-concentration analytes in micro-samples for cost-effective, real-time, and on-site analysis in the fields of life science, pharmaceutical chemistry, medical science and criminal investigation.

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1. Introduction

Because DNA is of great value in many important fields, including genetics [1], medical and healthcare diagnostics [2, 3], and criminal investigation [4], DNA sequence detection is highly important [5,6]. However, most common DNA detection approaches require the labeling of target molecules [7,8]. Prior knowledge of the presence of target molecules and their labeling limits is required for on-site or real-time detection, both of which are important for point-of-care DNA testing applications.

Many methods have been used to avoid analyte labeling and to develop label-free sensing technologies [9–11]. In particular, many researchers have focused on fiber optic biosensors that use label-free detection because of their potential sensitivity, detection speed, small size, variability and low cost. Recently, DNA detection has been demonstrated with some fiber-optic biosensors, such as long-period fiber grating [12,13], photonic crystal fiber [14,15], a microfiber grating biosensor [16], a tapered fiber sensor [17], and a tilted fiber Bragg grating [18]. For a biosensor, high sensitivity is of critical importance because the sensor typically must be able to detect only a few molecules. Thus, to achieve high sensitivity, the common method used in these sensors is the excitation of a strong, externally exposed evanescent field that can interact with molecules. The length of the sensing region can be increased to allow the light to contact sample target molecules repeatedly. Therefore, these sensors often have a relatively long, thin and fragile sensing head. Unfortunately, fiber thinning is often associated with reduced mechanical strength, a roughening of the fiber surface, unstable spectra and a fabrication process that is difficult to standardize, all of which compromise the performance and practicality of these refractive index (RI) sensors [12,16,17]. Increasing the length of the sensing head is another important method used to increase sensitivity. A longer sensing head has a larger surface area to bind molecules and allows the light to contact sample target molecules repeatedly. However, these fiber sensors are a few centimeters or longer in length, which makes the sensors fragile, difficult to load and difficult to wash [14–17]. Although fiber-optic sensors with centimeter-order sizes are relatively miniaturized compared with other sensor types, their applications as point sensors for the accurate measurement of small amounts of samples are still limited.

In this letter, we present a compact optical biosensor for the detection of label-free DNA molecules with high sensitivity. Instead of fiber, the key sensing area of our biosensor is the tubing wall, which is spliced between two single-mode fibers (SMFs) as the sensing element. Thus, the stable transmission of the excitation light with a high signal-to-noise ratio and a strong evanescent field are achieved in this fiber-optic sensing system, which effectively reduces the length and thickness of the sensing element while retaining its high sensitivity and mechanical strength. The overall length of the sensor element is just 1.3 mm, which is much

shorter than many other reported fiber DNA sensors [12–18]. Meanwhile, omitting the complex process and expensive facilities required for other sensors results in a simple manufacturing process and a low cost. The biosensor provides an evanescent field that is sensitive to added dielectric material on the surface. Because the molecules bound to the surface of the tubing wall can interact with this evanescent wave and influence the effective RI of the high-order mode of the tubing wall, the sensors can detect bio-molecules after surface modification. We used the silica surface of a capillary wall functionalized with a special single-stranded DNA (ssDNA) probe that binds to specific DNA sequences to demonstrate that the sensor combines multiple functions into one, such as sensitive and specific DNA sequence detection, the measurement of different analyte concentrations and single-base mismatch discrimination.

2. Sensor configuration and principle operation

A schematic of the micro-capillary-based biosensor for DNA detection is shown in Fig. 1. The sensing region of the biosensor reported here has a much shorter length (1.3 mm) than other reported sensors based on the principle of evanescent fields [12–18]. The inner and outer diameters of the fused-silica capillary used in this experiment are 250 and 350 μm , respectively (TSP250350 from Polymicro Technologies, LLC, Phoenix, Arizona), and the thickness of the tubing wall without the polyimide coating is 41 μm . The sensor was constructed with a short piece of the capillary and two standard SMFs that were used as lead-in and lead-out fibers with core and cladding diameters of 8.2 and 125 μm , respectively. Using a commercial fusion splicer (FSM-50s, Fujikura) in manual operation mode, the two SMFs were symmetrically spliced on the end faces of the tubing wall by carefully controlling the splicer parameters, including the motor movement steps and distances, the current for the electric arc (35 mA) and the arc duration time (700 ms). Because the tubing wall thickness of the capillary is smaller than the diameter of the SMF (128 μm), the step motor was controlled during the arc discharge process to ensure that the core of each SMF touched the end face of the capillary.

The specific steps are as follows: After stripping the coating, a short piece of the micro-capillary (about 2cm) was cut by optical fiber cleaver to access clean end faces. Then, the micro-capillary was put in fiber fusion splicer (FSM-50s, Fujikura) which was set in manual operation mode. Through controlling the motor movement, end face of a SMF was aligned and spliced to the end face of the micro-capillary. To ensure the fusion quality and avoid heat-induced collapse of the micro-capillary, the current for the electric arc and the arc duration time are set to 35mA and 700ms. Then, the extra part of micro-capillary was cut and only 1.3mm micro-capillary was left and connect with SMF. Another SMF was also spliced to the other side of the 1.3mm micro-capillary using the above method.

The capillary, which is spliced between two pieces of SMF, is used as the sensing element. The tubing wall functions as a waveguide to generate a strong evanescent field that is exposed to the external surroundings. Unlike a regular, center-symmetric and uniform structure-like fiber, the tubing wall is thin and curved and has a large surface area, all of which are helpful for effectively exciting a strong evanescent field over a short distance. Therefore, although the length of the capillary is short, the sensor is still highly sensitive. Moreover, the intact capillary structure leads to improved sensor robustness.

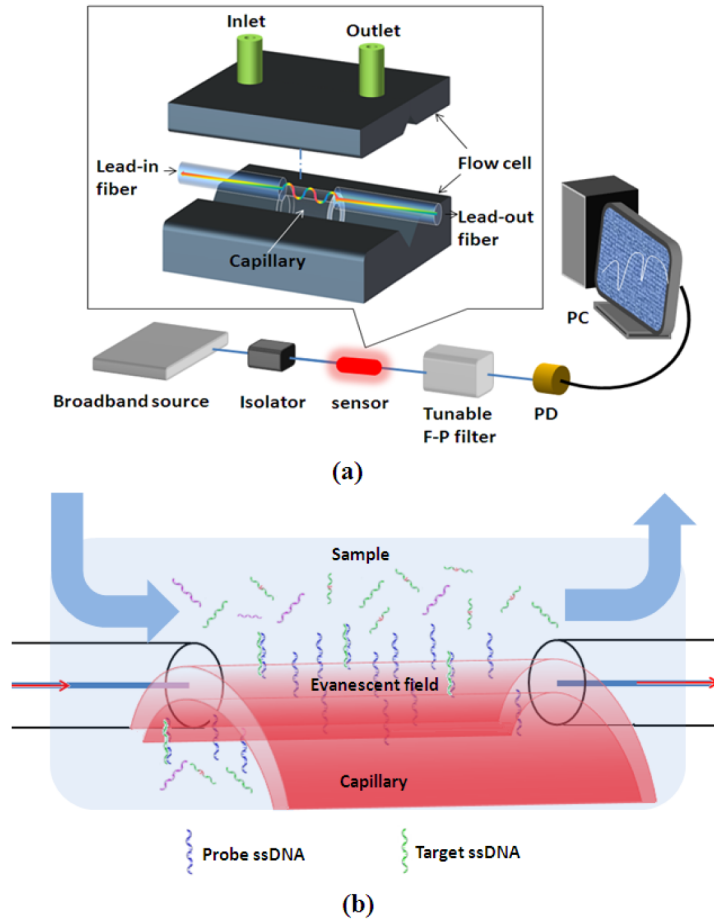


Fig. 1. Schematic diagram of the micro-capillary-based sensor. (a) Schematic diagram of the micro-capillary based biosensor. (b) Hybridization of the target DNA in a micro-flow cell.

The tubing wall was immersed in aqueous solutions packaged in a micro-flow cell (at a volume of 80 μL) with a peristaltic pump for the introduction of samples. Because the lead-in fiber launches light into the tubing wall, the light is partially confined inside the silica tubing wall and presents a lamellar distribution [Fig. 2(a)] but also excites the evanescent field and permeates into the liquid environment [Fig. 2(b)]. Thus, the light interacts strongly with the molecules captured on the tubing wall surface in a manner similar to that of optical microcavity sensors [19]. As a result, the tubing wall can be sensitized with a bioprobe affixed to its silica surface to capture specific molecules. This binding interaction creates a redshift of the resonance wavelength that can be monitored in real time.

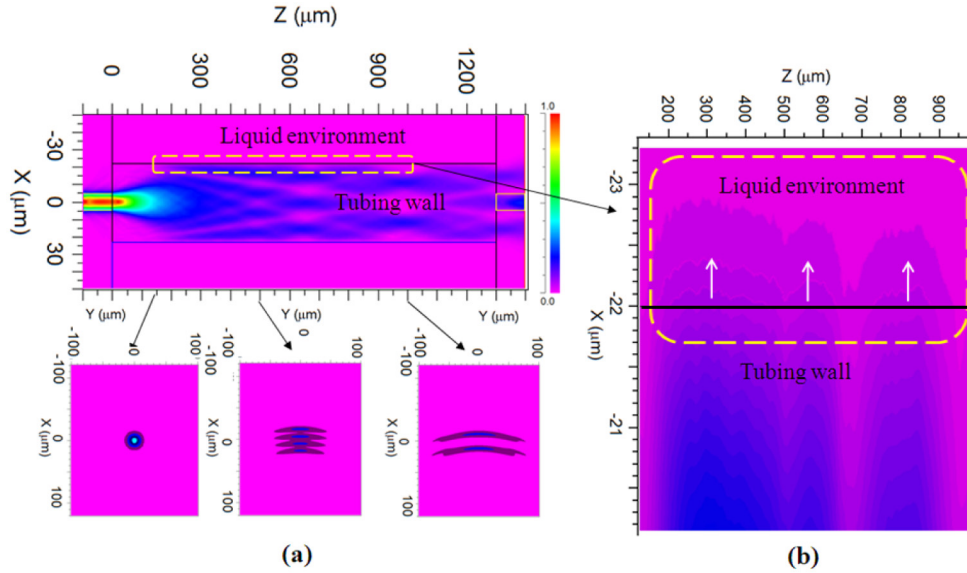


Fig. 2. Simulation result of the micro-capillary-based sensor with a 1.3-mm-long capillary immersed in water. (a) Optical field distribution on the X-Z plane and the X-Y plane. (b) Although the majority of the optical field resides in the tubing wall, a portion of the field evanesces into the environment (indicated by the white arrows). The operating wavelength used in the simulation is 1550 nm.

To choose the appropriate length of micro-capillary, several sensors with different micro-capillaries were tested. We find that a micro-capillary with too short length is hard to obtain interference fringe while micro-capillary with too long length has a large transmission loss and easily influenced by noise interference or shape distortion. We choose the micro-capillary sensor with 1.3mm length which provides a sharp fringe and stable spectrum.

The transmission spectrum of the capillary-based sensor with a 1.3-mm-long capillary is shown in Fig. 3. The transmission spectrum was measured by a homemade fiber-optic wavelength demodulation system that consists of a scanning laser and a photodetector with a minimum wavelength resolution of 0.004 nm. Several dips are present in the transmission spectrum. These dips not only indicate the presence of several modes that are excited by the fundamental core mode from the SMF coupling to the tubing wall, but they also generate an evanescent field that is highly sensitive to changes in RI and the molecules on the tubing wall surface. Because evanescent fields extend outside the tubing wall boundary, the effective index of the high-order mode is highly dependent on the RI of the surrounding medium; the change in the RI of the external medium can influence the resonance wavelength of the modes. When the external environment of the tubing wall surface changes due to the reaction between the sample and the evanescent field, the resonance positions of the corresponding high-order modes change accordingly.

Through the use of sucrose solutions with different RIs, we tested the sensitivity of the dips corresponding to the modes excited in the tubing wall. The response of the dips to the RI of the sucrose solutions is plotted in Fig. 3(b). As the RI increased, the resonant wavelengths of these dips shifted to a longer wavelength. When the RI changed from 1.328 to 1.369, the wavelength shifts for the first and second dips (dip1 and dip2) were 25.788 nm and 18.608 nm, respectively, and the corresponding sensitivities were 628.975 and 453.853 nm/RIU, respectively. Dip1 has a higher sensitivity and a larger redshift than dip2, indicating that the evanescent field of dip1's corresponding high-order modes overlaps maximally with the RI perturbation. Therefore, in this DNA biosensor, we chose the dip1 wavelength as the measuring signal for DNA detection.

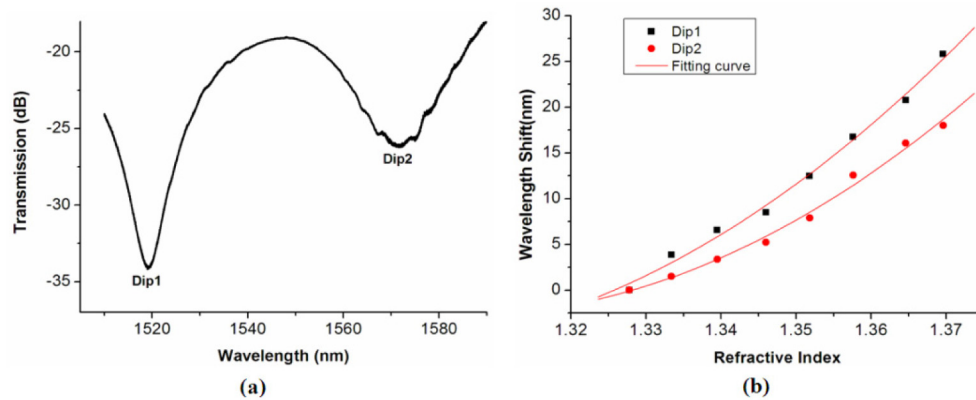


Fig. 3. RI response of the micro-capillary-based sensor. (a) Transmission spectrum of the sensor in water. (b) Resonance wavelengths with different external RIs for dip1 and dip2.

3. Results and discussion

The experimental setup of the proposed sensor is illustrated in Fig. 4. The lead-in SMF and lead-out SMF are connected to a homemade fiber-optic wavelength demodulation system. The wavelength range of the system is 1510-1590 nm and is determined by the scanning laser. During the experiment, the transmission spectrum of the sensor was recorded by a computer connected to the demodulation system in 30-s intervals. During the experiments, each individual sensor was packaged in the micro-flow cell with a volume of 80 μ L to minimize potential environmental influences during biosample measurement while maintaining enough space to allow the sensing region tubing wall to make contact with the biosample. Biosample solutions were injected into the micro-flow cell via a peristaltic pump with a constant velocity of 0.1 mL/min.

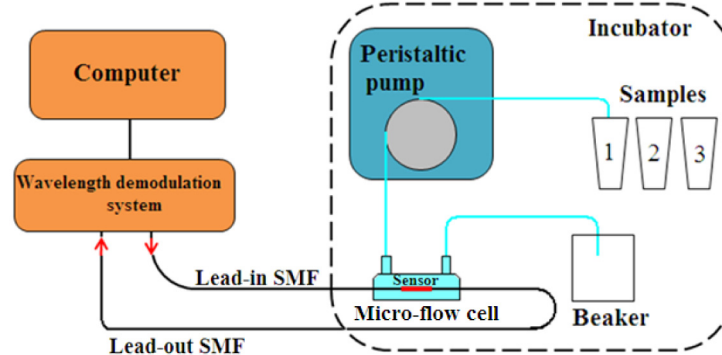


Fig. 4. Micro-capillary-based biosensor for a DNA detection system.

A control experiment is carried out on the DNA detection system. We packaged a micro-capillary sensor without surface-modified tubing into flow cell. Then, PBS Buffer, probe ssDNA sample and target ssDNA sample were pumped into flow cell in turn. The response of the sensor was recorded in real time. The experiment shows that there is no significant wavelength shift when the PBS Buffer, probe ssDNA sample and target ssDNA sample flow through the sensor. The reason is that micro-capillary sensor without surface-modified tubing can only perceive bulk RI change. The probe ssDNA sample and target ssDNA sample were prepared by PBS buffer. In these DNA samples, the contribution of DNA molecules to bulk RI is too little to change the RI. Thus, the bulk RI of the PBS Buffer, probe ssDNA sample and target ssDNA sample are nearly the same.

The general surface functionalization and probe-target sensitization procedure is shown in Fig. 5. Probe ssDNA immobilized on the sensor surface is necessary for detecting target ssDNA. Because the probe ssDNA cannot be effectively bound onto the bare silica surface of the tubing wall, poly-L-lysine (PLL) is used in our sensor. PLL adsorbs probe ssDNA with a negative charge because its amino group has a positive charge. The tubing wall, i.e., the sensing region, was cleaned in an ultrasonic acetone bath for 10 min and washed with ultrapure water. Then, the tubing wall was immersed in a mixture consisting of 3 volumes of 30% H_2O_2 and 7 volumes of concentrated H_2SO_4 (Piranha solution) for 1 h. It was then washed with ultrapure water and dried at room temperature, resulting in a clean and negatively charged silica surface. The cleaned sensor was then packaged in a micro-flow cell with a volume of 80 μL . To form a monolayer of PLL on the tubing wall surface, a PLL solution (0.1% w/v in water, with a molecular weight of 150,000-300,000 g/mol, Sigma) was pumped into the flow cell and allowed to react for 2 h. Then, PBS buffer (0.01 M Na_2HPO_4 , 0.15 M NaCl, pH 7.4) was flowed through the sensor for 30 min to wash away any PLL that had not been immobilized on the surface of the tubing wall. The probe ssDNA (5 μM in PBS buffer; 5'-GAT CAT GGA CTC GAA GAT GT-3') was then injected into the flow cell for 100 minutes at room temperature and immobilized on the surface of the PLL layer. Subsequently, PBS buffer was again used to wash the sensor surface. To examine the effectiveness of the probe and evaluate the specificity of the proposed sensor, target ssDNA (5'-ACA TCT TCG AGT CCA TGA TC-3') and non-complementary ssDNA (5'-CAC CCA GAG GAT AGC A-3') were each tested. Figure 6 presents the wavelength responses of the surface-functionalized sensors with a tubing wall 1.3 mm long. Because the probe ssDNA hybridizes to the target ssDNA, the RI of the fiber surface gradually increases. The dip wavelength of the high-order mode will shift to a longer wavelength. From Fig. 6, the maximum shift of the dip wavelength is 0.112 nm. Note that the wavelength demodulation system used in the current experiment has a resolution of ± 0.004 nm. Thus, a change of 0.1-0.2 nm is significant. However, for non-complementary ssDNA, because no bonding occurs between the non-complementary ssDNA and the probe ssDNA aside from weak, nonspecific adsorption, the RI surrounding the functionalized surface of the tubing wall is quite similar to that of PBS buffer alone and induces a negligible wavelength shift.

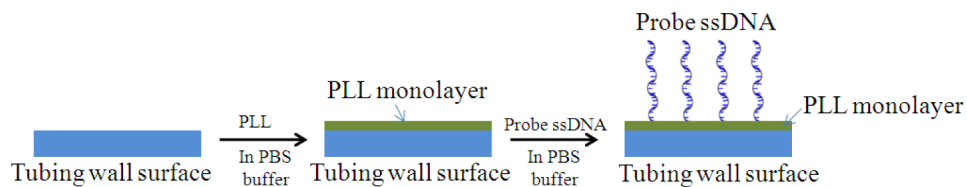


Fig. 5. Depiction of the functionalization steps on the tubing wall surface. The ssDNA probe was immobilized on the surface of the PLL layer on the tubing wall.

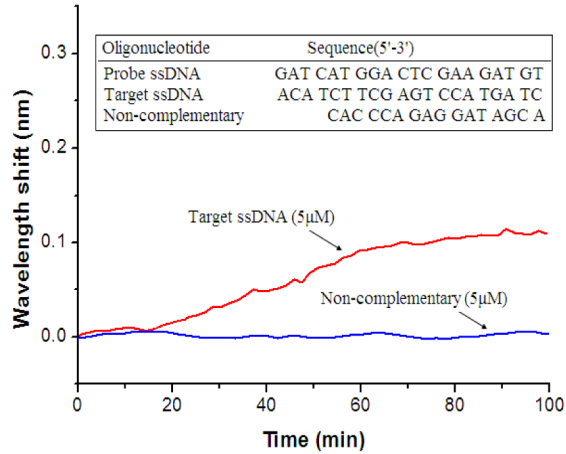


Fig. 6. Resonance wavelength monitoring during the hybridization of a target ssDNA and a non-complementary ssDNA.

To determine whether complementary target DNA at different concentrations can be detected and discriminated in situ, target DNA complementary to the ssDNA probe was tested with increasing concentrations. As in the previous test with 5- μ M target ssDNA (Fig. 6), typical hybridization responses of the complementary strands were obtained at three concentrations (2.5 μ M, 5 μ M, and 10 μ M) (Fig. 7). Each sample was prepared in PBS buffer, and PBS buffer alone was assayed to check the stability of the baseline before every sample test. The results are displayed as wavelength shift vs. time, where the dip in wavelength response over time for each sample introduced is recorded. The responses to 2.5 μ M, 5 μ M, and 10 μ M complementary DNA were 0.054 nm, 0.088 nm, and 0.128 nm, respectively. The changes in dip wavelength shifts at different concentrations demonstrate that DNA hybridization can be detected and discriminated at low concentrations. The baseline noise of the sensing system is also observed and supported by the results in Fig. 7, as monitored by the sensor response to the flow of PBS buffer alone. The fluctuations in wavelength were less than 0.01 nm over the period of observation. Thus, the changes in wavelength observed due to the presence of analytes at various concentrations are significant enough to be detected by the sensor. The detection limit was calculated as triple the baseline noise standard deviations at nanomolar concentrations.

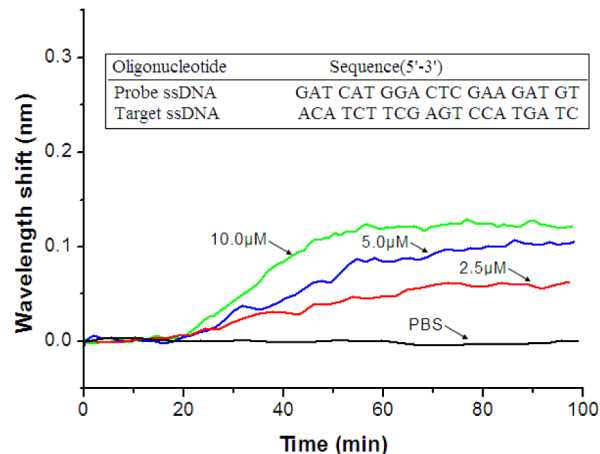


Fig. 7. Resonance wavelength shifts during the hybridization of a target ssDNA at different concentrations.

A corresponding experiment was performed to test the effect of target DNA concentrations for both complementary ssDNA and ssDNA with a single-nucleotide mismatch. The experimental procedure was similar to that used for the detection of complementary target DNA with differing concentrations. After the tubing wall surface was functionalized with probe ssDNA, PBS buffer was pumped into the flow cell for 20 min. Then, a target ssDNA with a single mismatch (5'-ACA TCT TCG CGT CCA TGA TC-3') was introduced at a concentration of 1 μ M. When this test stage was complete, the complementary target was also pumped into the flow cell for comparison. Due to weaker specific binding, the single mismatch ssDNA probe should result in a smaller maximum wavelength shift than the complementary target ssDNA. The hybridization response of the single-mismatch ssDNA at a concentration of 1 μ M as well as that of the complementary ssDNA is shown in Fig. 8. As seen in Fig. 8, the response of the single-mismatch sequence was smaller than that of the complementary ssDNA. Except for the response amplitude of the signal, we can also use the equilibrium to discriminate mismatch ssDNA from fully complementary target ssDNA [20]. Comparing the wavelength response of target ssDNA and mismatch ssDNA with concentration of 1 μ M, we can find the single nucleotide mismatch ssDNA achieved equilibrium faster than fully complementary ones. Through observing the response amplitude and equilibrium from the binding profile, we can identify the target ssDNA and mismatch ssDNA. This result demonstrates the ability of the sensor to distinguish single-nucleotide mismatches in the middle position of the target sequence. The single-nucleotide mismatch ssDNA may hybridize with the probe ssDNA in one of the following ways: it may hybridize over only half of its sequence, it may fully hybridize with a small bulge in the middle of the sequence, or it may cross-hybridize between two neighboring probes [20] (Fig. 9). All of these inexact hybridizations lead to the formation of a weaker bond with the complementary strand and occupy more space than the fully hybridized complementary ssDNA. Thus, a smaller amount of target ssDNA bound to the surface results in a smaller RI change on the tubing wall surface.

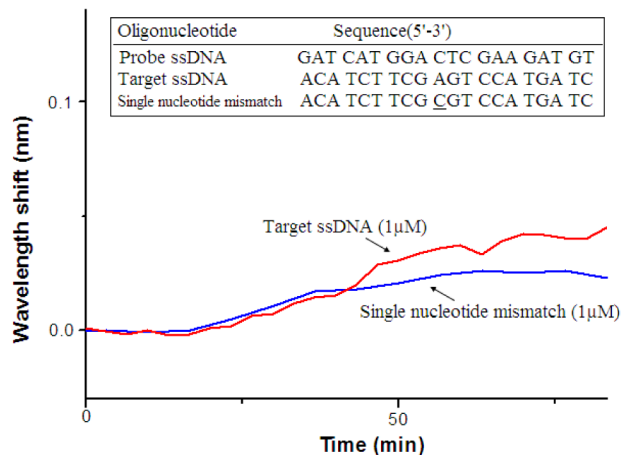


Fig. 8. Comparison of wavelength responses between a mismatch ssDNA and a target ssDNA.

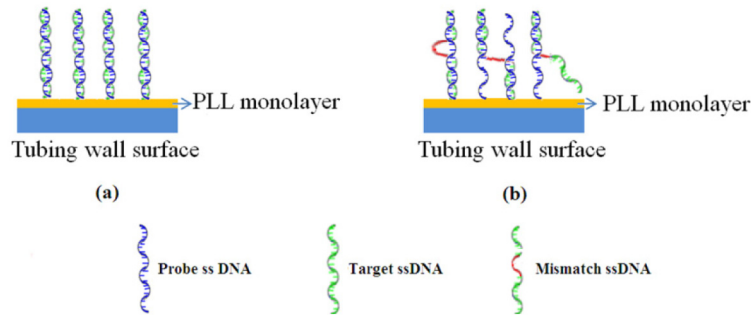


Fig. 9. Surface sensitization of the biosensor for hybridization of target ssDNA (a) or mismatch ssDNA (b).

4. Conclusions

In conclusion, we proposed and demonstrated a high-sensitivity evanescent field biosensor that allows for the cost-effective, rapid, and sensitive detection of specific DNA sequences. By functionalizing the surface of the tubing wall with probe ssDNA sequences, label-free DNA detection is achieved. The micro-flow cell is used during the detection process to precisely control the sample in a volume as small as 80 μL . The surface-functionalized biosensors respond to DNA hybridization with a shift in wavelength that can be monitored in real time. The experiments show that complementary DNA with concentrations ranging from 2.5 to 10 μM can be distinguished. Moreover, this sensor is also able to distinguish complementary DNA from target DNA with a single mismatch. Although other fiber-optic sensors can be used for DNA detection, this micro-capillary-based evanescent wave biosensor requires no analyte labeling and is easy to functionalize. Compared with other high-sensitivity fiber-optic DNA biosensors, our biosensor has a much shorter sensor probe and a simple manufacturing process. This biosensor operates at room temperature and is capable of performing label-free hybridization detection, measuring different analyte concentrations and detecting nucleotide mismatches through a single sensing device. This sensor has the advantage of a simple manufacturing process, standardized production control, reliable quality, low cost and an economic demodulator. Therefore, this biosensing detection system is a rapid, cost-effective, highly sensitive, real-time and on-site detection tool for micro-samples of analytes at low concentrations. In addition, its compact nature and miniature size lends to its potential application in the fields of life science, pharmaceutical chemistry, medical science and criminal investigation.

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