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# Molecular beacons immobilized within suspended core optical fiber for specific DNA detection

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**Abstract:** We propose and experimentally demonstrate a new class of sensor for specific DNA sequences based on molecular beacons (MB) immobilized on the internal surfaces of suspended core optical fibers (SCF). MBs, a type of hairpin structured DNA probe, are attached on the surface of the SCF core using a fuzzy nanoassembly process used in conjunction with a biotin-streptavidin-biotin surface attachment strategy. The proposed DNA sensor detects complementary DNA sequences (cDNA) while discriminating sequences differing from the target by just one base. This enables the detection of DNA in unprecedentedly small sample volumes (nL scale) and is, to the best of our knowledge, the first specific DNA detection using a DNA probe immobilized within a microstructured optical fiber.

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## 1. Introduction

Over the last two decades, many approaches for using optical fibers in sensing applications have been explored, including a variety of approaches to biochemical sensing [1]. Among biosensors, DNA sensors are an important subclass as DNA detection and/or profiling is a fundamental step in numerous applications in Medical, Pharmacy, Forensics, and Archaeology. Attempts to use optical fibers, including microstructured optical fibers (MOFs) for DNA detection have been reported [2–7]. The intrinsic optical fiber based DNA sensors developed to date, in which the fiber itself serves as a sensing element, have largely been based on the use of label-free sensing principles [2–7]. In this case, localized variations in the refractive index of a DNA sensitive layer (the probe), immobilized on the surface [2–4] or at the end [5] of an optical fiber upon hybridization with its cDNA sequence (the target), induce a wavelength shift [2–4] or optical path length change [5]. Consequently the presence of the target sequence binding with the immobilized probe can be inferred. The optical transduction mechanism used for label-free detection is typically a fiber grating [2–4], a Fabry-Perot interferometer [5], or surface plasmon resonance [6]. For DNA sensing label-free approaches have the drawback that they do not generally work at room temperature. It is known that during hybridization sequences that differ by only a few bases will hybridize in addition to complementary sequences, even when care is used in controlling both the temperature and solvent, thus giving false positive results [7]. The use of DNA mimics like peptide nucleic acid (PNA) probes could help overcome such non-specific binding problems in DNA detection [8], however the label-free technique remains less advantageous for multiplexing different probes on a single optical fiber to detect multiple DNA targets. The PNA probes should have to be separated not only in the wavelength domain (e.g. different probes need different wave bands) but also in the spatial domain by immobilizing probes at different locations so that DNA targets can be differentiated. This is necessary because different DNA sequences are different from each other mainly in the form of sequence coding rather than composition. DNAs of very different sequence coding can have the same chemical composition and consequently give, in principle, very similar localized refractive index change upon binding with probes. The later becomes even more challenging for multiplexing a number of different PNA probes within MOFs. Therefore, labeled DNA detection based on fluorescence is still the preferred approach to DNA sensing.

Since their invention in 1996 [9], MOFs have been investigated for a variety of sensing applications, including biological and chemical applications. Of particular interest is the

suspended-core MOF, which can provide strong interaction between the guided mode and samples loaded within the fiber voids in addition to simple filling characteristics, while being simple to fabricate [10]. The SCF has been demonstrated for a variety of biochemical sensing applications based on fluorescence measurements such as selective detection of biomolecules [11], chemicals [12], and real-time distributed measurements using exposed-core SCF [13]. SCFs are hollow fibers with a solid core supported by a few thin struts (3 or 4 struts depending on the design) reflecting the name “suspended core fiber”. By drawing the fiber such that it has a core that is comparable to or smaller than the wavelength of light guided in the fiber, the portion of the light guided by the fiber that is located within the air voids can be significantly enhanced. Solutions under examination can be loaded into the air holes of the SCFs for direct interaction with this portion of the guided light, leading to the potential for high sensitivity [10]. Recently, SCFs have also been used for DNA sensing by immobilizing a PNA probe on the core of a SCF for specific DNA detection using the high selectivity of PNA for its cDNA sequence [14]. However, this approach still requires the cDNA to be labeled before detection, and the work does not extend to the detection of DNA using the PNA-immobilized SCFs; instead a characterization of PNA coating upon filling the fiber with DNA solutions using fluorescence imaging of the SCFs with excitation light incident on the fiber side rather than being coupled in the suspended core was reported [14]. While SCFs have been demonstrated to be a promising candidate for biosensors, particularly those based on fluorescence approaches, to the best of our knowledge there has been no report to date on DNA detection through SCFs or MOFs immobilized with a specific DNA probe on the surface of the fiber core.

Molecular beacons (MBs) are single-stranded oligonucleotide hybridization probes that form a stem-and-loop structure [15]. The loop contains a probe sequence that is complementary to a target sequence, and the stem is formed by the annealing of complementary arm sequences located on either side of the probe sequence. A fluorophore is covalently linked to the end of one arm and a quencher is covalently linked to the end of the other arm. In the absence of target DNA, the probe is dark, because the stem places the fluorophore so close to the quencher that they transiently share electrons and the fluorescence is efficiently quenched. When the probe encounters a target molecule it forms a probe-target hybrid, which is longer and more stable than the stem hybrid. Consequently, the molecular beacon undergoes a spontaneous conformational reorganization that forces the stem hybrid to dissociate and the fluorophore and the quencher to move away from each other, restoring fluorescence [15]. MBs are well known to be highly specific and capable of real-time monitoring of DNA amplification during a polymerase chain reaction [15] and are thus widely used as a probe for DNA detection in various applications [16], including immobilizing MBs on the surface of an etched optical fiber for selective DNA detection [17] or in conjunction with an optofluidic ring resonator laser [18]. However, since MBs are sophisticated DNA probes, they are typically synthesized with relatively low yield compared to their linear DNA probe counterpart and thus have a higher cost, particularly when a large amount of material is required for detection and/or analysis. In this aspect, the low-volume sensing capability of SCF as a DNA sensing platform is a critical advantage as it would allow massive reduction of the material cost in sensor fabrication as well as during hybridization.

In this work we report, for the first time to our knowledge, a suspended core fiber (SCF) onto which a molecular beacon is immobilized as a platform for specific DNA detection. Once MBs are successfully attached on the SCF core and excited through the evanescent field the fibers guided modes, clear enhancement of backscattered fluorescence upon filling the air-hole of the immobilized fiber with a solution containing cDNA was obtained. Fluorescence enhancement was negligible or much less if the solution contained non-complementary sequences (nDNA) or oligonucleotides differing by a single base (oDNA), thus demonstrating that the functionalized SCFs can serve as a highly specific DNA sensor.

## 2. Experimental procedures and measurement setup

### 2.1. Immobilization of MBs on the surface of the SCF core

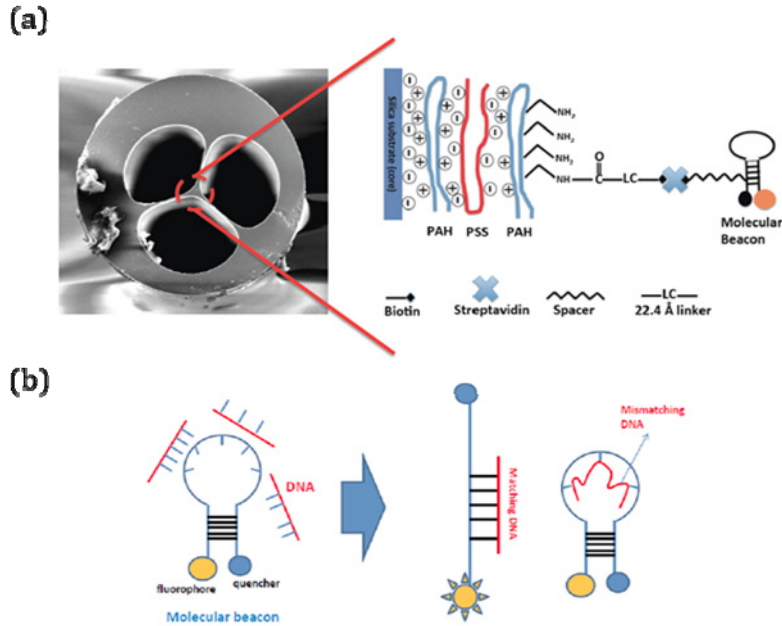


Fig. 1. (a) A scanning electron microscope (SEM) image of the cross-section of the SCF used in the functionalization experiment and the schematic diagram of the final surface state of the MB functionalized SCF and (b) Molecular beacons go through conformational change upon hybridizing with cDNA while remaining in closed form upon hybridizing with nDNA

The immobilization of MBs on the surface of the SCF core was carried out using a combination of the fuzzy nanoassembly technique [19] and the biotin-streptavidin binding mechanism [17]. Figure 1a shows the cross section of the SCF used in this work and a sketch of the final state of the SCF core surface after immobilization. The SCF was a silica glass SCF made in-house with a core diameter of approximately 10  $\mu\text{m}$ . We chose to use a relatively large core SCF principally to reduce the coupling instability during fluorescence measurement. First, positively charged poly(allylamine) hydrochloride (PAH, 2mg/mL in 1M NaCl solution, Sigma Aldrich) and negatively charged poly(sodium 4-styrene sulfonate) (PSS, 2mg/mL in 1M NaCl solution, Sigma Aldrich) were deposited alternately onto the fiber core surface using the layer by layer deposition technique described in [17], ending with a PAH layer (PAH/PSS/PAH) which provides amino groups for immobilization of biotinylated MB through a biotin-streptavidin-biotinylated MB link [17]. In between depositing each layer, the sensor was rinsed extensively with deionised (DI) water. The deposition was carried out by flowing corresponding solutions through the air-holes of the SCF using a pressure pump. NHS-LC-Biotin (0.5mg/mL, Thermo Fisher) was prepared freshly and flowed through for 2 hours, followed by extensive rinsing using phosphate buffer solution (PBS, Sigma Aldrich) to remove unbound biotin on the surface. Non-specific blocking solution (Candor) was flowed through the fiber for 2 hours and rinsed by PBS. Streptavidin (0.5mg/mL, Thermo Fisher) was flowed through for 40 minutes at room temperature then left inside the air-holes overnight and then rinsed thoroughly with PBS. A biotinylated MB solution (1  $\mu\text{M}$ , Midland Certified Reagent Company Inc.) was flowed through for another 40 minutes, rinsed with PBS and DI water and then dried with  $\text{N}_2$  for 15 minutes. The functionalized fiber was cut into several pieces of 70 mm length each and measured immediately. A schematic diagram of MB's state upon hybridizing with cDNA or nDNA is shown in Fig. 1b.

## 2.2. Measurement setup

A schematic diagram of the measurement setup for fluorescence measurement of the immobilized SCF is shown in Fig. 2. Excitation light from a 532 nm laser (Crystal Laser, equipped with an electronics shutter which was synchronized with the spectrometer) was directed into the SCF core through a 20X microscope objective, which also serves as the collecting objective for the backscattered fluorescence from the fiber. Note that the fiber core is not a perfectly symmetric triangle due to fabrication imperfections and thus does not have three-fold rotational symmetry and supports non-degenerate modes (i.e. the fiber is birefringent) [20]. Therefore, a 45° angled quarter-wave plate is inserted in front of the objective to convert the linearly polarized laser light into circularly polarized light so that coupling into the fiber is not affected by the SCF orientation. The coupling efficiency (measured by comparing the maximum transmitted power through a bare SCF and the incident power, where negligible fibre loss for the 70 mm is assumed) is approximately 70% and power coupled into the fiber is limited at approximately 800  $\mu$ W using optical attenuators. The same mode coupling in each measurement was achieved by means of maximizing the power transmitted through the SCF core. The backscattered fluorescence is directed through a dichroic filter (Iridian), which blocks the residual pump, and directed to a spectrometer through two parallel mirrors, a 20x objective, and a large core multimode fiber.

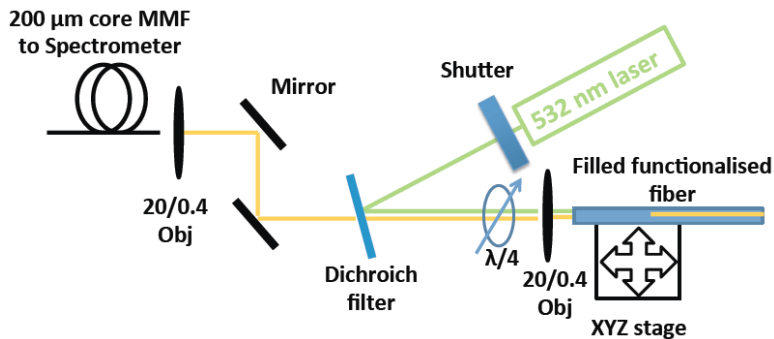


Fig. 2. Experiment setup for fluorescence measurement of the immobilized SCF filled with DNA solutions

## 2.3. DNA hybridization

The hybridization test between the SCF immobilized with MBs and the cDNA, oDNA, and nDNA were carried out in a manufacturer-recommended buffer solution containing 20 mM Tris-HCl, 5 mM MgCl<sub>2</sub>, and 50 mM KCl, and at room temperature (26°C). The background of each piece of fiber under test was recorded first and all the fluorescence spectra were normalized with their own backgrounds to extract the fluorescence enhancements. The filling length for all the fiber pieces was approximately 40 mm. The MBs and DNA sequences used in this work are given in Table 1. For the purpose of demonstrating the proof-of-concept, in this work the MBs and the corresponding DNA sequences were arbitrarily designed by the manufacturer with a quoted discriminating capability (for in-solution measurement) between cDNA and oDNA of more than 10 times. However, once the immobilization of MBs on the SCF core is achieved, virtually any sequences of practical interest could be detected by immobilizing the correspondingly designed MBs on the SCF core.

Table 1. Molecular Beacons and DNA Sequences used for Testing the Immobilized SCF<sup>a</sup>

Molecular Beacon		
5'-(HEX)AGCGGATGTTAAAGACCTATGCCGC(BHQ1-dT)(spacer 18)(3'-Biotin)-3'		
cDNA	oDNA	nDNA
5'-CATAGGTCTTTAACAT-3'	5'-CATAGGTTTTAACAT-3'	5'-TTAACGATCAGACTAT-3'

<sup>a</sup>Samples were synthesized by the Midland Certified Reagent Company Inc.

### 3. Results and discussions

Upon immobilizing a biological probe to a solid substrate such as the SCF core, the first thing we need to verify is whether the MBs were successfully immobilized on the SCF core through the intended biotin-streptavidin link rather than via simple physical adsorption, and whether the MB still retains its characteristics (e.g. showing fluorescence enhancement once encountering its cDNA). When DNA probes are immobilized on the solid substrate it is always advantageous to have the probe connected to the substrate through a spacer of certain length so that the immobilized probes have more flexibility and can hybridize with DNA sequences suspended in the solution more easily [17]. That is, when immobilizing a DNA probe on a solid substrate, one should try to make the immobilized DNA probe exist in as flexible a state as possible so that it resembles, to an extent, the same characteristics as when suspended in a solution.

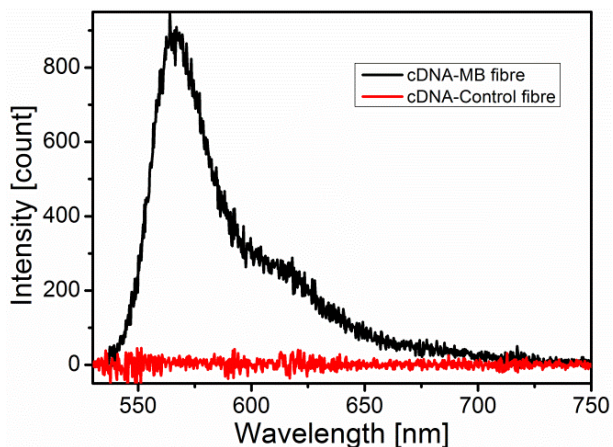


Fig. 3. Fluorescence enhancements of the MB functionalized fiber and that of the control fiber upon hybridization with cDNA.

Another SCF that serves as the control fiber was put through the same immobilization process as described in Sec. 2, except that the biotin-streptavidin linking step was omitted. The two fibers, MB immobilized fiber and the control fiber, were loaded with cDNA solution with a concentration of 4  $\mu$ M and the fluorescence was measured one minute after loading the solution into the SCF holes. As can be seen in Fig. 3, it is clear that the MBs are successfully immobilized on the surface of the SCF core through the biotin-streptavidin link as the fluorescence of the MB immobilized fiber increased significantly upon hybridizing with cDNA while that of the control fiber remains approximately unchanged. This indicates that without the intended biotin-streptavidin link, biotinylated MBs cannot form a stable chemical attachment to the fiber surface and are removed when rinsed. The use of the non-specific blocking layer helps to ensure that biotinylated MB binds only to the surface through the biotin-streptavidin link and not directly to the surface due to physical adsorption, which would be too close to the surface and thus might be associated with high steric hindrance.



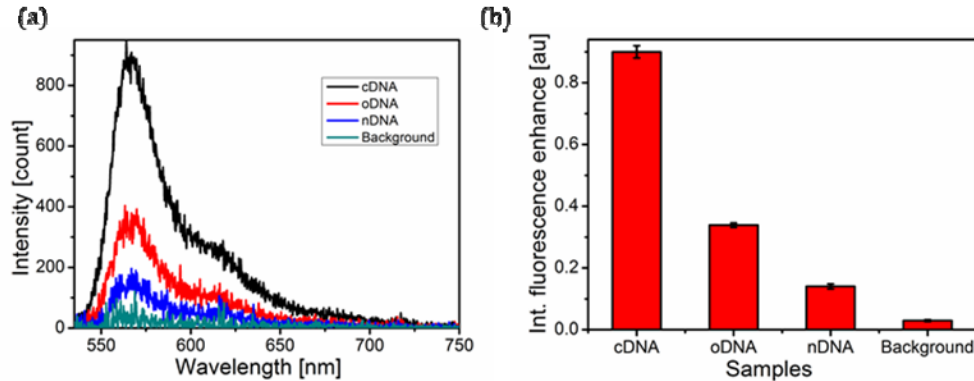


Fig. 4. (a) Fluorescence enhancement of the MB functionalized SCF upon filling with buffer solutions containing cDNA, oDNA, nDNA or buffer only (see legend) and (b) Integrated and normalized fluorescence enhancements with error bars for three consecutive measurements.

The results of the hybridization test, in which MB immobilized SCFs were loaded with different DNA solutions and fluorescence enhancements were recorded, is shown in Fig. 4a. When the fiber was filled with a buffer solution containing cDNA, fluorescence increased significantly compared to when the fiber was filled with buffer solutions containing either nDNA, oDNA (all solutions were prepared at 4  $\mu$ M concentration), or the buffer only, as can clearly be seen in Fig. 4. Therefore the proposed MB immobilized fiber clearly functions as a specific DNA detection platform. Figure 4b shows the integrated and normalized plot of the fluorescence enhancement with error bars for consecutive measurements. It can be seen that the detection process is optically stable during the measurement, which is primarily due to the use of a relatively large core SCF. However, it should be noted that the oDNA also induced a certain amount of fluorescence enhancement of approximately 30% compared with that induced by the cDNA. The discrimination ratio for the cDNA/oDNA pair measured with the MBs immobilized SCF in this experiment is approximately 3, which is reduced relative to measurements performed in-solution. Nevertheless, direct comparison between the solution-solution and solution-surface cases is generally inappropriate due to differences in their kinetics [17]. Another factor affecting the fluorescence results is that in the relatively large core SCF used in this work, a relatively small proportion of the guided field is located in the voids compared to other reported SCFs [14] and will not excite the fluorophore of the MB if it is located too far from the surface. That is, a significant proportion of the MBs may not be effectively excited in this experiment. The use of smaller core SCFs could mitigate this effect at the expenses of coupling stability and loss induced by surface functionalization.

On the biological side, the specificity of the sensor can be improved through design of the MBs since all factors such as the loop length, the base composition, and the position of the mismatched base within the sequence all have a significant impact on the selectivity [21]. Further improvements can be made by refining the buffer solution condition as well as optimizing the immobilization process. It is interesting to note that due to the fact that the immobilized SCFs can detect oDNA along with the cDNA, with careful calibration this can become a advantageous feature of the proposed SCFs as it allows detection of single nucleotide polymorphism, that is, DNA sequences being different by just a single base from each other can be detected and discriminated, which is the most frequent type of variation in the human genome [22].

#### 4. Conclusions

This work proposed and demonstrated an MB immobilized SCF for specific DNA detection for the first time as both excitation and fluorescence collection was performed through guided



light launched in the fiber core. The results confirmed the proper attachment of MBs on the SCF core through a biotin-streptavidin link and the usage of the functionalized SCF in selective DNA detection. The concentration of DNA solutions being detected in this work is 4  $\mu\text{M}$  but it can always be greatly reduced further by using smaller core SCF, longer length of immobilized SCF or both. Modifying buffer condition or design of molecular beacons can also lead to a better detection limit. Optimization of the SCF design and the immobilization process is being investigated to further improve the sensitivity and the selectivity of the proposed approach.

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