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XXI RINEM

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FIBER-BASED BIOSENSORS FOR DNA DETECTION

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Abstract

This paper describes a bio-sensor based on a functionalized microstructured optical fiber with a Bragg grating, for specific DNA target sequences detection. The inner surface of the fiber has been functionalized using PNA probes that allows hybridization of the target DNA. To achieve sensing enhancement, functionalized gold nanoparticles were used. Experimental measurements show the high selectivity and sensitivity of the bio-sensor.

Index Terms – Biosensors; fiber sensors; label-free DNA detection.

I. INTRODUCTION

The need for new, fast, and cheap technologies for medical and healthcare diagnostic equipment has been driving interest and investment in biosensor technology and research [1]. Among the different principles of detection, optical fiber-based bio-sensing is one of the most widely investigated transduction methods. Optical bio-sensors can be classified in two main categories: labelled sensors [2], and label-free sensors. While in a labelled detection scheme the target molecules are modified by adding a tag element, a label-free sensor allows the direct sensing of the target DNA without markers. Most of the label-free detection schemes exploit a refractive index change related to the target presence/concentration.

In particular, microstructured optical fibers (MOFs), in addition to intrinsic advantages of the standard fiber-based sensors (small and flexible shape, in situ sensing, etc.), allow the infiltration of the solution to be tested inside their capillaries. In this way, small biological samples inside the holes can be used. In general, for DNA detection, a *functionalization* process, i.e., a chemical treatment of the fiber surface, is needed, so that the optically sensitive surface is able to bind the specific DNA sequence (*DNA hybridization*). In this work we summarize results obtained with a DNA sensing approach based on a peptide nucleic acid (PNA)-functionalized MOF Bragg grating.

II. EXPERIMENTAL SET-UP

Two MOFs with a Bragg grating inscribed were used: a Penta fiber (Fig. 1a) and an LMA-10 (Fig. 1b). Penta is a multimode fiber whose grating reflects few modes [3], while LMA-10 is single mode fiber [4]. Both fibers have been functionalized with a PNA sequence complementary to the target DNA, according to the procedure described in [4]. The experimental setup (Fig.1c), consisted of an amplified spontaneous emission (ASE) source connected to a fiber optic circulator. Light was coupled to the MOFs and the signal reflected by the grating was sent to an optical spectrum analyzer (OSA). The fiber was placed on two tri-axial micro-positioning devices, to keep it fixed during the measurements and to allow a good coupling in air with the light. From one fiber end, DNA solution was infiltrated through a syringe pump. The system was finally connected to a high pressure nitrogen line, to empty and dry the fiber after infiltration.

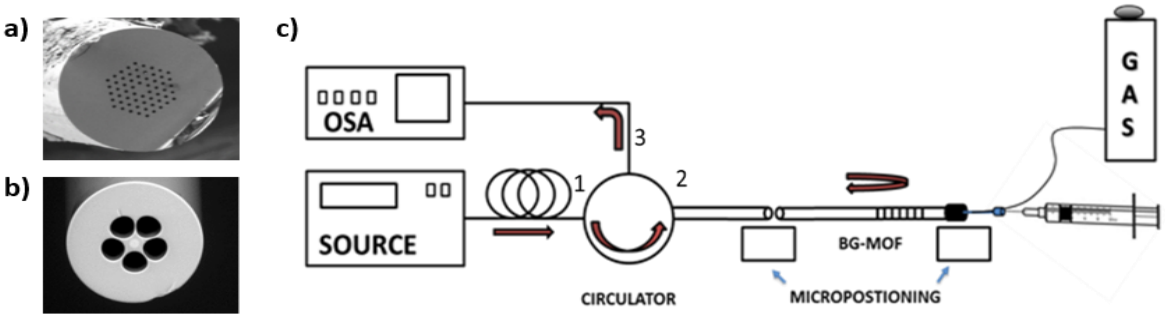

FIG. 1 – SEM images of: (a) Penta fiber (b) LMA-10 suspended-core PCF. (c) Schematic experimental Set-up

II. APPLICATIONS: DNA LABEL-FREE DETECTION USING PNA PROBES

PNAs are artificial nucleic acid mimics in which the natural nucleobases are linked to a poly-amidic backbone instead of the sugar-phosphate scaffold naturally present in DNA or RNA systems. The advantages of using PNA instead of DNA or other oligonucleotide mimics are linked to their high DNA affinity, which allows properly designed PNA molecules to perform strand invasion of the double stranded DNA [4], and sequence-specificity, in particular their ability to discriminate even a single base mismatch.

In recent years, we explored the possibility to combine the advantages provided by PCF (small analysis volumes; possibility to perform label-free detection) with the great performances in the PNAs (sequence selectivity and stability), for the realization of systems able to perform qualitative or quantitative analysis. Two example applications are described in the following, along with the obtained experimental results. The first example developed in our laboratories, focused on the detection of the single point mutation W1282X, associated with cystic fibrosis (CF) disease, was performed using a multi-mode Penta fiber with a Bragg grating inscribed within its core [3]. The sandwich-like scheme of detection adopted in this study, derived from previous studies conducted with different optical

techniques, was designed in order to avoid the use of labelled target DNA as well as a system to enhance the spectral shift exploiting the higher refractive index of gold compared to that of air or organic layers. The label -free detection experiment was performed using longer oligonucleotides sequences having the point mutation target sequence (full matched DNA) or the sequence of the wild type gene (single mismatched DNA). After a subsequent nanoparticles infiltration step, the reflected spectra were analyzed showing a significant shift in the high order Bragg mode only were target DNA was used (Fig. 2b and Fig. 2c).

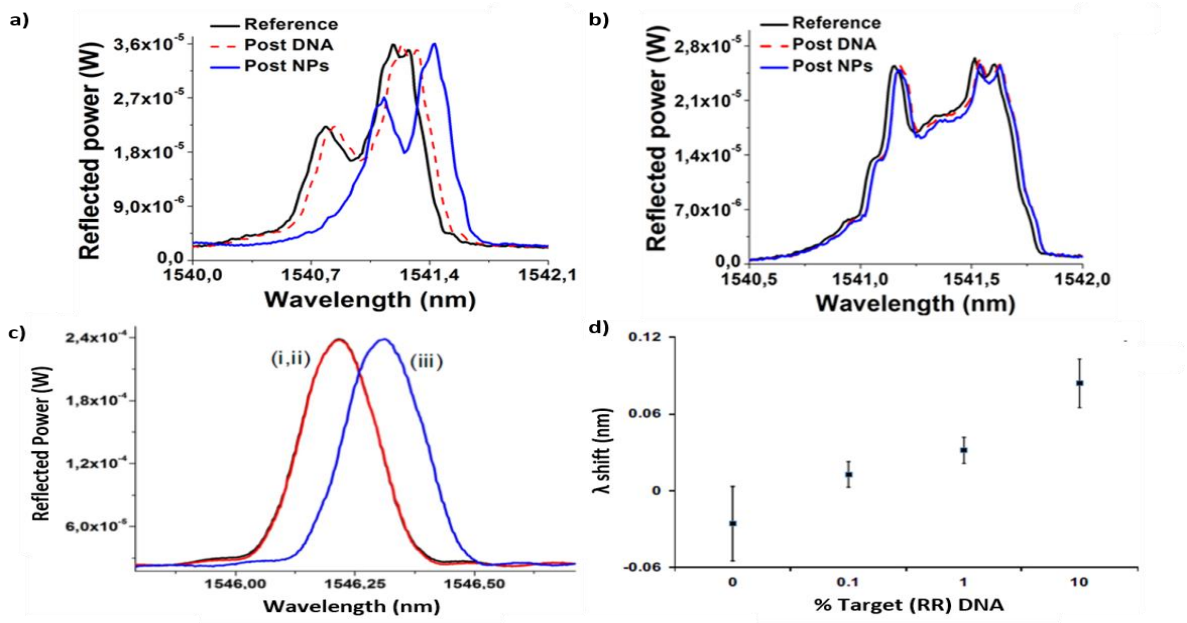

FIG. 2 – (a) shift in the high order band induced by infiltration of target sample; (b) shift in the high order band induced by infiltration of non-target sample;(c) wavelength shift in the reflection mode obtained for the LMA10-PNA fibers (i) before (blackline),(ii) after DNA(redline), and(iii) after ON-AuNPs infiltration (blueline),using10% GMO DNA sample; (d) optical shifts obtained using DNA at the same concentration but with different GMO%(vertical bars represent standard deviation).

Later we moved our attention to the possibility to apply this approach to a PCR-free detection for the identification of Read up Ready Soy GMO contaminants in soy flour [4]. In this case, a single mode LMA-10 fiber (with a Bragg grating inscribed within its core) and DNA samples directly extracted from food matrices were used. Compared to the previous case in which Penta fibers were used, this new type of fibers has the advantage of having only one reflected mode, so that the evaluation of the variation induced by the recognition events was clear and sharp. The detection scheme was the same as applied before and it was possible to detect small percentages of target DNA (1% and 10%) in the presence of a large excess of non-target DNA, using only a small amount (3 ng) of sample. Fig. 2d shows the spectral shift obtained using a 10% target containing sample and the shift obtained using solution with different percentages of target. Selectivity of the analysis was assessed using a blank soy flour sample as well as an unrelated calf thymus

sample, resulting in no significant shift. In this latter experiment, we exploited the great stability of the PNA: DNA complexes for the realization of quantitative detection of contaminants in food matrices. The great sensitivity eliminates the need for Polymerase Chain Reaction (PCR), typically required to *multiply* small amounts of DNA to a detectable level. This reduces the time and costs required for the analysis and enables the application of this kind of devices for a faster screening of contaminants in raw materials or in the food processing chain. In both studies the reproducibility of the detection was confirmed by repeating the measurement after a preliminary washing step, and only a small memory effect was observed in accordance with previous experiments, confirming the possibility to apply this kind of detection in the realization of devices.

III. Conclusion

In this work, fiber-based biosensors for DNA detection has been presented. A small shift of the reflected high order Bragg mode observed when DNA molecules, complementary to the PNA probes, were infiltrated into the MOF; however, by "decoration" of the captured DNA with gold nanoparticle, a significant shift was observed only in the case of full match DNA. This first demonstration proves the feasibility of realizing a sensor for biological measurements by observing the optical signal reflected by a Bragg grating, utilizing the fiber itself as a probe. The fibers used in the experiment shows a good compromise between size of the holes, sensitivity and relative ease of inscription of the grating. Other recognition elements are possible, that are able to bind target analytes, such as proteins or contaminants, using the same strategy as described here, thus making this technology suitable for powerful and versatile bio-sensing platforms.

IV. References

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