Optical Fiber Sensors for Label-free DNA Detection

Optical Fiber Sensors for Label-free DNA Detection

M. Barozzi, A. Manicardi, A. Vannucci, A. Candiani, M. Sozzi, M. Konstantaki, S. Pissadakis, R. Corradini, S. Selleri, and A. Cucinotta

***Abstract*—Optical fiber-based biosensors are an emerging field of research with an extremely broad area of possible applications and a disruptive potential to turn the paradigm known as *lab-on-fiber* into reality. In the past few years, a variety of system choices has been explored, ranging from the type of sensing fiber, to the optical transducing element, to possible sensing amplification strategies. We revise some of the possible approaches to the design of a biosensor, highlighting their advantages and disadvantages, based on previous literature and on the experience of our research groups. The discussion is focused onto DNA sensing systems, especially in a *label-free* format, where the hybridization and recognition of the sought DNA sequence is translated directly into a modification of the optical fiber properties.**

***Index Terms*—Biosensors, Fluorescence, Optical fiber applications, Optical fiber devices, Optical fiber sensors, Optoelectronic and photonic sensors, Sensor phenomena and characterization, Sensor systems and applications, Biophotonics, Optofluidics**

I. Introduction

The last decade has witnessed a surprisingly fruitful meeting of two broad research fields: namely, the field of life and medical science, including biological and pharmaceutical chemistry, on one side, with that of photonic technologies, including optical fibers and devices that are typically studied and employed in optical transmission systems, on the other side. The research and development of optical biosensors brought together these scientific contexts, apparently far from each other. In particular, a challenging and versatile task that can be accomplished through optical biosensors is that of analyzing and detecting analytes and biomarkers for environmental, biomedical, forensic and food analysis. One advanced subject in this research area is the possibility to selectively detect specific DNA sequences in a cost-effective and sensitive way, paving the way to identification of biological materials and products based on genome information [1]–[6].

Optical fibers have served to this purposes, being a widely-used, relatively accessible, and highly flexible platform, enabling to transmit an optical signal as a result of a recognition event. Optical fiber sensors are already now available for the detection of many different chemical species.

In general, in order to generate a physical signal, the surface of a biosensor requires a modification with materials (*probes*) able to change their status upon interaction with the molecule (*analyte*) to be detected. In the case of DNA detection, a *functionalization* process, i.e., a chemical treatment, or *derivatization*, is needed, so that the optically sensitive surface is able to bind the specific DNA sequence, a phenomenon that is referred to as DNA *hybridization*.

Single-mode optical fibers couple light from a broadband source to the sensing area and from this to a receiver where the wavelength-selective behavior of the sensor is monitored. Measurements of analyte concentration and single-nucleotide mismatch discrimination can thus be achieved [2], [4]-[6].

Interestingly, besides carrying light to and from a separate sensing area, in which case it is called an *extrinsic* fiber-based sensor, the fiber itself can provide the sensing area, either through its external surface or, in the case of *holey fibers*, through the capillary holes running along its length. In this case an *intrinsic* sensor is realized. In such a device, a functionalization of the external or of the internal fiber surface with DNA sensing probes, and a mechanism for the transduction of the binding event into an optical signal are needed.

A variety of wavelength monitoring subsystems can be applied, at the receiver side of the sensing system, ranging from a traditional (and expensive) optical spectrum analyzer to novel microwave photonics signal processing techniques [1]. The objective is to detect DNA hybridization by measuring the change on the surrounding refractive index, due to molecular interactions occurring on the functionalized sensing area. A resolution in the order of 10^{-5} was achieved, for the refractive index change, which was shown to be directly proportional to the shift of the notch frequency of a microwave photonics filter realized with a microfiber Bragg grating [1].

In intrinsic sensors, the sensing effect can occur onto the fiber surface, onto gratings (either long-period, tilted, or etched fiber gratings) or through other fiber-based frequency-selective devices, such as Fabry-Perot or Mach-Zehnder interferometers [3], [6]-[8] which typically act as the *sensing element*, i.e. as the element enabling transduction between the biochemical activity (DNA hybridization) and the optical effect (i.e. change of the refractive index, hence of the frequency

M. Barozzi, A. Vannucci, S. Selleri, and A. Cucinotta are with the Dipartimento di Ingegneria dell'Informazione, Università degli Studi di Parma, Parco Area delle Scienze 181/A, 43124, Parma (Italy) (e-mail: annamaria.cucinotta@unipr.it)

A. Manicardi and R. Corradini are with the Dipartimento di Chimica, Università degli Studi di Parma, Parco Area delle Scienze 17/A, 43124, Parma (Italy) (e-mail: alex.manicardi@unipr.it)

A. Candiani, and M. Sozzi are with DNAPhone s.r.l., Parma (Italy) (e-mail: alessandro.candiani@dnaphone.it)

M. Konstantaki and S. Pissadakis are with the Foundation for Research and Technology-Hellas, Institute of Electronic Structure and Laser, Heraklion 70013, Greece (e-mail: pissas@iesl.forth.gr)

response). The technological approach to integrate these frequency-selective transduction elements into the sensing fiber is an inventive and ever-expanding field of research. As an example, an interferometric structure was recently realized by splicing a short (178 mm) segment of microfiber between two single mode fibers with a lateral offset, so as to produce an U-shaped microcavity where the axial mismatch between fibers yields a modal interference process [3]. Moreover, since propagation in the microfiber section is mainly supported by the evanescent field, which extends into the functionalized microcavity, this structure ensures a strong coupling between the evanescent field and the biosample.

All of the above described features implicitly apply to the so-called *label-free* detection schemes. More traditional DNA sensing schemes, instead, required to *label* the target DNA with fluorescent groups and to measure fluorescence, as the signal related to the occurrence of hybridization, with dedicated excitation light sources and fluorescence detectors. Besides the need for extra instrumentation, the functionalization process becomes more complicated and costly, in this case. Label-free DNA detection, instead, allows a real-time in-situ hybridization detection, while permitting a compact and flexible fiber-based system for a highly sensitive rapid response.

Ease of remote control is another desirable feature in label-free optical fiber biosensors. Microstructured optical fibers (MOFs) can be seen as optical as well as microfluidic devices, simultaneously, offering the possibility to infiltrate the biosample into their capillary holes [9], [10]. An extremely interesting possibility is opened up by the recent demonstration of light-driven fluidic actuation [11]. While the variation of the light guiding properties of the fiber as a function of the infiltrated fluid sample is clearly at the core of a MOF-based biosensor, the inverse actuation, namely the all optical control of fluid filling and draining of the MOF capillaries opens up the possibility to realize remotely controlled optofluidic lab-on-chip devices, which was demonstrated in [11] based on the interactions of a ZnO layer within the MOF, activated by either UV (248nm) or visible green (532nm) light.

The field of optical fiber sensors is one example of how photonics could be applied to improve the miniaturization and simplification of analytical and diagnostic tools. This technology can allow reaching a portable and easy-to-use platform in formats such as “lab on fiber” or “lab in a needle” [12], [13]. As recalled above, each of the devices/subsystems of the optical fiber-based sensing platform is subject to a different design choice and brings along pros and cons, as well as further design constraints. In this work, we wish to revise the key elements of a fiber-based biosensing system, discussing the implications of the main design options. Special attention is paid to label-free DNA detection systems, with explicit reference to the previous experience of our research groups.

The paper is organized as follows: in Sec.II, optical fiber based bio-sensing is introduced, discussing various types of possible sensing fibers in Sec. II.A; the process of functionalization of the sensing area is described in Sec. II.B. Sec. III is devoted to different techniques to detect the analyte, e.g., DNA hybridization, from fluorescent labels (Sec. III.A) to transductions elements, such as various types of gratings (Sec. III.B). The opportunity to enhance the sensitivity of the opto-chemical transduction elements by the use of nanoparticles, is discussed in Sec. III.C. Sec. IV reports a couple of meaningful applications of DNA detection, based on our recent experience. Detection of the single-point mutation related to cystic fibrosis is an example of application in the field of medical diagnostics, while food safety and analysis was explored in the identification of a genetically modified organism (GMO) in soy flour. Finally, we summarize the discussion and suggest some future directions of exploration in this fascinating and ever growing field of research.

II. Optical Fiber-Based Bio-Sensors

Biosensors are a particular class of devices, where the sensing unit is constituted by a biological recognition element, able to recognize in a highly specific manner a selected target or a class of compounds. A transducer element then converts the biological recognition event into a signal which can be detected. Different principles of detection have been studied, including electrochemical, mechanical, calorimetric, and optical.

Among these, optical-based biosensing is one of the most widely investigated transduction methods. In particular, optical fiber sensors offer the main advantage of small and flexible shape, connecting a remote light source to a small in situ sensing element; they are able to give rapid and sensitive detection of the target in real time, especially if the detection is performed using a label-free scheme.

In general, optical fibers have long been used as sensors to perform mechanical measurements, for example of force, pressure, strain, displacement, acceleration, vibration, temperature - where typical applications include, e.g., monitoring of oil or gas pipes - as well as sensors/transducers for electric and electromagnetic measurements [14]-[17]. More recently, chemical, biological and biomedical measurements have broadened the field of their application beyond the traditional ones, e.g. civil, aerospace or naval engineering, to the medical field or that of food safety.

The advantages of using optical fibers for the realization of sensors are related to their properties: a) high sensitivity over a very large bandwidth, b) exemption from electromagnetic interference, c) resilience to hazardous environments e.g. high temperature, corrosion, high pressures, etc., d) electrical and chemical passivity, e) low volume and weight, and f) suitability for multi-point and multi-parameter sensing. Moreover, being the elective medium for long distance transmission, thanks to their low power loss, optical fibers are inherently capable of remote sensing (distance of km) and offer the possibility to integrate telemetry through a data-link allocated in a dedicated transmission sub-band, not used for sensing. Contrary to these advantages, some kinds of fibers can be costly and certain sensing systems can be highly complex, involving measurement subsystems that are hard to develop. Moreover, many final users can have little familiarity with this technology, depending on the field of application.

As in most optical communication systems, fiber-based sensing systems can be typically composed of a light source,

providing the optical signal to be sent through the fiber, a *transducer* and a receiver able to detect and monitor the variations of the transmitted signal. The transducer (typically, an optical fiber or a fiber-based device) acts as a modulator which imposes a variation of the optical parameters sensitive to the physical variable that has to be sensed/measured.

A. Fibers for Bio-Sensors

In general, the recognition of the target in the sample induces a modification of the guiding properties of the fiber, hence – in terms of linear systems theory – of its transfer function. In any case, the frequency response of the fiber to any input signal is a function of the parameter to be measured like the presence and/or the quantity of a certain substance. It is thus convenient to adopt a 'spectral sensing,' i.e., to monitor the output spectrum with an optical spectrum analyzer (OSA), especially when the input signal has a very large bandwidth, so as to get as much information as possible on the modifications of the spectral response of the fiber. Coherent or incoherent broadband light sources, like an amplified spontaneous emission noise source or a supercontinuum source, are thus preferable within this scheme.

The principle of operation of a physical, chemical or biological sensor can be based on the measurement of absorbance, reflectance, fluorescence, luminescence and variations of the refractive index and, more in general, of the frequency-selective behavior of the fiber thereby induced.

Within the field of chemical and biological sensing, photonic crystal fibers (PCFs), also called microstructured optical fibers (MOFs), have been deeply studied in recent years because of a number of unique features [18], [19]. A proper design of the PCF geometry, i.e., of the size and displacement of the air holes that run along the fiber, allows an unprecedented control of its light-guiding properties [18].

The two broad categories of PCFs, hollow- and solid-core fibers, provide different coupling methods between guided light and the sample. In the former case, the sample can be infiltrated directly into the low-refractive index core, providing maximal overlap between the sample and the field intensity profile, while in the latter case the cladding air-holes are filled and the interaction with light occurs only in the evanescent tails of the guided modes. Despite the drawback of a weaker light-analyte interaction, however, solid-core fibers are normally preferred because of their lower confinement loss, over a broad wavelength range.

Both standard fibers and PCFs have been employed to realize DNA-detectors. By employing a standard fiber, the target molecules are recognized on the external surface of the fiber. Hence, they can be detected through an interaction with the cladding modes. As a consequence, a DNA-sensor realized with a standard fiber needs a transduction element able to excite the cladding modes (e.g., a long period grating or a tilted fiber Bragg grating) and the system can be read only in transmission mode, as discussed in Sec. III. Moreover, a liquid handling tubing system must be used for packaging the fiber and for keeping the solution close to it; this way, milliliters quantities of analyte are needed [5], [20],[21].

Using PCFs many of these limits can be overcome since the microstructured fibers can be used for light guiding and as a fluidic channel at the same time. Due to the presence of air holes running along its entire length, these fibers have the ability to accommodate the samples in gaseous or liquid form in the immediate vicinity of the fiber core (solid-core) or even inside the core (hollow-core) where the analyte can be detected through interaction with the core modes. In principle, this allows the analysis of volumes in the order of nano- to microliters, orders of magnitude lower than the previous approach. The use of extremely small analyte volumes is of particular interest for biochemical applications, like protein or DNA recognition [22], [23].

In hollow-core fibers, a guided wave sensing occurs, since the light-analyte interaction takes place in the core region where the maximum of the transverse section of the field is concentrated. Finite element methods (FEM) are commonly used in simulating the mode field distribution, to assist the design of a novel PCF structure or to analyze its performance, e.g., to calculate the light power fraction in the region of interest. In FEM calculation, Maxwell's equations are typically decoupled and numerically solved, on a proper grid discretized mesh of points, in terms of the magnetic field, which ensures the absence of field discontinuities along the interface between materials with different refractive indices. As an example, Fig. 1 reports the FEM solution within a *Bragg fiber*, in which light guiding is provided by concentric layers of dielectric materials with different refractive indices. Conceived long ago [24], Bragg fibers can today be fabricated with PCF technology, where the cladding is realized with thin concentric silica rings surrounded by air and connected by thin silica bridges, as shown in the diagram in Fig. 1.

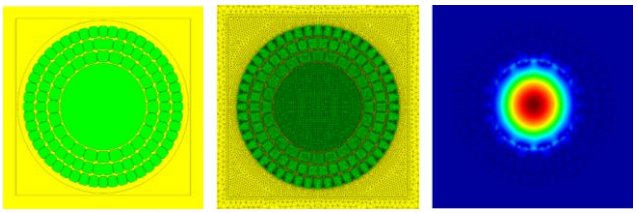

Fig. 1. A model of Bragg fiber cross-section (left) with the corresponding discretized mesh of points (triangular second order elements, center) and the computed magnetic field distribution for the fundamental mode (right). Picture taken from [22].

The same problem of evaluating the mode field distribution is faced when solid core PCFs are chosen. Since the sample is infiltrated in the air holes of the microstructured cladding, sample-light interaction will necessarily occur with the exponentially decaying tails of the optical wave thus providing an *evanescent wave sensing*. As seen in Fig. 2, the size and arrangement of air holes in the cladding can make up very different types of suspended core fibers. In the figure, a *Penta* fiber, thus called for the number of holes, is shown on the left, where the five non-circular holes effectively make up an air cladding, except for the five thin silica bridges that hold the suspended core, accommodating a relatively large volume to infiltrate the sample. A totally different pattern and size of holes characterizes the commercially available large mode ar-

ea (LMA-10) fiber, visible on the right of Fig. 2. These fibers are more properly called *triangular lattice fibers*, from the regular arrangement of the holes, where one or more missing holes in the center of the pattern provide the solid silica core. As stated, the transverse distribution of the optical field is crucial for the effectiveness of sensors like these, based on the evanescent wave, since the detection mechanism typically relies on the spectral measurement of transmitted or reflected light.

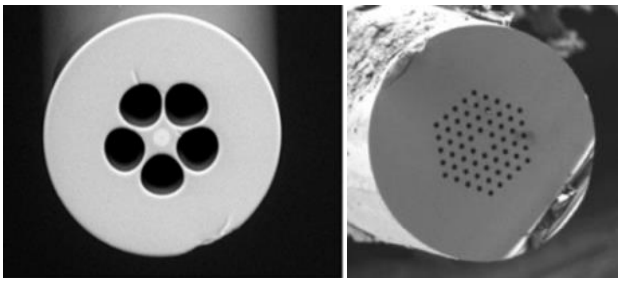

Fig. 2. Scanning Electron Microscope images of Penta and LMA-10 suspended-core PCFs.

One possible approach is that of measuring the absorption spectrum, which characterizes the specific analytes to be sensed. The well-known Beer-Lambert law (1) states that the logarithmic absorption spectrum is proportional to the sample-filled fiber length L as:

$$A(\lambda)[dB] = 10\ \varepsilon(\lambda)\ C\ \Phi(\lambda)\ L \quad (1)$$

where C is the concentration of the sample and ε(λ) is its characteristic molar absorbance spectrum, while Φ(λ) is the fraction of light power that overlaps with the sample, which should be optimized, by fiber design, so as to maximize the sensitivity of the sensor, for a given sample and a given length.

Another issue related to the transverse distribution of the optical field is that of light coupling, from the light source to the sensing fiber. An effective coupling, able to convey all light power into the sensing fiber core, thus avoiding coupling losses, is clearly more difficult to achieve for evanescent wave sensing fibers, where the core is smaller.

Besides silica fibers, microstructured polymer optical fibers can be used for biological sensing and have been demonstrated to perform DNA detection [25]. In addition, photonic quasi-crystal fiber have been proposed as a sensing platform [26]. Recently, nanoslotted parallel quadrabeam photonic crystal cavity have been used as sensory systems, showing an improvement of >2000 in the sensor figure of merit, which allowed to sense streptavidin at 10 ag/mL [27].

B. Fiber Processing for Recognition Elements

An essential part of the process for transforming an optical fiber into a biosensing device is the derivatization of outer or inner surface with biorecognition elements, which can selectively "capture" the analytes to be sensed. Derivatization procedures make use of liquid solutions which are brought in contact with the outer or inner surfaces of the fibers, as schematically depicted in Fig. 3. External derivatization can be achieved by immersion of the sensing area of the fiber into reagents' solutions (Fig. 3a), whereas inner modification is performed by infiltration of reagents inside the holes of a microstructured optical fiber by applying pressure (Fig. 3b).

Chemical reactions are common to both these approaches. Hundreds of microliters to millilitres of derivatizing solutions are necessary for external derivatization, whereas dozens of nanoliter-to microliter volumes are, in principle, necessary for the inner derivatization. Also, the external derivatization is a static process, whereas the infiltration is a flow process, which ensures constant renewal of the solution coming in contact with the surface, and this enhances the rate and homogeneity of surface derivatization. The use of a micro-dip coater can enhance the efficiency of external coating, since it allows to dip the end part of the fiber into the reagents and then withdraw it with a programmed velocity [28]. Extensive washings are necessary for both procedures.

One disadvantage of outer derivatization is that the fiber polymeric coating must be removed, thus leaving the bare fiber, which is rigid and fragile, and thus the sensing part can be broken during processing (Fig. 3a). Inner derivatization can be performed without removing the coating, or at least by removing only the terminal part of the fiber coating to allow coupling with a liquid handling system (Fig. 3b). On the other hand, the set-up for external derivatization is simpler, whereas the infiltration of reagents and analytes into microstructured fibers requires a liquid-handling system similar to microfluidic devices, with a pumping system, a reservoir, and ferrule or other sealed joints. Correct coupling of these elements and check for possible leakage are necessary for appropriate derivatization and analysis. Inner derivatization of the microstructured fibers also allows optical processes to be more confined and generally produces a more regular distribution of the probes, with less contaminants, as shown in Fig. 3c from our own experiments.

Absorption of a layer of organic material to coat the surface of the sensor can be used as a strategy to obtain functional optical fibers: poly-Lys, streptavidin, avidin or neutravidin and

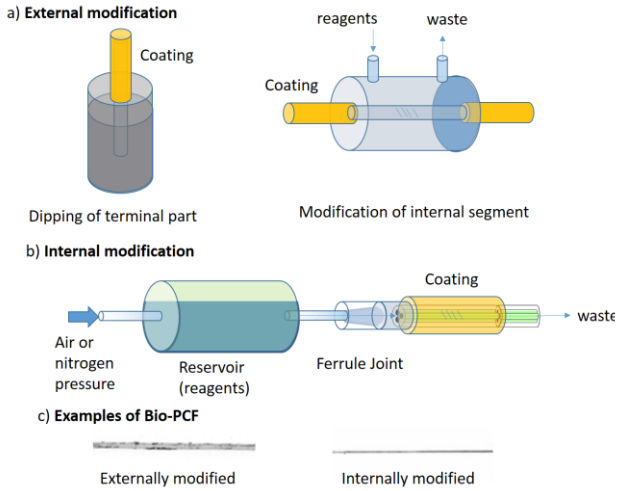

Fig. 3. Scheme of internal (a) and external (b) functionalization of fibers. c) Fluorescent signal of externally or internally modified fibers.

charged polymers can be used for this purpose [29]–[31]. Layer-by-layer deposition can be used to change the overall charge of the layer exposed to the solution. Covalent or non-covalent bonding to this layer can be used to attach specific biorecognition elements (receptors). However, non-covalent coating can be more easily removed by effect of chemical as well as mechanical treatments, while covalent functionalization will increase the stability of the device, opening the possibility to reuse it after a properly designed washing step [21]. External coating with a metal layer has been widely used for surface plasmon resonance (SPR) detection [13]. Inner coating with metal nanoparticles has also been reported [32].

Covalent linking the biosensing elements to the surface of the optical fiber creates a more robust device. While a broad range of different protocols are now present in the literature, for silica or siliceous material fibers well established procedures, available from glass surface chemistry, are based on silanization of the surface. (3-aminopropyl)triethoxysilane (APTES) have been widely used, both to create a positively charged layer (NH_2 groups are protonated at neutral pH), and to provide an anchoring group (exploiting the nucleophilicity of amino groups) for subsequent attachment of other functional molecules and biorecognition elements. A series of different silanes bearing different functionalities are commercially available and these allow to choose between many different options for both adsorption and covalent linking of biorecognition elements.

Silanization is normally preceded by a washing/activation step performed either with strong acid (e.g. HCl in methanol) or with a combination of acid and oxidant (e.g. with piranha solution, composed of sulfuric acid and hydrogen peroxide); this treatment allows to eliminate residual organic components and to activate the silica surface creating silanol groups, which are necessary for the silanization step.

These procedures have been shown to be compatible with deposition of proteins, DNA, or fluorophores that can be denatured, or are chemically or photochemically labile. Using a silanization protocol with an apparatus similar to that in Fig. 3a, we showed that suspended-core PCF could be externally modified with a fluorescent molecule such as tetramethyrhodamine (TAMRA) achieving fluorescence properties, as detected by laser scanning [33].

DNA recognition elements can be introduced in this way; small DNA segments, i.e. oligonucleotides, or molecules mimicking their properties can be used for this purpose, and the recognition scheme is the pairing of the nucleotide sequences following the well-known Watson-Crick base pairing scheme (Fig. 4B). In our work, we exploited the intrinsically superior properties of peptide nucleic acids (PNA), which are polyamidic analogs of DNA (vide infra and Fig. 4A for DNA vs PNA structure); these molecules can be conveniently linked to the optical fiber inner and outer surfaces through this strategy. Optical fibers made of polymeric materials such as poly(methyl methacrylate) (PMMA) can also be modified using coating techniques [28]. Covalent modification of these surfaces require more elaborated techniques such as chemical or photochemical oxidation [34].

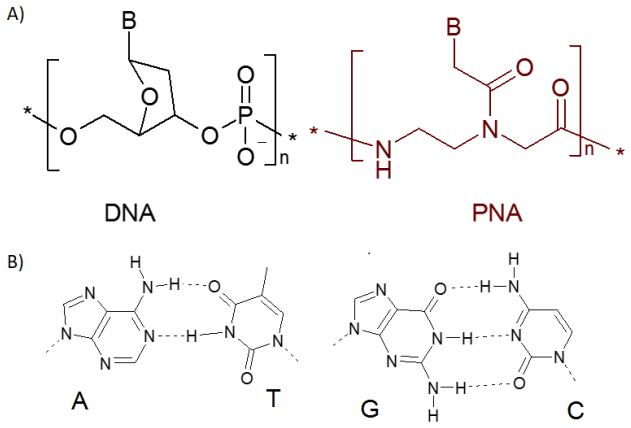

Fig. 4 A) chemical structure of probes based on DNA and PNA; B) Watson-Crick base pairing used to recognize the target complementary nucleic acids.

III. Detection Techniques and Sensing Amplification

Sensing the presence of a DNA target sequence can be achieved when *hybridization* occurs between the DNA strand and a sensing probe, a short oligonucleotide or a nucleotide mimic, with a sequence that is complementary to it. This interaction can be converted by the transducer element into a detectable signal that can be recognized and measured. A number of different working principles for fiber-based biosensors exist in the literature, focusing on different detection protocols.

Common optical techniques detect target DNA by exploiting: i) fluorescent markers; ii) the change in the refractive index achieved when target analytes bind to the biorecognition molecules; iii) the spectral response in transmission or reflection; iv) the spectral response given by Bragg, long period and relief gratings that can be inscribed into the fiber core or capillary surfaces [35].

Optical bio-sensor can be classified in two main categories, depending on the method applied to detect the recognition event: labelled sensors [33], [36]-[38] and label-free sensor [31], [39]. In a labelled detection scheme, the target molecules are modified by adding a *tag* element that is easy to detect, while in a label-free detection scheme, the target molecules are detected with no need for preliminary modification steps.

A. Sensing Formats for Labelled Detection

Fiber-based biosensors for labelled detection scheme, typically rely on the use of fluorescent markers. Thus, a traditional method to check that (and how much) hybridization has taken place, is to measure the fluorescence signal output by the *labelled* DNA strands. The presence of a specific signal connected to the fluorescent dye used indicates the presence of the target molecules, while its intensity depends on the concentration of the captured molecules. This approach has been widely exploited in microarray technologies [40], [41], where the hybridization of target DNA occurs on functionalized glass slides, and the reading process is performed with dedicated instrumentation equipped with specific lasers for fluorophore excitation and filters for the selection of the emission wave-

length.

A broad variety of fluorophores and protocols has been developed during the years, and labelling kits are now commercially available with affordable prices. By carefully choosing the molecules (with special attention to the excitation and emission wavelengths) and the derivatization protocols, it is also possible to design multi-labelled systems that enable multi-component detection or increase the sensitivity of the system [42].

Labelling of nucleic acids can be achieved by reaction of terminal groups with specific derivatization kits, but it is most widely done using incorporation of fluorescently labelled monomers or oligomers during polymerase chain reaction (PCR) or other DNA amplification strategies, i.e. protocols used in order to increase the number of copies of a specific DNA sequence. Since fluorescent labelling has been widely used for PCR and DNA sequencing (including some of the most important "next generation sequencing" technologies), a wide variety of labels and schemes are available [43]. Some of the most used fluorophores (i.e. fluorescent groups to be attached to the analyte) which have been used are fluorescein and rhodamine derivatives (e.g. FAM and TAMRA), cyanines (e.g. Cy3 and Cy5), and Alexa fluorophores; Alexa are generally preferred when laser excitation is used, due to their low photobleaching. Fluorescent nanoparticles, such as quantum dots and fluorophore-loaded inorganic nanoparticles are also emerging as labelling agents and have considerable advantages in biological applications [44]–[48]. A general scheme of fiber modification and detection of labelled complementary DNA is shown in Fig. 5.

The need for modification of the DNA analyte for fluorescence detection can be avoided using two strategies: using a so called "sandwich" approach, or a "molecular beacon." In a sandwich approach, the DNA is bound by a "capture probe" on the sensor surface through a target sequence; a second target sequence, flanking to the first one, is then used to capture a complementary DNA oligonucleotide which is labelled with the desired fluorophore. Molecular beacons are oligonucleotides, or oligonucleotide analogs, in which a fluorophore and a quencher are held close to each other by a stem-loop structure; in the free probe the fluorescence is therefore quenched; interaction with target DNA induces separation of the two units and the fluorescence is then "switched on." Beacon probes used on a surface allows to directly obtain a fluorescence signal upon interaction with the unlabelled target DNA.

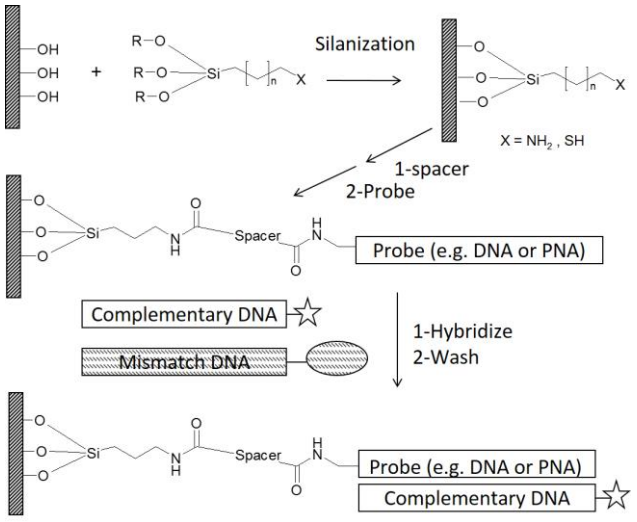

Fig. 5. Schematic representation of silanization of fiber surface, probes deposition and hybridization with nucleic acids. Ideally, only complementary DNA should bind to the probe attached to the fiber surface.

B. Label-free Sensing

Compared with labeled sensors, a label-free sensor allows the direct sensing of the target DNA without a previous functionalization step for the introduction of the marker. To this purpose, a change in the specific properties of the device, induced by the hybridization of the DNA, is exploited.

Typical optical fiber label-free biosensors measure the change of a resonance - typical of a grating, a surface plasmon, or a Fabry-Perot etalon - that is introduced by the presence of a given analyte. Most of the label-free detection mechanisms exploit the change of refractive index induced by molecular interactions, which is related to the target presence/concentration and can be achieved with very low sample volumes. This implies simpler sample treatment and detection protocols.

In our previous experiments on fiber-based bio-sensors, we focused upon the use of gratings as transducer elements. A grating consists of a periodic change, i.e. a modulation, of the refractive index of the fiber core. Such a periodic structure results in a wavelength filter which affects the spectrum of the signal propagating through the fiber. The properties of the filter depend on the period, the length and the index profile of the grating. In general, gratings are used in many fields of application because of their sensitivity to temperature, stress, pressure, and, as stated, changes of the refractive index. The changes of the refractive index, in the area surrounding the grating, modify the grating properties, hence the filtering of light that propagates through the fiber. Thus, the DNA recognition event can be identified and measured by monitoring the spectral response of the grating, either in transmission or in a reflective configuration, depending on the grating type.

As stated in Sec. II, in a biosensor realized with a standard fiber, the analyte is deposited onto the external surface of the fiber, hence the changes in the refractive index occurs at the cladding-analyte interface. As a consequence, a grating able to excite the cladding modes has to be employed.

Examples of gratings that satisfy the above condition are Long Period Gratings (LPG) and Tilted Fiber Bragg Gratings (TFBG). Both have been employed to realize label-free biosensors [5], [49], [50], also by our research groups [20], [21]. LPGs are characterized by a "long" period, ranging from 100 μm to 1 mm, which allows the coupling between the fundamental core mode and a discrete number of cladding modes. As a consequence, the transmitted signal spectrum shows a series of attenuation bands, located at discrete wavelengths. The central wavelength of the attenuation bands is directly proportional to the difference between the core and cladding

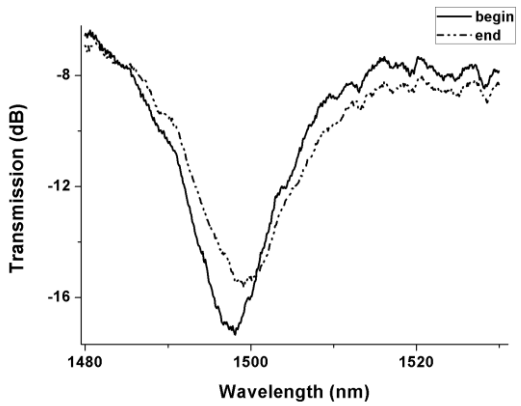

Fig. 6. Wavelength shift of the transmission spectrum of a LPG induced by the DNA-PNA binding, at the beginning/end (after 1h) of the hybridization process (from [20]).

effective indices, i.e., $\lambda_{att}=\Lambda(n_{core}-n_{clad})$, through the grating period Λ. A change in the effective index of the cladding, due to the presence of the target DNA captured by a specific probe, yields a shift of the central wavelengths of the attenuation bands.

Fig. 6 shows the shift in the resonant peak of the first (and most visible) among the five attenuation bands in the transmission spectrum of a 20 mm long LPG (with period Λ = 407 μm) inscribed in a standard single-mode fiber [20]. The transmission spectra were monitored throughout the hybridization process, with a 120 nM solution of DNA binding to the PNA active sites, which seems to reach saturation after one hour. The spectra in Fig. 6, at the beginning and end of this 1h interval, show a remarkable difference, $\Delta\lambda_{att}$ = 1.2 nm, in the resonant peak of attenuation of the LPG.

In TFBG, an angle between the plane of the grating and the transverse section of the fiber produces a complex multi-coupling interaction between the fundamental co-propagating core mode and the counter-propagating core mode, the counter-propagating cladding modes, and the radiant modes. All of these interactions results in a highly variable transmission spectrum, showing a large number of attenuation bands.

In particular, in [21] we have implemented a DNA label-free sensor based on the double tilted fiber Bragg grating (DTFBG) shown in Fig. 7, as a detector element. A resonant Fabry-Perot cavity, with a given length *d*, is created by inscribing two identical blazed gratings into the fiber core (diagonal stripes at the edges of Fig. 7). Resonance occurs at the Bragg wavelength due to the standard Fabry-Perot interference, where light is back reflected into the fiber core at each of the two gratings. Resonance occurs at the Bragg wavelength due to the standard Fabry-Perot interference, where light is back reflected into the fiber core at each of the two gratings. In addition to this, the blazed gratings let part of the energy in the core modes couple with cladding modes and vice-versa. This phenomenon occurs for wavelengths lower than the Bragg wavelength and can create resonances. As explained in Fig. 7 in terms of ray optics, part of the energy that back-propagates as cladding modes can be coupled back into the core modes at the opposite grating.

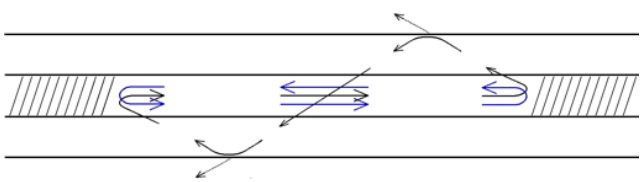

Fig. 7. Schematic diagram showing the core and cladding modes in a double tilted fiber Bragg grating (DTFBG), where the mode-coupling between core and cladding is provided by the two blazed gratings, in addition to the standard core-to-core mode Bragg scattering.

Clearly, the guiding properties of the cladding modes depend on the refractive index of the external part of the fiber, i.e., the one in contact with the sample to be sensed. A sufficient index step is needed for the cladding modes to be weakly guided, i.e., the external refractive index has to be lower than the effective refractive index of the cladding. On the contrary, since the DNA attachment layer has a refractive index similar to that of silica [51], the external index increases and approaches that of the cladding. Hence, guiding is broken and energy leakage from the cladding modes becomes significant. Thus, the onset of resonances due to an effective interference between the core and cladding modes directly depends on the external refractive index, which can be measured from the spectral fringes of interference. In particular, for wavelengths lower than the Bragg wavelength, there is a multiplicity of cladding modes. Although higher-order modes, i.e., those associated to much lower wavelengths, are more sensitive to the changes of the external refractive index, there is a highly resonant multiplicity of lower-order modes, associated with intermediate frequencies. These modes are collectively referred to as the *ghost mode*, that is very well identified in the Fourier spectrum and whose *visibility* is strictly related to the above-discussed resonant structure. A tilting of the gratings by only a few degrees (3° or 5°) provides an almost equal energy split between the (Bragg) core-mode and the cladding ghost modes, at any reflection.

Each spectral mode, with an original spectral amplitude $E(\lambda)$ from the light source, undergoes constructive or destructive interference that can be modelled as a multiplication by a sinusoidal function of wavelength, i.e., the received spectrum is

$$T(\lambda) = E(\lambda)\,[1 + V\cos(2\pi\sigma\lambda + \varphi)\,] \quad (2)$$

where V is the *visibility* of the fringes, σ is the frequency of the interference and φ is a phase term. In order to evaluate V, the portion of the received spectrum $T(\lambda)$ in the neighborhood of the ghost mode was processed through Fourier analysis, in [21]. Given that the bandwidth of the envelope of the ghost modes (1553.9-1554.6 nm, in [21]) is much larger than the period of the fringes, i.e., the inverse of σ, the modulus of the Fourier transform of (2) clearly shows three lobes that do not depend on φ, where the relative amplitude of the sidelobes is V/2, compared with the central one. Through the numerical processing described in [21], V is evaluated and its dependence on the presence of the target DNA is shown Fig. 8 as a

function of time. Consider that, as expected, no visibility variation was observed for an unfunctionalized reference sample.

The main advantage in using a fiber with a double TFBG in biosensing applications, as described above, is that it is almost insensitive to the variations of temperature or strain. In fact, both these effects produce a shift of the wavelength spectrum,

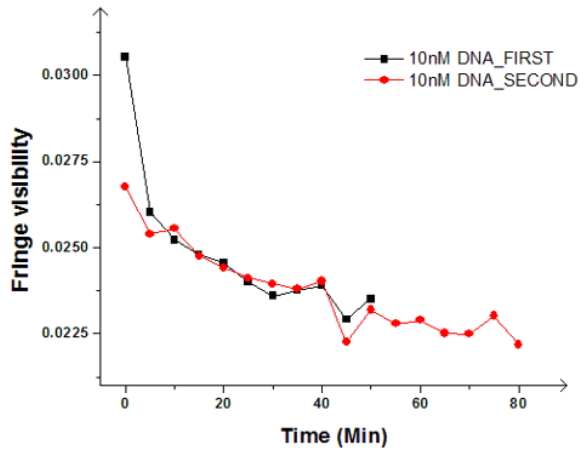

Fig. 8. Variation of the fringe visibility V of the ghost mode in a DNA sensor based on DTFBG (from [21]), as a function of time. The sensor was used twice, with 10nM DNA, to highlight possible memory effect.

hence of (2). Such a shift implies a modification of the phase spectrum, in the Fourier transform of (2), which is not considered, since the evaluation of the fringes' visibility V is based on the modulus of the transform of (2). On the contrary, sensors based on LPGs are highly sensitive to environmental conditions, including variations of temperature and strain [52], which strongly influence the Bragg mode propagating within the fiber core, so that thermal/strain variations can influence the drift of the resonant peaks of the LPG, as a parasitic effect.

Nevertheless, the possibility to have a direct readout of the resonant wavelength in real-time, on an OSA, constitutes an enormous advantage in favor of LPGs. In [20], for instance, such a real-time monitoring of the resonant wavelength shift permitted to verify that most of the hybridizations occurs between 20 and 35 minutes, before the onset of saturation. Of course, a similar continuous spectral monitoring is also feasible for sensors based on DTFBG, as shown in Fig. 8, provided that the measurement is obtained by an off-line processing of the spectrum. In general, the possibility of continuously monitoring the changes in the transmitted spectrum, throughout the whole hybridization process, is a positive feature of externally functionalized fibers. As depicted in Fig. 3, the part of the fiber that is sensitive to the analyte is closed in a sealed cell where the reagents flow, while both fiber ends are accessible and can accommodate both the light source and the OSA that continuously monitors the transmitted spectrum, during the functionalization and hybridization processes.

Furthermore, in Fig. 8 the reproducibility of the measurements, hence the re-use of the same fiber for several detections, is demonstrated. The two curves represent the visibility measurements after two hybridization steps implemented with the same amount of DNA (10 nM) and conditions. An extensive washing of the fiber, for more than 24 h, is needed in order to remove the bound DNA and allow the re-use of the fiber for another detection. Since this washing step has not been optimized in [21], the visibility measured in the second hybridization step (red curve in Fig. 8) starts from a value lower than that measured in the first hybridization step (black curve in Fig. 8). Such a “memory effect”, which has been seen also in [3], limits the possibility to re-use the sensor described above (and deeply analyzed in [21]) to 6 hybridization steps. In [5] the use of the same LPG for more than 20 complete assays has been reported.

A different measurement setup must be adopted for internally functionalized fibers, i.e., for the PCFs, whose features and advantages have been discussed in Sec. II.A. Since the functionalization takes place in a region in close contact with the fiber core, it is possible to employ a fiber Bragg grating (FBG) as a transduction element. A FBG inscribed into the fiber core directly interacts with the core modes, as a selective mirror, and is able to selectively reflect a fundamental mode as well as higher-order modes, located at their respective bandwidths. This feature offers the possibility to monitor the signal in reflection mode, hence to use the fiber itself as optical/mechanical probe. A spectral modification, such as a wavelength shift, of one or more of the reflected modes transduces the occurrence of the hybridization process into the measurable optical signal.

Although sensors based on PCFs with reflection mode FBGs can offer significant advantages, the need of an internal functionalization protocol prevents continuous monitoring during this steps (see Fig. 3b). Hence, the reflected spectrum can be measured only once the functionalization or hybridization process is finished. Applications of PCFs-based biosensors exploiting FBGs as transducer element to detect target DNA will be discussed in Sec. IV.

Of course, both the fabrication of a PCF and the inscription of an FBG into it are nontrivial tasks: the fiber cladding needs to be etched to expose the core and the direct writing of the grating, with dedicated lasers is a delicate process. In addition, grating inscription in photonic crystal and microstructured fibers is still challenging due to the presence of effects such as the side illumination scattering [53].

C. Sensing Amplification with NanoParticles

As mentioned above, label-free analysis does not require a pre-functionalization of the analyte for detection, so the sensing of the target is merely related to the variation of fiber properties induced by the recognition. However, the employment of refractive index variation based methods in label-free detection of DNA have to face with the low change of refractive index induced by the recognition of the target sequence in the sensing area. Moreover, when using hollow-core PCFs there is a good overlap between the recognition region and the light, whereas in solid-core PCFs only the evanescent modes interact with the sample, resulting in a lower sensitivity of the transducer to sample-induced variation. An easy way to en-

hance this effect on the refractive index, is to compare the variation induced in the sensing layer against the refractive index of air rather than the one of a solution, when performing the measurements.

When analyzing long DNA sequences, we can take advantage to the part of the target that is not involved in the recognition by the sensing element attached to the surface of the fiber. In this "sandwich" approach, the regions flanking the sequence recognized by the probe attached to the surface, the capture probe, are exploited for the decoration of the system with additional elements connected to a "decoration" probe. In this context high refractive index units can be used so that with this second recognition event is possible to change the character of the layer sensed by the device from pure organic (DNA) coating, to a mixture with this second component having the role of sensing enhancer.

In the so-called label-free techniques, amplification of the response given by the transducer element, obtained using a "sandwich" format, can be very useful, in particular when nanoparticles (NPs) are used [54]–[56]. In this context, the use of NPs can offer both the advantage of introducing a sensing amplifier together with a nanoscopic dimension that do not obstruct the micrometric holes composing the PCF. Fig. 9 shows a generic scheme for the realization of a "sandwich" system that employs NPs.

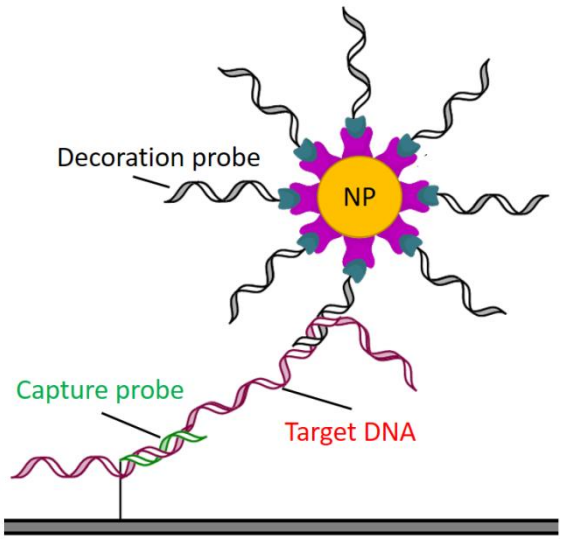

Fig. 9. Sandwich approach using nanoparticles.

In order to establish a specific interaction between the NP and the sequence captured on the surface, a second recognizing element have to be used to decorate the shell of the nanosystem and ensure the selective recognition. The chemistry that can be employed is strictly connected to the nature of the NPs and the description of system and chemistry that have to be used for the construction of this structure is beyond the scope of this review and can be easily found in the literature [57].

IV. Applications: DNA Label-free Detection Using PNA Probes

In order to build a device that is able to sense the presence of a specific DNA, we have to modify the fiber with some recognizing element that can interact in a selective way with the target sequence. In most of the device, this role is played by short DNA oligonucleotides, or analogs [2], [3].

Our experience in the development of sensors for DNA label-free detection was focused in the use of peptide nucleic acids (PNAs, Figure 4a) as a tool for the recognition of the target sequence. 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. They combine chemical flexibility and the sequence selective recognition of nucleic acid targets with a unique stability towards chemical and biological degradation [58].

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 [59], and sequence-specificity, in particular their ability to discriminate even a single base mismatch.

The application of this kind of systems in combination with an easy protocol to connect them covalently to the inner glass surface, as described in Sec. II.B, allow the production of robust sensing elements that can be easily reused several times, thus allowing the possibility to incorporate them in the realization of devices.

In recent years, we explored the possibility to combine the advantages provided by PCF in term of small analysis volumes (Sec. II.A) and with the possibility to perform label-free detection (Sec. III.B) with the great performances in the PNAs in term of sequence selectivity and stability, for the realization of systems able to perform qualitative or quantitative analysis.

In the former case the ability of the PNA to detect even a single base mismatch was exploited in the detection of M-W1282X single point mutations related to the onset of cystic fibrosis, thus a realization of an ON/OFF detection of the target sequence that can be of great interest for medical diagnosis, where detection of point mutations can help early prognosis of diseases. Detection of single nucleotide polymorphisms (SNPs) also enables to classify the origin of biological samples and raw materials.

In the latter case we exploited the great stability of the PNA:DNA complexes for the realization of quantitative detection of contaminants in food matrices, with particular focus on the development of a system able to perform a PCR-free detection, in order to reduce the time and costs required for the analysis and enable the application of this kind of devices for a faster screening of contaminants in raw materials or in the food processing chain.

The first example developed in our laboratories, focused on the detection of the single point mutation W1282X, associated with cystic fibrosis disease, was performed using a multimode Penta fiber with a Bragg grating inscribed within its core [60]. The sandwich-like scheme of detection adopted in this study, derived from previous studies conducted with different optical techniques [54], was designed in order to avoid the use of labelled target DNA as well as a system to enhance

the signal shift exploiting the higher refractive index of the gold compared to those of air or organic layer, as described in Sec. III.C.

The capturing abilities of the PNA inside the fiber capillaries were first tested by means of a fluorescent oligonucleotide probe (Fig. 10a) with the full complementary sequence. After this first verification step 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 signal were analyzed showing a significant shift in the high order Bragg mode only were target DNA was used (Fig. 10b and Fig. 10c).

Later we moved our attention on the possibility to apply this approach to a PCR-free detection for the identification of RadupReady Soy GMO contaminants in soy flour [61]. 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 the evaluation of the variation induced by the recognition events was clear and sharp.

The detection scheme was the same applied before and it was possible to detect small percentages of target DNA (1% and 10%) in presence of a large excess of non-target DNA, using only a small amount (3 ng) of sample. Fig. 10 shown the signal shift obtained using a 10% target containing sample (Fig. 10d) and the shift obtained using solution with different percentage of target (Fig. 10e). Selectivity of the analysis was assessed using a blank soy flour sample as well as an unrelated

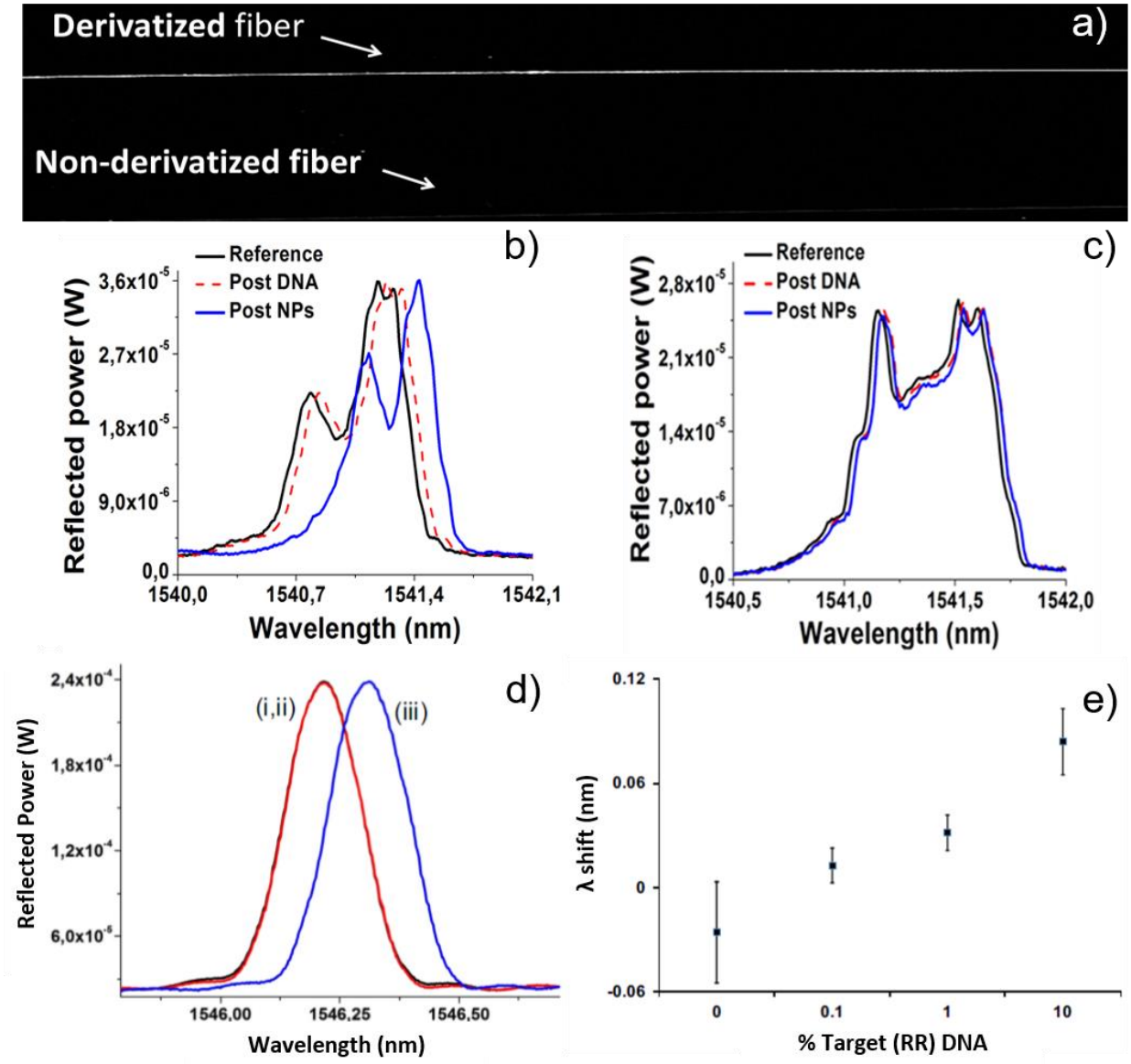

Fig. 10. a) Fluorescence image of PNA-modified and unmodified fibers after infiltration of fluorescent DNA; b) shift in the high order band induced by infiltration of target sample; c) shift in the high order band induced by infiltration of non-target sample; d) wavelength shift in the reflection mode obtained for the LMA10-PNA fibers (i)before(blackline),(ii) after DNA(redline), and(iii) after ON-AuNPs infiltration (blueline),using10%GMO DNAsample; e) optical shifts obtained using DNA at the same concentration but with different GMO%(vertical bars represent standard deviation).

calf thymus sample, resulting in no significant shift.

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 [21], confirming the possibility to apply this kind of detection in the realization of devices.

V. Conclusions and perspectives

Future perspectives in the development of this system will be focused at different levels. In particular, it is possible to act both on the "mechanical structure", i.e. on the fiber structure and on the transducer element, and on the "chemical structure" of the bio-sensor.

About the optical fiber that can be employed to realize biosensors, an ad-hoc design of microstructured optical fibers (MOFs) holes could be developed, both to enlarge the area where the analyte resides and to bring more signal power in that area. Moreover, the development of new techniques for structuring MOFs that allow to machine optical structures (Fabry-Perot, gratings, etc.) directly into the fiber core [62], could bring to a more specific and sensitive transducer element. Together, these enhancements could increase the interaction between the wave propagating into the fiber and the area where the target DNA is deposited, hence to a more sensitive bio-sensor.

From the chemical point of view, in the detection scheme employed for the experiments reviewed in Sec. IV (as depicted in Fig. 9), different parameters can be improved in order to increase the sensitivity and the robustness of the analysis. Compared to devices in which DNA is employed, the use of PNA as capturing element, as already described in Sec. IV, strongly increase the stability of the sensing device to both chemical and biological degradation, together with easier functionalization protocols.

On the other side of the "sandwich," the AuNPs have some drawback connected to the possible aggregation in the long period, with formation of clusters that cannot be employed in the analysis because of the risk to irreversibly clog the holes of the PCF. In addition, the biological nature of the molecules used for the decoration of the nanosystem, strongly decrease the possibility to store the solution for long times. In this perspective, the possibility to design new nanosystems based on more stable NP and the possibility to adopt chemical-based approaches for the linking of the decoration probe, will greatly increase the shelf life of the signal enhancer. Moreover, if the refractive index of the system is higher compared to the one of the AuNP, it is possible to forecast an increase in the sensitivity of the device.

Last, many experiments [21], [60], [61] [3] show that the reusability of the bio-sensor seems to be limited at 5/6 DNA detection processes, due to the "memory effect" discussed in Sec III.B. To increase the reusability of the bio-sensor new washing or calibration [5] techniques, for example with solutions stronger than PBS or with thermal techniques, that bring back the bio-sensor to the original conditions and minimize or even eliminate the "memory effect", are under investigation.

References

[1] Y. Cao, T. Guo, X. Wang, D. Sun, Y. Ran, X. Feng, and B. Guan, "Resolution-improved in situ DNA hybridization detection based on microwave photonic interrogation," *Opt. Express*, vol. 23, no. 21, pp. 27061–27070, 2015.

[2] Y. Liu, S. Chen, Q. Liu, and W. Peng, "Micro-capillary-based evanescent field biosensor for sensitive, label-free DNA detection," *Opt. Express*, vol. 23, no. 16, p. 20686, 2015.

[3] B. Song, H. Zhang, B. Liu, W. Lin, and J. Wu, "Label-free in-situ real-time DNA hybridization kinetics detection employing microfiber-assisted Mach-Zehnder interferometer," *Biosens. Bioelectron.*, vol. 81, pp. 151–158, 2016.

[4] R. Gao, D. Lu, J. Cheng, Y. Jiang, L. Jiang, J. Xu, Z. Qi, "Fiber optofluidic biosensor for the label-free detection of DNA hybridization and methylation based on an in-line tunable mode coupler", *Biosensors and Bioelectronics*, vol. 86, pp. 321-329, 2016.

[5] L. Moreira, H. M.R. Gonçalves, L. Pereira, C. Castro, P. Jorge, C. Gouveia, J.R. Fernandes, P. Martins-Lopes, "Label-free optical biosensor for direct complex DNA detection using Vitis vinifera L.", *Sensors and Actuators B: Chemical*, vol. 234, pp. 92-97, 2016.

[6] W. Yu, T. Lang, J. Bian, W. Kong, "Label-free fiber optic biosensor based on thin-core modal interferometer", *Sensors and Actuators B: Chemical*, vol. 228, pp. 322-329, 2016.

[7] L. V. Nguyen, K. Hill, S. Warren-Smith, T. Monro, "Interferometric-type optical biosensor based on exposed core microstructured optical fiber", *Sensors and Actuators B: Chemical*, vol. 221, pp. 320-327, 2015.

[8] X. Jiang, Y. Chen, F. Yu, L. Tang, M. Li, J. He, "High-sensitivity optical biosensor based on cascaded Mach–Zehnder interferometer and ring resonator using Vernier effect," *Opt. Lett.*, vol. 39, pp. 6363-6366, 2014.

[9] D. Psaltis, S. R. Quake, and C. Yang, "Developing optofluidic technology through the fusion of microfluidics and optics.," *Nature*, vol. 442, no. 7101, pp. 381–386, Jul. 2006.

[10] X. Fan and I. M. White, "Optofluidic Microsystems for Chemical and Biological Analysis.," *Nat. Photonics*, vol. 5, no. 10, pp. 591–597, Oct. 2011.

[11] I. Konidakis, M. Konstantaki, G. D. Tsibidis, and S. Pissadakis, "Light driven optofluidic switch developed in a ZnO-overlaid microstructured optical fiber," *Opt. Express*, vol. 23, no. 24, pp. 31496–31509, 2015.

[12] M. Consales, A. Ricciardi, A. Crescitelli, E. Esposito, A. Cutolo, and A. Cusano, "Lab-on-fiber technology: toward multifunctional optical nanoprobes.," *ACS Nano*, vol. 6, no. 4, pp. 3163–3170, Apr. 2012.

[13] A. Ricciardi, A. Crescitelli, P. Vaiano, G. Quero, M. Consales, M. Pisco, E. Esposito, and A. Cusano, "Lab-on-fiber technology: a new vision for chemical and biological sensing.," *Analyst*, vol. 140, no. 24, pp. 8068–8079, Dec. 2015.

[14] T. Shen, Y. Feng, B. Sun, and X. Wei, "Magnetic field sensor using the fiber loop ring-down technique and an etched fiber coated with magnetic fluid," *Appl. Opt.*, vol. 55, no. 4, pp. 673–678, 2016.

[15] S. Atakaramians, A. E. Miroshnichenko, I. V. Shadrivov, A. Mirzaei, T. M. Monro, Y. S. Kivshar, S. Afshar V., "Strong Magnetic Response of Optical Nanofibers," *ACS Photonics*, vol.6, pp. 972-978, 2016.

[16] S. Musolino, E. Schartner, G. Tsiminis, A. Salem, T. Monro, and M. Hutchinson, "Portable optical fiber probe for in vivo brain temperature measurements," *Biomed. Opt. Express*, vol. 7, pp. 3069-3077, 2016.

[17] L. Nguyen, S. Warren-Smith, H. Ebendorff-Heidepriem, and T. Monro, "Interferometric high temperature sensor using suspended-core optical fibers," *Opt. Express* vol. 24, pp. 8967-8977, 2016.

[18] F. Poli, A. Cucinotta, and S. Selleri, *Photonic Crystal Fibers*, vol. 102. Dordrecht: Springer Netherlands, 2007.

[19] P. S. J. Russell, "Photonic-Crystal Fibers," *J. Light. Technol.*, vol. 24, no. 12, pp. 4729–4749, Dec. 2006.

[20] M. Sozzi, A. Cucinotta, R. Corradini, R. Marchelli, M. Konstantaki, S. Pissadakis, and S. Selleri, "Modification of a long period grating-based fiber optic for DNA biosensing," in *SPIE BiOS*, 2011, p. 78940J.

[21] A. Candiani, M. Sozzi, A. Cucinotta, S. Selleri, R. Veneziano, R. Corradini, R. Marchelli, P. Childs, and S. Pissadakis, "Optical Fiber Ring Cavity Sensor for Label-Free DNA Detection," *IEEE J. Sel. Top. Quantum Electron.*, vol. 18, no. 3, pp. 1176–1183, 2012.

[22] R. Corradini and S. Selleri, "Photonic Crystal Fibers for Physical, Chemical and Biological Sensing," in *Photonic Bandgap Structures Novel Technological Platforms for Physical, Chemical and Biological Sensing*, BENTHAM SCIENCE PUBLISHERS, 2012, pp. 216–231.

[23] M. Skorobogatiy, "Microstructured and photonic bandgap fibers for applications in the resonant bio- and chemical sensors," *J. Sensors*, vol. 2009, p. 524237, 2009.

[24] P. Yeh, A. Yariv, and E. Marom, "Theory of Bragg fiber," *J. Opt. Soc. Am.*, vol. 68, no. 9, pp. 1196–1201, Sep. 1978.

[25] J. B. Jensen, G. Emiliyanov, O. Bang, P. E. Hoiby, L. H. Pedersen, T. P. Hansen, K. Nielsen, and A. Bjarklev, "Microstructured Polymer Optical Fiber Biosensors for Detection of DNA and Antibodies," in *Optical Fiber Sensors*, 2006, p. ThA2.

[26] M. S. A. Gandhi, S. Sivabalan, P. R. Babu, and K. Senthilnathan, "Designing a Biosensor Using a Photonic Quasi-Crystal Fiber," vol. 16, no. 8, pp. 2425–2430, 2016.

[27] D. Yang, S. Kita, F. Liang, C. Wang, H. Tian, Y. Ji, M. Lončar, and Q. Quan, "High sensitivity and high Q-factor nanoslotted parallel quadrabeam photonic crystal cavity for real-time and label-free sensing," *Appl. Phys. Lett.*, vol. 105, no. 6, p. 063118, Aug. 2014.

[28] A. Kulkarni, J.-H. Lee, J.-D. Nam, and T. Kim, "Thin film-coated plastic optical fiber probe for aerosol chemical sensing applications," *Sensors Actuators B Chem.*, vol. 150, no. 1, pp. 154–159, Sep. 2010.

[29] Z. He, F. Tian, Y. Zhu, N. Lavlinskaia, and H. Du, "Long-period gratings in photonic crystal fiber as an optofluidic label-free biosensor.," *Biosens. Bioelectron.*, vol. 26, no. 12, pp. 4774–4778, Aug. 2011.

[30] L. Rindorf, P. E. Høiby, J. B. Jensen, L. H. Pedersen, O. Bang, and O. Geschke, "Towards biochips using microstructured optical fiber sensors.," *Anal. Bioanal. Chem.*, vol. 385, no. 8, pp. 1370–1375, Aug. 2006.

[31] J. R. Ott, M. Heuck, C. Agger, P. D. Rasmussen, and O. Bang, "Label-free and selective nonlinear fiber-optical biosensing," *Opt. Express*, vol. 16, no. 25, p. 20834, Dec. 2008.

[32] C. Shi, C. Lu, C. Gu, L. Tian, R. Newhouse, S. Chen, and J. Z. Zhang, "Inner wall coated hollow core waveguide sensor based on double substrate surface enhanced Raman scattering," *Appl. Phys. Lett.*, vol. 93, no. 15, p. 153101, Oct. 2008.

[33] E. Coscelli, M. Sozzi, F. Poli, D. Passaro, a. Cucinotta, S. Selleri, R. Corradini, and R. Marchelli, "Toward A Highly Specific DNA Biosensor: PNA-Modified Suspended-Core Photonic Crystal Fibers," *IEEE J. Sel. Top. Quantum Electron.*, vol. 16, no. 4, pp. 1–6, Jul. 2010.

[34] D. Kim and A. E. Herr, "Protein immobilization techniques for microfluidic assays.," *Biomicrofluidics*, vol. 7, no. 4, p. 041501, Jan. 2013.

[35] R. Narayanaswamy and O. S. Wolfbeis, *Optical Sensors*. Berlin, Heidelberg: Springer Berlin Heidelberg, 2004.

[36] S. Afshar V., S. C. Warren-Smith, and T. M. Monro, "Enhancement of fluorescence-based sensing using microstructured optical fibres," *Opt. Express*, vol. 15, no. 26, pp. 17891–17901, Dec. 2007.

[37] A. S. Webb, "Suspended-core holey fiber for evanescent-field sensing," *Opt. Eng.*, vol. 46, no. 1, p. 010503, Jan. 2007.

[38] M. Purdey, J. Thompson, T. Monro, A. Abell, and E. Schartner, "A Dual Sensor for pH and Hydrogen Peroxide Using Polymer-Coated Optical Fibre Tips," *Sensors*, vol. 15, pp. 31904–31913, Dec. 2015.

[39] X. Fan, I. M. White, S. I. Shopova, H. Zhu, J. D. Suter, and Y. Sun, "Sensitive optical biosensors for unlabeled targets: a review.," *Anal. Chim. Acta*, vol. 620, no. 1–2, pp. 8–26, Jul. 2008.

[40] A. Simons, B. Sikkema-Raddatz, N. de Leeuw, N. C. Konrad, R. J. Hastings, and J. Schoumans, "Genome-wide arrays in routine diagnostics of hematological malignancies.," *Hum. Mutat.*, vol. 33, no. 6, pp. 941–8, Jun. 2012.

[41] R. Jaksik, M. Iwanaszko, J. Rzeszowska-Wolny, and M. Kimmel, "Microarray experiments and factors which affect their reliability.," *Biol. Direct*, vol. 10, no. 1, p. 46, Jan. 2015.

[42] R. T. Ranasinghe and T. Brown, "Ultrasensitive fluorescence-based methods for nucleic acid detection: towards amplification-free genetic analysis.," *Chem. Commun. (Camb).*, vol. 47, no. 13, pp. 3717–3735, 2011.

[43] R. T. Ranasinghe and T. Brown, "Fluorescence based strategies for genetic analysis.," *Chem. Commun. (Camb).*, no. 44, pp. 5487–5502, Nov. 2005.

[44] I. P.-J. Lai, S. G. Harroun, S.-Y. Chen, B. Unnikrishnan, Y.-J. Li, and C.-C. Huang, "Solid-state synthesis of self-functional carbon quantum dots for detection of bacteria and tumor cells," *Sensors Actuators B Chem.*, vol. 228, pp. 465–470, Jun. 2016.

[45] B. Priem, C. Tian, J. Tang, Y. Zhao, and W. J. M. Mulder, "Fluorescent nanoparticles for the accurate detection of drug delivery," *Expert Opin. Drug Deliv.*, no. ahead-of-print, pp. 1–14, 2015.

[46] E. Rampazzo, L. Prodi, L. Petrizza, and N. Zaccheroni, "Luminescent Silica Nanoparticles Featuring Collective Processes for Optical Imaging.," *Top. Curr. Chem.*, vol. 370, pp. 1–28, Jan. 2016.

[47] J.-Y. Zhao, G. Chen, Y.-P. Gu, R. Cui, Z.-L. Zhang, Z.-L. Yu, B. Tang, Y.-F. Zhao, and D.-W. Pang, "Ultrasmall Magnetically Engineered Ag2Se Quantum Dots for Instant Efficient Labeling and Whole-Body High-Resolution Multimodal Real-Time Tracking of Cell-Derived Microvesicles.," *J. Am. Chem. Soc.*, vol. 138, no. 6, pp. 1893–903, Feb. 2016.

[48] J. Y. Lee, J. S. Kim, J. C. Park, and Y. S. Nam, "Protein-quantum dot nanohybrids for bioanalytical applications.," *Wiley Interdiscip. Rev. Nanomed. Nanobiotechnol.*, vol. 8, no. 2, pp. 178–90, Mar. 2016.

[49] X. Chen, L. Zhang, K. Zhou, E. Davies, K. Sugden, I. Bennion, M. Hughes, and A. Hine, "Real-time detection of DNA interactions with long-period fiber-grating-based biosensor," *Opt. Lett.*, vol. 32, no. 17, pp. 2541–2543, Aug. 2007.

[50] S. Maguis, G. Laffont, P. Ferdinand, B. Carbonnier, K. Kham, T. Mekhalif, and M.-C. Millot, "Biofunctionalized tilted Fiber Bragg Gratings for label-free immunosensing," *Opt. Express*, vol. 16, no. 23, pp. 19049–19062, Nov. 2008.

[51] S. Arnold, M. Khoshsima, I. Teraoka, S. Holler, and F. Vollmer, "Shift of whispering-gallery modes in microspheres by protein adsorption," *Opt. Lett.*, vol. 28, no. 4, pp. 272–274, Feb. 2003.

[52] S. W. James and R. P. Tatam, "Optical fibre long-period grating sensors: characteristics and application," *Meas. Sci. Technol.*, vol. 14, no. 5, pp. R49–R61, May 2003.

[53] S. Pissadakis, M. Livitziis, and G. D. Tsibidis, "Investigations on the Bragg grating recording in all-silica, standard and microstructured optical fibers using 248~nm, 5~ps laser radiation," *J. Eur. Opt. Soc. Rapid Publ.*, vol. 4, p. 09049, Dec. 2009.

[54] R. D'Agata, R. Corradini, C. Ferretti, L. Zanoli, M. Gatti, R. Marchelli, and G. Spoto, "Ultrasensitive detection of non-amplified genomic DNA by nanoparticle-enhanced surface plasmon resonance imaging," *Biosens. Bioelectron.*, vol. 25, no. 9, pp. 2095–2100, 2010.

[55] R. D'Agata, R. Corradini, G. Grasso, R. Marchelli, and G. Spoto, "Ultrasensitive detection of DNA by PNA and nanoparticle-enhanced surface plasmon resonance imaging.," *Chembiochem*, vol. 9, no. 13, pp. 2067–2070, Sep. 2008.

[56] N. L. Rosi and C. A. Mirkin, "Nanostructures in biodiagnostics.," *Chem. Rev.*, vol. 105, no. 4, pp. 1547–1562, Apr. 2005.

[57] W. R. Algar, D. E. Prasuhn, M. H. Stewart, T. L. Jennings, J. B. Blanco-Canosa, P. E. Dawson, and I. L. Medintz, "The controlled display of biomolecules on nanoparticles: A challenge suited to bioorthogonal chemistry," *Bioconjug. Chem.*, vol. 22, no. 5, pp. 825–858, 2011.

[58] P. E. Nielsen, "PNA Technology.," *Mol. Biotechnol.*, vol. 26, no. 3, pp. 233–248, 2004.

[59] G. He, S. Rapireddy, R. Bahal, B. Sahu, and D. H. Ly, "Strand invasion of extended, mixed-sequence B-DNA by γPNAs," *J. Am. Chem. Soc.*, vol. 131, no. 34, pp. 12088–12090, 2009.

[60] A. Candiani, A. Bertucci, S. Giannetti, M. Konstantaki, A. Manicardi, S. Pissadakis, A. Cucinotta, R. Corradini, and S. Selleri, "Label-free DNA biosensor based on a peptide nucleic acid-functionalized microstructured optical fiber-Bragg grating.," *J. Biomed. Opt.*, vol. 18, no. 5, p. 57004, 2013.

[61] A. Bertucci, A. Manicardi, A. Candiani, S. Giannetti, A. Cucinotta, G. Spoto, M. Konstantaki, S. Pissadakis, S. Selleri, and R. Corradini, "Detection of unamplified genomic DNA by a PNA-based microstructured optical fiber (MOF) Bragg-grating optofluidic system," *Biosens. Bioelectron.*, vol. 63, pp. 248–254, 2015.

[62] S. C. Warren-Smith, R. M. André, C. Perrella, J. Dellith, M. Rothhardt, and H. Bartelt, "Direct core structuring of microstructured optical fibers using focused ion beam milling," *Opt. Express*, vol. 24, no. 1, pp. 378–387, 2016.