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Review

Fluorescence based fiber optic and planar waveguide biosensors. A review

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HIGHLIGHTS

- Principles, configurations and fluorescence techniques using fiber optic and planar waveguide biosensors are discussed.
- The biorecognition elements and sensing schemes used in fiber optic and planar waveguide platforms are reviewed.
- Some major and recent applications of fiber optic and planar waveguide biosensors are introduced.

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ABSTRACT

The application of optical biosensors, specifically those that use optical fibers and planar waveguides, has escalated throughout the years in many fields, including environmental analysis, food safety and clinical diagnosis. Fluorescence is, without doubt, the most popular transducer signal used in these devices because of its higher selectivity and sensitivity, but most of all due to its wide versatility. This paper focuses on the working principles and configurations of fluorescence-based fiber optic and planar waveguide biosensors and will review biological recognition elements, sensing schemes, as well as some major and recent applications, published in the last ten years. The main goal is to provide the reader a general overview of a field that requires the joint collaboration of researchers of many different areas, including chemistry, physics, biology, engineering, and material science.

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1. Introduction

Biosensors have been defined [1–3] as self-integrated devices capable of providing specific quantitative or semiquantitative analytical information on the species of interest using a biological recognition element (biochemical receptor), which is in direct spatial contact with the transducer element. Those biosensors based on the measurement of photons are classified as optical. This review is focused on optical fiber and planar waveguide fluorescence based biosensors, a type of device in which a waveguide (an optical transmitter) is used as a platform for the biochemical receptor as well as to transmit the excitation light and/or the resultant signal to a photodetector that converts the light into an electrical signal.

At present, optical biosensors are not competitive with bulky laboratory instrumentation, such as microplate array systems, for applications where a large number of samples need to be analysed simultaneously. However, they present some desirable features such as potential low cost, small size and ease of use and are well suited for some applications such as on-line monitoring or for the analysis of complex samples, as well as for the measurement of binding events in real time [4]. As a result, this is an active research area and a number of optical biosensing platforms are already in the market for application in specific areas including, environmental analysis, food safety or clinical diagnosis [5,6a,b].

Fluorescence is, without doubt, the most commonly used transducer signal in biosensors [7,8]. Several parameters can be recorded and applied for sensing including, fluorescence intensity, that can be measured at the given wavelengths of excitation and emission; decay time or emission anisotropy, which is a function of the fluorescence intensities obtained at two different polarizations, vertical and horizontal [8,9]. Therefore, a variety of possibilities exists to improve biosensor performance. For example, the excitation or the emission wavelengths of the lumiphore can be adequately tuned to improve method selectivity and, in addition, the emission kinetics as well as the anisotropy properties of the luminescent compound may add specificity to the measurement in comparison to other optical methods [10]. Recent biosensor reviews [11–15] confirm that fluorescence-based transduction, in whichever of its possibilities, is one of the most popular optical detection methods used in biosensing.

This paper focuses on the working principles of fluorescence-based fiber optic and planar waveguides biosensors and will review biological recognition elements, assay formats, as well as selected applications in different areas, published in the last ten years. The main goal is to provide the reader a general overview of this exciting field which requires the joint effort of researchers of many different areas, including optics, biochemistry, electronics and fluidics.

2. Light guiding in optical waveguides and biosensor configurations

Optical waveguides are dielectric structures that transport energy between its two extremes at wavelengths in the UV–Vis and IR region of the electromagnetic spectrum. Depending on their geometry they can be classified in two main groups: cylindrical and planar. Optical fibers are included in the first group and consist of a cylindrical central dielectric core clad by a material of slightly lower refractive index (by ≈ 1%). A planar waveguide is formed from a dielectric slab core sandwiched between two cladding layers with lower refractive indexes [16]. In both, light propagation and guiding along the core is based on the well-known optical phenomenon of total internal reflection (TIR) [5,16].

When light propagates along an optical waveguide (Fig. 1), such as an optical fiber or a planar waveguide, light is totally reflected at the interface between the optically more dense medium and the optically rare medium if the angle of refraction is larger than a critical angle (θc). At each point of reflection there is a finite decaying electric field across the interface that penetrates some distance into the lower refractive index medium. This field is referred to as the evanescent wave [17].

In emission spectroscopy, the evanescent wave can be used to generate luminescence or Raman scatter. The wavelength range extends from the UV to the far infrared depending on the quality of the waveguide. Higher order modes, i.e. those that propagate at angles close to the critical angle, contribute in a major extent to the power of the evanescent wave [5]. Therefore, the geometry of the sensing region must be properly designed in fluorescent biosensors based on evanescent field excitation (EFE) so as to increase the excitation power along the probe length, as well as to avoid the coupling of the emitted fluorescence into guided modes that fail to propagate in the clad fiber, what is known as V-number mismatch [5,18–20]. Tapering of the sensing region has been proven to be a
very effective way to minimize V-number mismatch and increase biosensor sensitivity when using partially clad fibers [5].

Several authors have studied different ways of increasing the performance of evanescent wave fiber optic biosensors. Design characteristics, taper parameters, as well as intrinsic fiber characteristics have been explored in order to increase depth and magnitude of the evanescent field [21,22]. For optical fibers to be used in evanescent field sensing, they must first be de-clad, at least partially, and most times tapered (either the core only, after de-cladding, or the complete fiber maintaining the cladding), so that they can be coated with the sensing element forming a uniform layer and thereby permitting that the evanescent field can interact with the fiber’s surroundings [23–25]. All three processes (etching, tapering and coating) are not simple and require of several physical and chemical operations to be optimized [18,23–26]. One of the main advantages of EFE biosensors is the selective excitation of fluorophores attached either to the waveguide surface or within the penetration depth of the evanescent field [82]. The excitation of unbound interfering species present in bulk solution is avoided since the de is typically in the order of 100 and 200 nm, for visible light, which allows their operation in absorbing, particulate or turbid media, as is the case in many biological samples [27]. This approach works extremely well for the detection of relatively small micron-size objects, as cells or bacteria, require the use of sensing probes with increased penetration depth such as reverse waveguides, based on low refractive index substrates (below 1.33), or metal-clad waveguides that employ a thin metal layer between the substrate and the thicker waveguide layer to increase the probing depth [28].

Fiber optic (FO) biosensors can be based on two different configurations that rely on TIR for light propagation and guiding [17]: a) the opt(ri)de and, b) the evanescent wave configurations. In the former one, optical fibers are used to guide the excitation light from the light source to the distal end of the fiber where the recognition element is immobilized. In fluorescence based biosensors, the emitted light is guided back through the same fiber, or a different one, to the detector for signal generation. In the second configuration, the biorecognition layer is immobilized onto the longitudinal surface of the optical fiber core and is excited with the evanescent wave. In the latter approach, the penetration depth of the light is much lower, but the interrogated area is larger, than for optodes of the same diameter.

Planar waveguide (PWG) fluorescence biosensors are based on the localized excitation of the fluorophores at the surface of the waveguide upon evanescent field excitation. The emission phenomenon is known in this case as Total Internal Reflection Fluorescence (TIRF), but some papers based on EFE waveguides also call the emission simply evanescent wave fluorescence [29]. PWGs are not usually applied for guiding back the emitted light to the detector but instead, as an advantage over optical fiber devices, they are used in combination with imaging systems (charge-coupled devices (CCDs) or CMOS camera) for multianalyte detection [30].

Planar slab waveguides can be classified in two main groups, bulk waveguides and integrated optical waveguides (IOWs) [29]. The diameter of bulk waveguides, also known as “internal reflection elements” (IREs), is higher than the wavelength of the reflected light and sensing hot spots can be clearly distinguished, at the points of reflection, on the waveguide surface. Integrated optical waveguides (IOWs) are prepared by depositing a very thin layer, usually in the order of the light wavelength or less, of a high refractive index material (usually metal oxides such as SiO₂, TiO₂, Ta₂O₅, or Nb₂O₅) on a glass substrate [29–31]. The sensitivity of IOWs has shown to be much higher in fluorescence based studies than for IRE-based waveguides, however they are more difficult to prepare and light coupling into the optical waveguide is more difficult requiring the assistance of prism or grating arrangements [29,32,33].

Planar waveguides have gained ground on optical fibers, especially when using EFE, for the development of fluorescence-based biosensing platforms in applications where the flexibility and remote capability of optical fibers are not essential. Other advantages of planar waveguides include their mechanical robustness and ease of fabrication, the potential for integration with optoelectronic components for compactness, the relatively easy patterning of the biological reagents needed for the assay, or the possibility to control the penetration depth of the evanescent field by controlling the incident reflection angle [34,35].

The development of sensing microarrays, which enable multiple and simultaneous analyte detection, using both coherent fiber optic bundles, also referred to as optical imaging fibers [36–38], as well as integrated optical planar waveguides, hold great potential for fluorescence techniques in biosensing with FO and PWG. Imaging fibers are high density coherent optical arrays, formed by thousands of micron-sized optical fibers, bundled in a coherent way, so that the position of each fiber in one end is identical to the other end. Thus, the light is guided through each individual fiber from one end of the bundle to the other and the fluorescence signal from each region can be spatially resolved, depending on the distance between two adjacent fibers, and detected with a CCD camera. Fiber bundles have been employed for imaging purposes or, for biosensor arrays using encoded bead libraries and a variety of biorecognition elements [39,40].

3. Fluorescence techniques in biosensing with fiber optics and planar waveguides

The fluorescence phenomenon is multifaceted and has several features that can be the basis of a biosensing design. Its versatility includes intensity-, kinetic-, wavelength- and anisotropy-based measurements [10].

3.1. Fluorescence intensity

After molecular excitation with appropriate light wavelength, spontaneous emission (fluorescence) of excess energy occurs at longer wavelengths, due to an electronic transition between the lowest excited singlet and the ground state. Fluorescence occurs if
this deactivation process is favoured by the molecular structure of the compound and by the molecular environment (temperature, solvent characteristics, pH, etc.). Only if these factors make this relaxation mechanism the fastest and most efficient return to the ground state is fluorescence observed. The intensity of the emitted light \( (I_f) \) at the analytical wavelength is directly related to the concentration of the fluorophore, according to the following equation (Eq. (1)), valid only for diluted solutions where \( ebc \leq 0.02 \) [41,42],

\[
I_f = \kappa \Phi H \lambda \epsilon bc
\]  

where \( \kappa \) is a constant related to the efficiency with which the instrument collects and detects the emitted light, \( \Phi \) is the fluorescence quantum yield (ratio of fluorescing to excited molecules; it can also be described in terms of light quanta or rate constants), \( H \) is the intensity of the incident light and \( ebc \) are the parameters involved in the Lambert-Beer molecular absorption law.

In the case of fluorescence from an (optically) thick polymer film doped with a fluorescent dye, scattering from the solid material has to be also taken into account. Under this situation, very common in fluorescent sensors where the recognition and transducing layers are placed at the distal end of an optical fiber, only the reflectance mode can be used for fluorescence analysis so that the fluorescence intensity as a function of the immobilized fluorophore concentration (\( c \)) for an “infinite” layer thickness is given by Eq. (2) [43],

\[
I_f = \frac{a}{a_b} \Phi \lambda I_0 (1 - R_{\infty \lambda}) = c
\]

where \( a \) and \( a_b \) are the absorption coefficients of the fluorophore and the “background” material of the film, respectively, at the analytical wavelength; \( R_{\infty \lambda} \) is the diffusely reflected part of the incident radiation by the background, and the other symbols have the same meaning than those in Eq. (1).

For a given fluorophore sensitivity, as well as selectivity, is due mostly to instrumental design. First of all, the detector only views light when the dye in the transducing layer emits; therefore, fluorescence “turn-on” sensors are preferred as long as sufficient discrimination of the emitted and the excitation light is provided (usually with interference filters as monochromators are only used for benchtop spectrofluorometers). Detection limits are in the range of \( 10^{-9} \) and \( 10^{-10} \) \( M \) or in the pP levels [41]. In fact, under very strict conditions, the detection of a single molecule may be attained [44,45]. On the other hand, the use of the two wavelengths involved in the process (excitation and emission) provides extra selectivity since either of them or both can be controlled [8,10].

Although not all compounds have intense native fluorescence, this disadvantage can be reverted, sometimes simply by their derivatization into a fluorescent compound, by making them part of a chemical reaction where a fluorescent compound is ultimately produced [42] or by attaching them with a fluorescent label that indicates their presence [46]. Fluorescent dyes, quantum dots (QDs) [47] and green fluorescent protein (GFP) have been used as fluorescent tags that have enabled researchers to study molecular events and the nature and accessibility of binding sites in biological macromolecules [48–50]. In the field of biosensing, again fluorescence is ideal since there are many naturally fluorescent biochemical substances or they can be easily tagged [51].

3.2. Luminescence lifetime

The luminescence lifetime (\( \tau \)) represents the reciprocal of the rate constant of the (first order) emission decay that follows an “instantaneous” excitation of the fluorophore by a flash of light [10]. Lifetime measurements can be carried out using a pulse light of radiation with a width typically shorter than the decay time of the luminophore. Upon excitation, the digitized emission decay profile is fitted to Eq. (3):

\[
I_t = I_0 e^{-t/\tau}
\]

where \( I_0 \) corresponds to the amplitude, i.e. the fluorescence intensity at the end of the “instantaneous” excitation flash [52].

Alternatively, the luminescence lifetime can be evaluated by phase fluorometry (or phosphorimetry) measuring the phase shift between the sinusoidally modulated exciting light and the emitted light [53] (Eq. (4)):

\[
\tan \phi = 2 \pi f \tau
\]

where \( \phi \) is the measured shift between the excitation and the emission sinusoidal signals and \( f \) is the excitation modulation frequency (Hz) [10].

Luminescence lifetime measurements have been extensively applied to the development of gas sensors, especially for oxygen measurements, and also in the development of enzymatic biosensors based on oxygen transduction [8] or in whole cell biosensors for biological oxygen demand (BOD) monitoring [54]. Its application to affinity sensors has been limited by the availability of probes whose decay time changes upon biomolecular interactions.

3.3. Fluorescence quenching

Quenching, in a very simplistic manner, can be described as any bi-molecular interaction by which the fluorescence intensity of the fluorophore molecule is diminished. One of the greatest difficulties of fluorometry is that it suffers from great environment-dependent quenching. Yet, what was originally considered a nuisance, if the fluorophore is the analyte, has been converted into a major application of fluorescence-based biosensors. Many are designed on the basis of the variation the quencher (in this case, the analyte or a third party related to it) produces on the fluorescence of an indicator dye. Although the many mechanisms of fluorescence quenching are beyond the scope of this paper, the process generally follows the Stern-Volmer equation [Eq. (5)] regardless of the quenching mechanism [9,41]:

\[
\frac{r_P}{\tau_F} = \frac{r_P}{\tau_f} = 1 + K_{SV} C_Q
\]

where \( r_P \) and \( h_k \) and \( r_P \) and \( \tau_f \) are, respectively, the fluorescence intensities and the fluorescence lifetimes of the indicator dye in the absence and presence of the quencher (Q) at concentration \( C_Q \); \( K_{SV} \) is the so-called Stern-Volmer quenching constant which is related to the efficiency with which Q quenches the indicator fluorescence. Eq. (5) is only valid in the case of “dynamic” quenching, i.e. when the fluorophore and the quenching molecules are not pre-associated before the former absorbs a photon and Fick’s diffusion laws apply. If quencher and dye are pre-associated to a certain extent, instantaneous “static” quenching occurs within the fluorophore-quencher complex, and Eq. (5) is only valid for the \( r_P/\tau_F \) ratio but not for the \( r_P/r_f \) ratio because only the free quencher follows the dynamic quenching rules. If there is practically no free quencher (i.e. the association constant is high enough), the emission lifetime ratio will not change with the quencher concentration, but the intensity ratio will obey Eq. (5) with \( K_{SV} \) being now the mass concentration \( K_{SV} \) [10]. Because the fluorophore’s signal depends on the quencher concentration, Q (or the third party) can be determined by its quenching action, in a sample that contains, or to which is added, a fluorescent compound.
Numerous procedures based on the quenching process have been devised for biosensing and, in this case, the biorecognition event causes a decrease in fluorescence. In some cases molecular recognition leads to fluorescence quenching of QDs used as markers [55] or of fluorescent dyes, such as certain chelates, which are of common use in this field [56,57].

Quenching of fluorescent dyes and QD by metallic nanoparticles (NP) can occur at larger distances than those related with molecular quenchers [58,59], and although NP have increased their application in quenching-based biosensing, there are examples of PO sensors based on the fluorescence enhancement of low-emission-quantum-yield dyes near certain metal surfaces or NPs (“metal-enhanced fluorescence” or MEF) [60]. Several authors have studied, explained and applied to biosensing this phenomenon, which is due to the formation of a mirror dipole on the metallic NP surface plasma that itself radiates with high efficiency, resulting in enhanced radiative deactivation of the fluorophore excited state [61–65]. Contribution from the enhanced absorption of light by the fluorophore due to increased electric field between and around the metal NPs has to be taken into account as well. MEF has been shown to be more pronounced for larger particles, more efficient with metals possessing high free electron densities (Ag, Au) and at distances between 2 and 100 nm from the metal surface [60]. At shorter distances, fluorescence quenching due to the competing non-radiative energy or electron transfer to the metal NP surface prevails over the MEF effect [66].

3.4. Förster Resonance Energy Transfer (FRET)

Förster Resonance Energy Transfer, commonly (but incorrectly) called “Fluorescence” Resonance Energy Transfer, is a particular manifestation of fluorescence quenching that occurs when two different species, one (a donor) with a fluorescence spectrum that overlaps the excitation spectrum of the other (the acceptor), are close enough, usually less than 80 nm [67]. Thereby, the radiative-excited donor can transfer energy non-radiatively to the acceptor; so that the fluorescence intensity of the former is partially quenched, no matter if the acceptor is fluorescent or not. The electronic energy exchange is due to the dipole-dipole resonance interaction between the two molecules, not requiring a collision. Nevertheless, the FRET rate constant ($k_F$) not only depends strongly on the donor-acceptor distance and on the extent of the spectral overlap between their respective spectra, but also on the relative orientation of the donor and the acceptor species [Eq. (6)] [68].

\[
k_F = \frac{9(\ln 10) k^2 \Phi_D}{128 \pi^3 n^4 N_A \tau_0 R_0^6} \int_0^\infty F_D(\nu) e_A(\nu) d\nu
\]

where $k^2$ is the orientation factor, $\Phi_D$ is the donor fluorescence quantum yield, $F_D(\nu)$ is the spectral distribution of the donor fluorescence (normalized to unity in the wavenumber scale), $e_A(\nu)$ is the molar absorption coefficient (spectral distribution) of the acceptor, $N_A$ is the Avogadro’s number, $n$ is the solvent refractive index, $\tau_0$ is the excited state lifetime of the donor in the absence of any quencher (including the acceptor), and $R$ is the distance between the donor and the acceptor (in cm). Eq. (6) if often displayed as Eq. (7) after defining $R_0$ as the so-called “Förster distance”, i.e., the donor-acceptor distance at which $k_F = 1/\tau_0$, so that at that particular distance, the probability of emission of the excited donor is equal to the probability of energy transfer to the acceptor.

\[
k_{\text{tr}} = \frac{1}{\tau_0} \left( \frac{R_0^6}{R^6} \right)
\]

Evidently, the molecular recognition of the analyte must alter in some way the FRET process, thus producing a change that signals its presence. The most straightforward sensing application is obtained when the distance or orientation between the donor and acceptor changes in the presence of the target.

Several strategies have been used in FRET biosensing; the simplest, when the target itself is the acceptor-quenching moiety. Another one is that this second species also be fluorescent and thereby uses the transferred energy to emit its own fluorescence at a much longer wavelength, thereby dramatically increasing the emission Stokes shift. In this way, the primary excitation of the donor, whose fluorescence is quenched, will give rise to the fluorescence of the acceptor. When this is the case, it is possible to advantageously use a two-wavelength ratiometric method to sense the binding event [69–72].

According to some authors [9], only in rare, lucky cases is one of the host/guest partners fluorescent. Therefore, labeling them with one or two organic dyes (or other type of label) is usually necessary. Also, FRET mechanism is not limited to the use of fluorescent organic dyes, QD and noble metal NP are now being used as either donors or acceptors, respectively [73–76].

Intramolecular FRET in single stranded nucleic acids that form a stem-and-loop structure is the basis of molecular beacons [77], also known as molecular hairpins (see section 4.3). Very recently, FRET between luminescently-labelled (donor) NPs and a fluorescently-tagged (acceptor) analyte has been successfully used in a competitive assay format based on molecularly-imprinted polymer nanoshell coatings on the NPs [78].

4. Applications of fluorescence based fiber optic and planar waveguide biosensors

Recognition elements have become an extraordinary tool in recent years playing a successful role in the implementation of optical biosensors. Typical recognition elements are macromolecules or molecular assemblies that provide specific analytical information upon the recognition of the target molecule (ligand or analyte) in the course of a reaction.

The chemical information derived from the driven interactions is optically trapped and transformed into an electrical signal for data acquisition and interpretation. The recognition receptors feature inherent binding capabilities that determine the specificity and sensitivity of any proposed biosensor. In general, based on the nature of this binding event, recognition elements can be classified in [79–81]:

(A) Catalytic Recognition Elements: The metabolic properties of these molecules, with natural or artificial origin, rely on catalyzed reactions whose progress is related to the concentration of the analyte, which can be measured by monitoring the changes in the product rate formation, reactant rate consumption or the inhibition of the reaction. The first biosensors described in literature were based on these recognition elements. Examples of catalytic recognition elements are: isolated enzymes, subcellular organelles, whole cells, tissues and/or organisms, etc. Moreover, engineered DNAzymes and catalytic antibodies (Abzymes) should also be included.

(B) Affinity Recognition Elements: The recognition is typically driven by many weak interactions between the analyte and the receptor in a non-catalytically controlled process. It covers natural recognition elements such as antibodies, receptors, nucleic acids, bacteriophages, whole cells, etc. and genetically engineered or artificial recognition elements, such as recombinant antibodies, aptamers, engineered...
whole cells and/or organisms, as well as molecularly imprinted polymers (MIPs). Although most devices based on affinity interactions cannot work in a fully reversible way, they have been termed as biosensors for decades by the scientific community [51].

Biological, engineered and biomimetic recognition elements, employed for fiber optic and planar waveguide (bio)sensor development, have been described in detail and classified accordingly [8,82,83]. We will highlight herein those supported by a remarkable number of publications during the last years. Besides complementing the description of the (bio)recognition element, sensing schemes typically applied in optical fiber and planar waveguide based (bio)sensors will be reviewed.

4.1. Enzymes in biosensing with FO and PWG

Enzymes are the gold standard of the catalytic recognition elements used in biosensing development. Enzymes were the first receptors applied in biosensors [84] and they are still the basis of many devices in combination with different types of transducers (optical, electrochemical, etc). Enzyme-based biosensors proceed with extraordinary catalytic efficiency at small concentration levels. Interestingly, although major efforts to develop non-enzymatic sensors have been made, still there is a significant number of optical enzyme-biosensors developed in recent years [51,85].

Three different sensing schemes are applied in enzyme-based FO and PWG biosensors, considering the following principles (Fig. 2) [8,86]: (i) the analyte is assessed directly by monitoring the fluorescence properties in the course of the enzymatic reaction; (ii) the concentration of the target analyte is indirectly related to the signal generated by a fluorescence indicator (optochemical transducer) which responds to the changes in the biocatalytic process and (iii) the enzyme itself reports the presence of the analyte by variations in its luminescence properties.

Based on the first sensing scheme, Orellana et al. [87] have developed a fiber optic dosimeter for the analysis of carbamate pesticides. The authors have synthesized a red emitting Ru(II)polypyridyl complex containing a 4-acetoxyphenantroline ligand whose luminescence is strongly decreased upon hydrolysis by the enzyme acetylcholinesterase (ACHE). The pesticide inhibits the enzymatic activity, and the luminescence intensity or the lifetime of the indicator dye can be used to monitor its concentration. The reduced form of nicotinamide adenine dinucleotide (NADH), a coenzyme involved in many oxidation or hydrogen transfer reactions, can be monitored via its emission at $\lambda_{\text{max}}$ 450 nm. For example, Kudo et al. [88,89] have developed a fiber optic set-up for formaldehyde sensing (bio-sniffer) via the increase in the fluorescence of NADH using either formaldehyde dehydrogenase (FALDH) or the system aldehyde dehydrogenase (ALDH)/formate dehydrogenase (FDH) immobilized in a polytetrafluoroethylene (PTFE) membrane coated with a phospholipid polymer at the tip of an optical fiber. The reported biosensor enables real-time formaldehyde monitoring with a limit of detection of only 0.75 μg L$^{-1}$. As mentioned above, the second sensing scheme, mostly used for these enzymatic biosensors, is based on the indirect detection of the target analyte by monitoring the signal generated by a fluorescent indicator (optochemical transducer). In most cases, the concentration of the target compound is related to the oxygen consumption, pH change, hydrogen peroxide or ammonia production in the course of a biocatalytic reaction that is measured with the corresponding optochemical transducer near the immobilized enzyme. Recent advances in this field include the development of miniaturized devices that are applied to in vivo analysis. For example, an optical fiber microsensor, using fluorescence lifetime measurements, has been applied to the continuous measurement of glucose in subcutaneous tissues at concentrations up to 20 mM [90]. The hybrid sensor system consists of two oxygen optodes, one of them containing immobilized GOx to monitor glucose, and the second compensating for oxygen fluctuations in the intercellular fluid as well as for slight temperature changes. In a different approach, Pospíšilová et al. [91] have developed a fiber optic biosensor with incorporated magnetic microparticles for the determination of biogenic amines. The device consisted of a lens coated with a UV-cured hybrid polymer that incorporates magnetic microparticles whose cores contain an optical oxygen indicator, Ruthenium tris-(bathophenanthroline) dichloride $[\text{Ru (BaPhen)}_3]$Cl$^2$+, 2Cl$^-$, and their shells are modified with diamin oxide. The measurement is performed by obtaining the quenched fluorescence lifetime of the ruthenium complex. The proposed system features a limit of detection of 25 μmol L$^{-1}$ for putrescine and 30 μmol L$^{-1}$ for cadaverine.

Stein et al. [92] have proposed an in vivo biochemical monitoring platform using what they call “smart tattoo” sensors. The biosensors consist of fluorescent microspheres that can be implanted intradermal and interrogated noninvasively using a custom optical fiber. The sensor particles were prepared using Pt(II) octaethylporphine (PtOEP), as oxygen indicator, incorporated into hybrid mesoporous alginic-silicate microspheres of ca. 10 μm, followed by loading and subsequent covalent immobilization of glucose oxidase. The surface of the nanoparticles was assembled with multilayer nanofilms doped with rhodamine B to provide a reference signal. The particles showed a fast ($t_{1/2} = 84$ s) and reversible response to glucose levels in the range of 2 and 10 mg dL$^{-1}$, which is below the accepted clinical range of 40–350 mg dL$^{-1}$ required for in vivo monitoring. However, the adequate selection of the surface adsorbed nanofilm thickness,ionic strength of assembly conditions and capping layer allowed to tune the sensor response to cover the hypoglycemic levels (>120 mg dL$^{-1}$) [93].

Zheng et al. [94] have described an optical fiber based nanosensor for the analysis of extracellular lactate at single cell level. The nanobiensor that contains covalently immobilized lactate dehydrogenase at the fiber tip is parked on the plasma membrane of the cell and the evanescent field from the nanotip illuminates a spatially confined region. The enzyme catalyzes the conversion of lactate into pyruvate, and the fluorescence of the byproduct NADH is monitored as analytical signal. The device has been applied to the analysis of extracellular lactate temperature changes in single HeLa, MCF-7 and human fetal osteoblast cells, allowing to distinguish the high extracellular lactate levels of cancer cell lines as compared to the normal cell line. The same instrumental set up has also been

**Fig. 2.** Classification of enzymatic assays: (A) Analyte/product is assessed directly, (B) The analyte is assessed by the monitoring of an optochemical transducer, (C) the enzyme reports itself the presence of the analyte by variations in its luminescence.
applied to the detection of telomerase, a general cancer biomarker, at single cell level, but using selective antibodies as recognition elements [95]. Single cell analysis using fiber optics and different recognition elements has been recently reviewed by Vo Dinh and Zhang [96].

Environmental monitoring has also been a growing area for biosensor application in the last decade, as these devices allow a simple, rapid and reliable measurement of various contaminants. For example, the analysis of 1,2-dichloroethane (DCA), a widely used halogenated organic released into the environment in large quantities, has been accomplished using halokaline dehalogenase, DhlA, in whole cells of Xanthobacter autotrophicus GJ10 immobilized in calcium alginate on the tip of a fiber optic fluoresceinamide-based pH optode [97]. The biosensor had a response time of 8–10 min, mainly limited by DCA transport across the cell membrane, and provided a rapid, reagentless, continuous and simple measurement of DCA in water. In a further development [98] Rhodococcus sp GJ70 expressing DhlA were immobilized on the tip of the optical fiber, along with the fluoresceinamide dye, allowing the detection of 1,2-dibromoethane in the range of 1–10 μg L⁻¹.

Fluorescent nanoparticles, such as quantum dots (QDs), are being increasingly applied in combination with enzymes for biosensor development. QDs exhibit unique photophysical properties reflected by size-controlled fluorescence, high fluorescence quantum yields, stability against photobleaching and sensitivity to surface ligands. Therefore they offer an interesting alternative to common fluorophores for biosensor interrogation. In a recent example they have been applied to the development of microarray-based biosensors for the determination of phenol [99] fabricated by photopatterning of a solution containing PEG diacrylate (PEG-DA), tyrosinase, and CdSe/ZnS QDs. The entrapped enzyme catalyzes the oxidation of phenol to produce quinones, which subsequently quench the fluorescence of QDs within hydrogel microarray allowing 1 μM detection limits. Other applications of QDs are based on their photoluminescence quenching by H₂O₂ [100]. The production of this compound during the GOx biocatalysed reaction allowed glucose monitoring with a detection limit of 1.8 μM and the sensing platform has been adapted to 96-well plates for blood and urine multisample analysis.

Following the third sensing mechanism, Portaccio et al. [101] have characterized the intrinsic fluorescence of GOx immobilized in monolithic silica gel supports, in the UV and visible regions, using steady state and time resolved measurements, and they have further developed a fiber optic biosensor for glucose monitoring in the range from 0.2 to 10 mM. De Marcos et al. [102] have proposed a chemical modification of the enzymes using fluorescent labels, such as fluorescein derivatives, to increase the sensitivity and selectivity of direct enzyme based biosensors. GOx has been labelled with fluorescein-5(6)-carboxamidocaproic acid N-succinimidyl ester (GOx-FS) and entrapped in a polyacrylamide gel. The fluorescence intensity of GOx-FS is increased in the presence of glucose, with a linear response range between 300 and 2000 mg L⁻¹ that has been attributed to an inner filter effect [8]. New trends using this approach deal with the synthesis of more sensitive devices by application of molecular genetics techniques, although their application in combination with fiber optics or planar waveguides is still limited. For example, a fluorescent molecular biosensor for the analysis of methyl parathion (MP), an organophosphate pesticide, has been described based on the construction of the fusion protein between a green fluorescent protein mutant (E²GFP) and the enzyme methyl parathion hydrolase (MPH). In the presence of the pesticide, the H⁺ ions released during the enzymatic reaction decrease the fluorescence intensity of E²GFP. Biosensor sensitivity was greatly enhanced by the integration of the E²GFP-MPH fusion protein biosensor structure into protein nanowires through self-assembly of Sup35 protein of Saccharomyces cerevisiae. Concentrations as low as 0.26 ng mL⁻¹ MP, have been detected [103].

Enzyme immobilization on the transducer surface is of paramount importance for biosensor performance and several physical or chemical immobilization techniques can be applied to obtain a sensitive, robust and stable sensing layer [86,104]. Doong and Shih [105] have proposed the preparation of titanium dioxide sol-gel matrices by vapor deposition for the co-immobilization of a fluorescent pH indicator, carboxy seminaphthorhodamine-1-dextran (SNARF-1-dextran), and glutamate dehydrogenase (GLDH). The sensing layers were deposited by pin-printing onto a glass-slide and evaluated using fluorescence microscopy allowing application to the determination of glutamate in water and biological samples. In a further development, the same approach has been applied to the development of an optical array biosensor for the simultaneous monitoring of glucose, urea and glutamate in serum samples [106]. Urease, glucose dehydrogenase (GDH), and GLDH have been coupled with the pH indicator SNARF-1-dextran and the change in the solution pH was evaluated by measuring the fluorescence ratio of the dye at 585 and 630 nm. Dynamic ranges of 2–3 orders of magnitude with LODs of 3.1–7.8 μM, were obtained in serum samples without cross-interference from other species. Additional representative examples of fluorescence based enzymatic biosensors are depicted in Table 1.

4.2. Antibodies in biosensing with FO and PWG

Antibodies have been for years one of the most used affinity recognition elements in areas as clinical medicine or the environmental and biotechnological fields. The combination of high affinity and high specificity together with relatively good stability makes antibodies suitable biosensor probes and thus, they have been applied to the development of fluorescent FO and PWG immunosensors. These devices exploit the principles of solid-phase immunoassays, such as ELISA, where selective bio-affinity interactions between an antibody and a specific compound are commonly observed on a solid support. Table 2 shows some examples of the trends in recent years in fluorescence immunosensing using FO and PWG.

The most widely used schemes of the aforementioned optical platforms can be classified according to four different assay formats [107,108] (Fig. 3): (i) direct immunoassay — the antigen is incubated with excess amounts of immobilized antibody and the interaction is detected through, for example, a luminescence property of the antigen; (ii) competitive immunoassay — the preferred format for detecting small target molecules which contain only one epitope. Two modes of competitive assay are widely used: in one mode, the competition for the binding sites of the immobilized antibody is carried out between the target molecule and a labelled target. In the second mode, a target derivative is immobilized and the antibody is labelled so any target molecule in the sample is able to inhibit the binding of the antibody to the immobilized target; this mode is also known as the “binding-inhibition” assay; (iii) sandwich immunoassay — the preferred format for detecting targets with, at least, two epitopes in their structure. The assay involves the capture of the target molecules in solution by an immobilized primary antibody. Afterwards, a secondary labelled antibody (detection or reporter antibody) is added and binds the target molecule. Finally, the resultant complex is measured after washing the excess of immunoreagents; (iv) displacement immunoassay — in this flow assay configuration, the active sites of an immobilized antibody are previously saturated with an excess of labelled antigen. After a
| Analyte(s) | Material | Sensor platform | Scheme LOD | Analytical performance | Ref. |
|-----------|----------|-----------------|------------|------------------------|-----|
| **Direct sensors** | | | | | |
| Glucose | Glucose oxidase chemically modified with fluorescein-5(6)-carboxamidocaproic acid N-succinimidyl ester (GOx-FS) | • GOx-FS in polyacrylamide films FI | – | Working range: 300–2000 mg L⁻¹ | [102] |
| | | • Applied to the analysis of glucose in orange and pineapple juices | | Response time: 80 to 240 s | |
| | Lactate dehydrogenase | • Tapered multimode optical fibers FI sputtered-coated with 100 nm aluminium were silanized with 5% (v/v) APTES, activated with 5% (v/v) glutaraldehyde and incubated with lactate dehydrogenase. | – | Working range: 0.06–1 mM | [94] |
| | | • The nanoprobe was slowly and precisely located on the cell plasma membrane, whereby the released lactate under catalysis of the enzyme produced fluorescent NADH. | | Response time: 1 s | |
| L-Leucine | Leucine dehydrogenase immobilized in a PTFE membrane with PMEH polymer | The sensing membrane was placed FI on the edge of optical fiber and UV-LED excitation system. | 1 μM | Working range: 1–10000 μmol L⁻¹ | [201] |
| | | • Analytical signal: fluorescence of NADH, produced by enzymatic reaction between NAD⁺ and L-leucine. | | | |
| | | • Clinical application to the determination of maple syrup urine disease (MSUD) | | | |
| Sorbitol | Sorbitol dehydrogenase from *Flavimonas* immobilized in a PMEH membrane | The UV-LED excitation system is FI coupled to the detector by a Y-shaped optical fiber. | – | Working range: 1–1000 μmol L⁻¹ | [202] |
| | | • Analytical signal: fluorescence of NADH, produced by enzymatic reaction between NAD⁺ and D-sorbitol. | | Response time: 1.5 min | |
| **pH transduction** | | | | | |
| Glutamate, urea and glucose | Enzymes: glutamate dehydrogenase, urease and glucose dehydrogenase Indicator: SNARF-1-dextran | • The sensing films were prepared by FI pin-printing the reagents solution on a glass slide using the vapor deposition method. | Glutamate: 7.8 μM Urea: 3.1 μM Glucose: 5.4 μM | Serum samples Glutamate: 40–8000 μM Urea: 2–300 μM Glucose: 20–10000 μM | [106] |
| | | • Applied to the analysis of serum samples. | | | |
| | Organophosphate pesticides | Organophosphate hydrolase and carboxynaphthofluorescein | • The enzyme was immobilized onto EW the surface of a glass slide modified with TiO₂. The surface was derivatized using neutravidin-biotin chemistry. CNF was functionalized with NHS ester and bound to the slide surface. | Paraaxon 2.5 μM Parathion 10 μM | Working range: 0.00125–0.1 mM | [203] |
| | | • Total analysis time 2 min. | | | |
| | Halogenated aliphatic hydrocarbons | Haloalkane dehalogenase co-immobilized with 5(6)-carboxyfluorescein conjugated to bovine serum albumin | • The enzyme and the pH sensitive dye FI were co-immobilized at the fiber tip. | 0.133 mM for DBE 0.014 mM for CCMP | 30 min incubation | [204] |
| | | • Hydrolytic dehalogenation of halogenated aliphatic hydrocarbons, catalyzed by haloalkane dehalogenase, originates a pH change that is monitored with the pH sensor | | | |
| | O₂ transduction | Uric acid | The enzyme was crosslinked with FI glutaraldehyde and immobilized in a polyurethane hydrogel next to the oxygen probe, entrapped in Ormosil beads. | B1: 50 μM B2: 20 μM | B1: 5–600 μM B2: 5–800 μM Response time: 1.5 min | [205] |
| | | | • Membrane thickness was in the order of 12 μm. | | | |
| | | | • The sensor was applied to blood analysis. | | | |

**Note:** APTES = (3-aminopropyl)triethoxysilane; CCMP = 3-Chloro-2-(chloromethyl)-1-propene; CNF = carboxynaphthofluorescein; DBE = 1,2-Dibromoethane; EW = evanesence wave; FI = fluorescence intensity; Ir(ppy)₃ = Tris(2-phenylpyridyl)iridium(III) complex; PVA = Polyvinylalcohol; PMEH = 2-methacryloyloxyethyl phosphorylcholine; PTFE = polytetrafluoroethylene; Ru(dpp)₂ = Tris(4,7-diphenyl-10-phenanthroline)ruthenium(II) cation; Ru(dpp)₂TMS₂ = Tris(4,7-diphenyl-1,10-phenanthroline)ruthenium(II) trimethyl 3-(trimethylsilyl)-1-propanesulfonate; SNARF-1-dextran = Carboxy seminaphthodihamine-1-dextran.
| Analyte(s) | Material | Sensor platform | Scheme | LOD | Analytical performance | Ref. |
|------------|----------|-----------------|--------|-----|------------------------|------|
| C reactive protein, interleukin-6 (IL 6) and procalcitonin, in hospitalized patients | Telomerase, in MCF-7 serum protein, in human nucleocapsid plasma and FV, in blood | Sandwich type format. | FL (EW) | For IL-6: 0.08 ng mL$^{-1}$; for PCT: 1 ng mL$^{-1}$; for CRP: 0.35 ng mL$^{-1}$ | 35 min assay | [32] |
| Paralytic shellfish toxins: saxitoxin (STX), neosaxitoxin (NED) produced by marine dinoflagellates in coastal waters | Telomerase, in MCF-7 cell nucleus | Enzymatic sandwich type format. | FL | – | – | [95] |
| Capalstatin, C reactive protein, Analyte(s) Material Sensor platform Scheme LOD Analytical performance Ref. | Escherichia coli O157:H7, in spiked food matrices | Sandwich type format. | FL (EW) $5 \times 10^4$ cells mL$^{-1}$ | <30 min assay | [110] |
| SARS coronavirus nucleocapsid protein, in human serum | Capture antibody on surface of declad portion of plastic OFs | Combination of sandwich type format with localized surface plasmon technique. | FL (SP coupled) | Working range: 0.1 pg mL$^{-1}$ to 1 ng mL$^{-1}$ | [116] |
| Factor V leiden (FVL) and FV, in blood plasma | Capture antibodies, conjugated to donor fluorophore, on tips of tapered OF | Sandwich type format. | FL | – | Working range: 0–12 ng mL$^{-1}$ | [118] |
| VEGF; IP-10; IL-8 EGF; MMP-9; IL-1β in saliva samples | Capture antibody-functionalized microspheres loaded in microwell array etched on the distal end of imaging OF bundle | Sandwich type format. | FL | VEGF (6 pg mL$^{-1}$), IP-10 (26 pg mL$^{-1}$), IL-8 (4 pg mL$^{-1}$), EGF (3 pg mL$^{-1}$), MMP-9 (1311 pg mL$^{-1}$), IL-1β (5 pg mL$^{-1}$) | Working range: VEGF (202 – 16000 pg mL$^{-1}$), IP-10 (99 – 16000 pg mL$^{-1}$), IL-8 (24 – 10000 pg mL$^{-1}$), EGF (6 – 10000 pg mL$^{-1}$), MMP-9 (1311 – 800000 pg mL$^{-1}$), IL-1β (378 – 4000 pg mL$^{-1}$) | [124] |
| Carcinobenign iron oxide (IF) | Capture Ab attached to phospholipid bilayer film joint to surface of a thin SiO$_2$ deposited on a PWG | Sandwich type format within FL (EW) evanescent field. | <0.5 pM | 15 min assay | [206] |
| Paralytic shellfish toxins: saxitoxin (STX), neosaxitoxin (NED) produced by marine dinoflagellates in coastal waters | Capture antibodies anti-STX and anti-NEO labelled with Alexa Fluor® 647 carboxylic acid | Inhibition type format. | FL (EW) | STX: 12 pg mL$^{-1}$ | STX: (CCJ): 20 pg mL$^{-1}$; (CCV): 11% | [207] |
| (continued on next page)
stable signal is achieved, the addition of the target molecule produces the displacement of the labelled antigen whose signal is proportional to the amount of target molecule added.

Several simple conclusions can be drawn from the information presented in literature. Although the binding-inhibition configuration has been broadly used, the sandwich type format has been, by far, the preferred detection set up [107]. On the other hand, most of the fluorescent labels used for marking of reporter antibodies are

Table 2 (continued)

| Analyte(s) | Material | Sensor platform | Scheme | LOD | Analytical performance | Ref. |
|------------|----------|-----------------|--------|-----|------------------------|-----|
| Microcystin LR (MCLR) | Planar waveguide (borosilicate microscope slides) patterned with dextran-BTL2/MCLR conjugates for increased loading capability | | FI (EW) | For MCLR: 0.007 ± 0.001 ng L⁻¹ | Working range: 0.09–136.6 ng L⁻¹ - 3 h assay | [208] |
| | | | | MCLR: 0.78 ng mL⁻¹ | MCLR: (CCb): 1 ng mL⁻¹ (SCV: 12.6%) Dynamic range (IC10–IC90) of 0.22–5.12 ng mL⁻¹ | [209] |
| Yersinia pestis, in infected mice | Capture antibodies on polystyrene FO probes | | FI (EW) | – | Working range: 6.10³ – 6.10⁸ CFU mL⁻¹ 20 min assay | [210] |
| C reactive protein (CRP) | Polypeptide with tailored binder on bulk glass slide | | FI (EW) | – | With low affinity binder: 1.9 x 10⁻⁹ | [211] |
| | | | | 5 x 10⁻⁸ M | With high affinity binder: 3.4 x 10⁻⁸–2 x 10⁻⁷ M | |
| Progesterone, in bovine milk | Progesterone-11-hemisuccinate on glass slide with bound aminodextran layer | | FI (EW) | 0.04 ng mL⁻¹ | 5 min assay | [212] |
| 2,4-dichloro-phenoxyacetic acid (2,4-D) and microcystin-LR (MC-LR) | Hapten–carrier conjugate 2,4-D-BSA or MC-LR-OVA on plastic-clad step-index silica optical fiber, uncladded and tapered on distal end. | | FI (EW) | for 2,4-D: 0.09 µg L⁻¹ (0.07) for MC-LR: 0.03 µg L⁻¹ | – | [213,214] |
| Melamine, in milk products | BSA-mel crosslinked by GMBS to a mercaptosilanized glass. | | EW | 6.6 µg L⁻¹ | 26.6–517.5 µg L⁻¹ 20 min assay | [215] |

FI = fluorescence intensity; EW = evanesence wave; SP = surface plasmon; BTL2 = Geobacillus thermocatenulatus lipase 2; VEGF = human vascular endothelial growth factor; IP10 = interferon gamma-induced protein 10; IL-8 = interleukin-8; EGF = epidermal growth factor; MMP-9 = matrix metalloproteinase 9; interleukin-1 beta (IL-1β); BSA = bovine serum albumin; GMBS = N-(4-maleimidothiophenoxysuccinimide).
either organic fluorophores, such as Alexa® Fluor or Dylight® dyes, or fluorescent proteins, such as R-phycoerythrin. The advantages presented by QD labelling are barely used as yet, and the phenomenon of fluorescence enhancement near metal structures, such as NPs, has not been sufficiently exploited in FO and PWG biosensors [51].

One representative and commercial example of FO immunosensor is RAPTOR device. The field portable system was used to detect simultaneously biological agents, such as B. anthracis, Ricin, Cholera toxin or Salmonella, thanks to the four individual channels corresponding to one optical fiber [8]. Two representative examples of PWG immunosensors are based on a binding-inhibition format [109]: the first prototype was River ANAlyser platform (RIANA) and the second, built to overcome the drawbacks of RIANA, was the Automated Water Analyser Computer Supported System (AWACSS) [8]. Both were used to determine pesticides, pollutants and hormones in different liquid samples (waters and milk). The AWACSS platform employs a fiber-pigtaled chip, driven by a semiconductor laser that consists of a waveguide circuit which distributes excitation light to 32 separate sensing patches on the chip surface. Besides, a micro-fluidic system handles automatically the sample injection over the sensor surface and a fiber-coupled detection array monitors the fluorescence signals.

Ligler and coworkers developed a multi-analyte TIRF biosensor (National Research lab (NRL) array biosensor), which is fully automated and extensively applied in different fields [110–113]. According to these authors [114,115], the system consists of a two-dimensional array of immobilized capture molecules on the surface of an optical waveguide (glass slide), forming parallel patterns of either stripes or spots; the assays are performed within the channels of a multiple-channel flow cell placed on the PWG and multiple samples can be simultaneously applied. This biosensor uses mostly a sandwich type format and has been used in screening environmental, food and clinical matrices, as well as in the investigation of binding kinetics.

The three abovementioned platforms, take advantage of the qualities of PWG (their ability to define separate patterns of immobilized sensing elements on a single surface and the ease of integration into microfluidic platforms) as well as all the advantages provided by the evanescent field excitation (basically, surface selectivity). Moreover, the success of these platforms is due to the possibility of multiplexing and semi or fully automated analysis.

Chou and co-workers [64,116,117] developed another group of immunosensors that combine localized surface plasmon techniques, to increase the sensitivity of protein immunoassays, and E/W excitation, to perform protein binding kinetics. Lastly, the group of K. Kang and colleagues [118] have determined several analytes of clinical interest and have studied the surface plasmon enhancement of cardiac marker detection.

In all the FO biosensing platforms that are presented in Table 2, waveguides are used as solid supports activated with the appropriate antibody to capture the target molecule during the assay. However, other works in the field of fluorescence immunosensing use optical fibres, usually in the form of bundles or arrays, simply to collect and transport emitted light [119–123] from antibody-based microspheres that report the presence of target analytes in the sample. In particular, microarrays which combine microsphere-technology and randomly ordered arrays have grown rapidly. For example, Nie and coworkers [124] reported a multiplexed protein microarray based on the fiber-optic bundles and microsphere-based antibody arrays for the detection of six salivary biomarkers for asthma and cystic fibrosis diagnosis. The potential to use the multiplexed protein array for respiratory disease diagnosis was fully demonstrated by the construction of an analyzer, SDReader, which combines non-invasive sample collection and fully automated analysis [125]. Using the fiber-optic bundles, the Walt group has also developed a single molecule immunoassay, which they called digital ELISA [126], that can detect subfemtomolar concentrations of proteins in serum. Digital ELISA, also known as single molecule array (or SiMoA), features not only greater sensitivity but also enables faster assays, minimizes (by dilution) interferences and allows smaller sample volumes to be tested [127].

Although monoclonal and polyclonal antibodies are sensitive to temperature and have a limited self-life, they remain to be the main probes for immunosensor development. It is interesting to note that, despite the increasing production of recombinant antibodies engineered to improve their affinity and stability (e.g. Fab, Fv, single chain Fv, VH or VHH fragments), scarce number of works are published operating them in combination with either FO or PWG platforms. For example, Murphy et al. [128] produced a novel bioconjugates that (scFv) recombinant anti-microcystin avian antibody for the detection of microcystin-LR. This antibody was used in a fluorescence inhibition immunoassay on a planar waveguide detection system, where microcystin LR (MC-LR)-OVA conjugates were printed onto portable optical-planar waveguide cartridges and Streptavidin-Alexa-647® was used to detect the presence of biotinylated-anti-MC-LR. The immunoassay allows the detection of free MC-LR with a limit of detection of 0.19 ng mL⁻¹ and a detection range of 0.21–5.9 ng mL⁻¹.

4.3. Nucleic acids in biosensing with FO and PWG

Single-core optical fiber sensors, fiber optic microarrays and PWG sensors become powerful platforms by virtue of using nucleic acids as affinity biorecognition elements [129–131]. Recent applications are based on diagnostic tests of infections [132], detection of genetic mutations such as single nucleotide polymorphisms (SNPs) [133] or fast microbial pathogen detectors [134]; some of them are illustrative examples and will be reviewed in this section.

Fig. 3. Classification of (bio)mimetic assays: (A) Direct immunoassay, (B) Competitive immunoassay, (C) Sandwich immunoassay and (D) Displacement immunoassay.
Besides, Table 3 presents the general characteristics of other fluorescence-based Nucleic Acid FO and PWG biosensors (see Table 3).

The typical transduction employed in this class of biosensors requires immobilization of a single strand oligonucleotide probe to the surface of the optical substrate and capture or hybridization of the target sequence of interest. Afterwards, the most frequent scheme of detection is based on the well characterized sandwich assay, in which a captured target sequence is allowed to hybridize with a labelled complementary target sequence (Fig. 4A). A second indirect transduction format for detection is the use of luminescence reporter molecules that bind target nucleic acids previously labelled (e.g., biotinylated) by PCR amplification with labelled PCR primers or by a post-amplification labeling process (Fig. 4B) [39]. In a third approach, the hybridization between the capture probe and the target sequence is queried by a fluorogenic dye whose emission is enhanced by its binding to the double strand oligonucleotide structure (Fig. 4C).

During the last years, the number of publications describing nucleic acid fluorescence biosensors based on single core fibers has decreased notoriously since microarray architectures on fibers and other substrates, such as planar guides, have become powerful tools for multiplexed sensing and fundamental studies of single molecules and cells [135]. However, several recent publications demonstrate that single core optical fibers are still the preferred platform to perform nucleic acid biosensing. For example, Massey and Krull [136] have recently explored a novel strategy for nucleic acid detection based on scaffolding duplexes bound to a silica fused optical fiber. The tandem consists of a capture DNA probe and a probe that turns circular when binds both capture DNA probe via Avidin Bridge. The optical fiber sensor was first aminated by immersion in a 2% (v/v) APTS acetone solution. The tandem is performed by hybridization of target DNA followed by a nicking reaction using a nicking enzyme. The optical fiber sensor was further extended by labelling with a biotinylated reverse primer that is extended via a biotin-DNA target gen labelled with conjugated streptavidin-QD nanocrystals with emission wavelength maxima at 705 nm. Effective sensor area at 3.45 mm² and a sensitivity of 5.6 - 1011 molecules/cm². The tandem consists of a capture DNA probe and a biotinylated reverse primer. The tandem is performed by hybridization of target DNA followed by a nicking reaction using a nicking enzyme. The tandem is performed by hybridization of target DNA followed by a nicking reaction using a nicking enzyme. The tandem is performed by hybridization of target DNA followed by a nicking reaction using a nicking enzyme.

Table 3

| Analyte(s) | Material | Sensor platform | Scheme LOD | Analytical performance |
|------------|----------|-----------------|------------|------------------------|
| HABs (Harmful Algal Bloom) A. fundyense, A. ostenceldii, and Pseudo-nitzschia australis | ssDNA capture probes (<23 mer) covalently attached to encoded microspheres | Sandwich type format. | FI <0.1 PM | 45 min [216] |
| Peanut allergens, Ara H1 in food matrices | Use as bioreceptor Ara H1 DNA aptamer, selected by CE-SELEX | Sandwich type format. | FI (FO-SPR) 75 nM | Concentration tested from 0 nM to 634 nM [217] |
| uidA gene of Escherichia coli | ssDNA-coated fiber probe (30 mer) | Biosensing platform based on QDs and internal reflection fluorescence. | FI (EW) 3.2 amol of bound target DNA | 10 min/assay [218] |
| mRNA abundances in FFPE tissue samples: Expression control of 14 genes markers of breast cancer | Spotted DNA array on slides coated with epoxylysine or polyvinylamine. DNA capture probes ranged between 53 and 71 mer. | Sandwich assay format. | FI (EW) <10 FM, No target amplification is necessary | 7–18 h/24 samples [219] |
| 17β-estradiol | DNA aptamer probe (76 mer) specific to 17β-estradiol | Inhibition assay format: β-estradiol 6-(O-carboxy-methyl)oxime-BSA conjugate & corticosterone | FI (EW) 2.1 nM (0.6 ng mL⁻¹) | 10 min, 50 min/assay [220] |
| Simultaneous detection of proteins (IL-6, IL-8) and gen encoding membrane protein, PⅢ, of Haemophilus influenzae | A fiber-optic protein-DNA microarray using microsphere-immobilized capture antibodies specific to IL-6 and IL-8 | Sandwich assay format | FI 100 fm for IL-3, 3.4 amol for IL-6 and IL-8, 1 pM for target DNA sequence | [221] |

EW — evanesence wave; FI — fluorescence intensity; FFPE — formalin-fixed paraffin-embedded; Phen — 1,10-phenanthroline and PHPIP — p-hydroxyphenylimidazo[1,10-phenanthroline; FO-SPR — Fiber optic surface plasmon resonance; CE — Capillary electrophoresis; SELEX — Systematic evolution of ligands by exponential enrichment.
detection of nucleic acid hybridization using optical fibers modified with QDs and co-immobilized with two different capture DNA probes. A sandwich assay is performed to detect the target gene sequence and the signal response is achieved upon hybridization through FRET-sensitized acceptor emission. To that aim, the detection probes are labelled at the 3’-end with Cy3 and Rhodamine Red-X dyes which are suitable acceptors of the emission upcoming from the QD film. Although the LOD of the proposed DNA-sensor is not sensitive enough, 10 nM, this fiber biosensor opens a new insight in the development of FRET multiplexed biosensors [137].

Since Ferguson and co-workers pioneered in the creation of a fiber optic array biosensor for the detection of seven DNA sequences [138], numerous FO microarray biosensors have been reported. Remarkable is the work of Walt and co-workers based on an innovative high density fiber optic bead-array platform, which has been applied for a variety of DNA and RNA biosensors in the last five years, highlighting very low LODs as well as low non-specific binding [39]. For example, Song [139] uses this platform for the simultaneous detection of Bacillus Anthracis, Yersinia pestis, Francisella tularensis and four more biological warfare agents (BWA) by a RNA assay where 50-mer capture probes are bound to uniquely identified microspheres. The authors reported LODs between 10 and 100 fM, and multiplexed assay with mixed samples indicated high specificity on the target detection. Gunderson and co-workers performed a DNA assay for SNPs detection using high density fiber optic array and beads decorated with highly specific ssDNA capture probes. The assay, which is based on the well-known Illumina GoldenGate Genotyping Assay [140], is able to interrogate up to 1536 SNPs per sample, minimizing time, reagent volumes, and material requirements of the process [141].

Rindorf and co-workers proposed a novel optical detection system incorporating a microstructured optical fiber (MOF) into an optic-compatible fluidic coupler chip. This hybrid biosensor operates towards the capture of specific DNA targets by functionalizing the MOF with an oligonucleotide capture probe in an optical sensing layer [134].

A novel emerging tool for bioassays is the use of single-molecule detection (SMD) techniques that, apart from the fascination of looking at individual biomolecules at work, offer the possibility of developing highly sensitive assays. Although SMD techniques were at the beginning a challenging task that required complex microscope apparatus, recent studies have been published about single molecule detection using optical fiber arrays [142,143]. A nucleic acid assay with SMD is described by Z. Li and co-workers [144] using arrays of femtoliter-sized reaction vessels and a fluorescent enzymatic detection system. By a sandwich assay format, the simple binary read-out based scheme allows DNA detection down to 1 fM and exhibits a high signal-to-noise ratio.

The ability of single molecule (fiber-optic) arrays (SiMoA) to literally count the number of protein molecules in an assay has also been exploited to detect bacterial genomic DNA at sub-femtomolar concentrations (Fig. 5). The SiMoA DNA assay, reported by Song et al. [145], directly detects target DNA molecules without molecular replication, such as the polymerase chain reaction (PCR). Furthermore, the method enables the quantification of DNA in river water without purification and authors claimed that it would be suitable for measuring bacteria in many other samples, such as bloodstream.

For significant progress in the development of DNA biosensors, it is essential to achieve rapid and accurate detections of specific DNA and RNA sequences. To that aim, it is important to obtain stable DNA/DNA duplex (or DNA/RNA) structures upon hybridization [146]. Thus, synthetic nucleic acids, such as Peptide Nucleic Acid (PNA) and Locked Nucleic Acid (LNA) (Fig. 6A), are being used as alternatives, for example, to the classical ssDNA-based capture probes [147,148]. Briefly, PNA are synthetic DNA analogs whose sugar phosphate backbone has been replaced by an uncharged N-(2-aminoethyl) glycine scaffold. The uncharged nature of PNA is responsible for a better thermal stability of PNA-DNA [149,150]. LNAs are tailor-made nucleotides in which the ribose moiety is locked by an extra bridge connecting the 2’-oxygen and 4’-carbon. The novel nucleic acid architecture enhances base stacking and backbone pre-organization. LNA was initially prepared as an ideal oligomer for recognition of RNA, but LNA also displays high affinity toward DNA single strands [146].

The first publications of PNA polymers as novel recognition elements predicted high impact in biosensor development; however, it has still not been observed. PNA exhibit disadvantages related to the cost of their synthesis and the high occurrence of unspecific binding events [151]. If these problems can be solved, PNA will represent an interesting alternative for the development of the next generation of nucleic acid biosensors. For example, Sozzi et al. recently report a label free DNA sensor based on the use of capture PNA probes immobilized on the surface of a grating-based fiber optic platform [152].

In a similar way as PNAs, the use of LNAs has increased in the last years knowing their improved affinity and specificity with respect to their DNA and RNA targets. However, the new reported biosensors based on LNA capture probes are mostly based on non-optical techniques and their use in optical biosensors has not extended so far [153]. Optical platforms demonstrating the usefulness of LNA-modified oligonucleotides are mostly chip-based biosensing platforms constructed by photolithography of glass surfaces which eventually attach LNA capture probes arrayed by a combinatorial chemistry approach [154].

Molecular beacons (MBs) are particular dual labelled single strand oligonucleotide probes, DNA or RNA, that have self-complementary ends to form a stem-loop structure (hairpin) in their native state. The hairpin forces reporter and quencher into contact. Upon hybridization of a matching target to the loop portion, reporter and quencher are separated through conformational reorganization and the molecular beacon becomes bright (Fig. 6B). The use of MBs as immobilized capture probes on the

![Fig. 4. Most frequent sensing schemes for nucleic acid detection: (A) Sandwich assay; a captured target sequence is allowed to hybridize with a labelled complementary target sequence. (B) Post-amplification labeling process. (C) Signal is queried by a fluorogenic dye whose emission is enhanced upon intercalation in the double strand oligonucleotide structure.](image-url)
optical substrate show two sensing schemes in which the signal is directly obtained upon both hybridization to the target sequence or ssDNA binding protein (Fig. 6B) [155]. The use of MBs has the advantage that no additional labelled species are necessary [156–159]. In the last years, the employment of MBs in the development of solid-surface based biosensors has not fully matured. The results, which initially underscored the potential of MBs as smart biorecognition elements in fluorescence based FO and PWG biosensors, never overcame some of their critical limitations, such as the poor stability of the stem-and-loop structure once a MB nucleotide is immobilized onto a solid substrate. Some examples of these biosensors are depicted in Table 3.

Biosensors using aptamers as biorecognition elements are referred to as aptasensors. In the same way as immunosensors, aptasensors can be applied in several sensing schemes to transduce the recognition process, such as, direct, competitive and sandwich assay formats. There are some interesting examples of fluorescence based optical aptasensors that have been reviewed recently [160]. One example of aptasensor, combined with PWG and fluorescence detection, is the evanescent wave DNA-aptamer-based biosensor for Hg (II) detection [161]. In this work, a hybrid DNA-aptamer probe is covalently immobilized onto an optical fiber. These DNA-aptamer probes have two different parts (Fig. 7): (i) a short sequence to 5′ for the hybridization to a complementary chain labelled with Cy5.5 and (ii) a sequence of T–T mismatch pairs that, in presence of Hg (II), promotes the folding of the probe into a hairpin structure forming a T-Hg(II)-T complex. The sensing scheme is a competitive assay, between the Hg (II) of the sample and a complementary DNA fluorescently labelled with Cy5.5. This platform has a detection limit of 2.1 nM and can be regenerated with a 0.5% SDS solution (pH 1.9) more than 100 times. The authors also evaluated the response of other metal ions and proved the high specificity of the sensor.

Aptazymes (RNAzymes and DNAzymes) are bioinspired materials with allosteric properties that transduce the recognition event of their targets into catalytically generated reporting signals. An advantage of using aptazymes is their stability to repeated denaturation processes without losing neither catalytic nor binding capabilities. Furthermore, their production is not expensive and they are suitable for a wide range of analytes [162]. Although these recognition elements seem to be smart tools for optical sensing, they have not been applied up to date in fluorescence based FO or PWG sensing. The only publication we have found is the work of Yildirim et al. [163] who developed a portable and rapid DNAzyme based sensor to monitor Pb (II). The presence of the metal produces the cleave of the DNAzyme and a fluorescent labelled fragment is released that hybridizes with the complementary strand immobilized on the optic fiber sensor surface. Fluorescent signals of the hybridized fluorescent labelled fragment allows the detection of Pb (II) in a range from 2 to 75 nM, with a detection limit of 1.03 nM (0.21 ng mL⁻¹). The sensor demonstrated good selectivity against other metal ions, and can be regenerated by treatment with a 1% solution of SDS (pH 1.9) to be used over 100 times.

4.4. Whole cells in biosensing with FO and PWG

Whole cells, including microorganisms (eukaryotic and prokaryotic cells), animal or plant tissues and cell receptors [164–167] are the choice biorecognition elements to obtain information regarding the bioavailability, general toxicity and genotoxicity of target analytes, as well as to understand the interaction between cells and target compounds. Non-genetically engineered whole cells have been applied to the analysis of the total amount of pollutants and hazardous substances, and sensors for the analysis of the biological oxygen demand (BOD) are already on the market [168].

Peña-Vázquez et al. [169] developed an optical fiber sensor for the detection of simazine using three microalgae (Dictyosphaerium chloroeloides (D.c.), Scenedesmus intermedius (S.i.) and Scenedesmus sp. (S.s.)). The three microbial species were entrapped in a silicate sol-gel membrane placed at the tip of an optical fiber to create the sensor probe that showed response to those herbicides that inhibit the photosynthesis at photosystem II (PSII). The intensification in the chlorophyll fluorescence signal was measured, and the results showed that D.c. has a lower limit of detection (3.6 μg L⁻¹) than S.s. (48. μg L⁻¹) and S.i. (310 μg L⁻¹). This biosensor is stable for at least 3 weeks. In a similar approach, Nguyen-Ngoc and co-workers prepared an optical biosensor using Chlorella vulgaris to detect the pesticide diuron [170]. Again, the distal end of an optical fiber is modified with a sol-gel membrane, containing the microalgae, and an increase of fluorescence is observed when the pesticide inhibits the photosynthesis of PSII. The detection limit of the biosensor is 1 μg L⁻¹ and maintains the activity after 5 weeks.

Another approach relies on the ability of genetically engineered whole cells to be used as sensing systems. In this case, both prokaryotic or eukaryotic cells could be modified by introducing a plasmid prepared by coupling a reporter gene with a sensing element, generally formed by an operon promoter region and a regulatory gene that encodes for a regulatory protein responsible of target binding (e.g. luxCDABE operon) [171]. Upon formation, the regulatory protein-target complex binds the promoter, and triggers the expression of the reporter gene responsible for the production of a luminescent reporter protein such as bacterial and firefly luciferases. These biosensors can be applied to the detection of a broad range of analytes including metals, organic pollutants, sugars, drugs, etc. [165]. For example, Ivask et al. [172] prepared two luminescent fiber-optic biosensors for the detection of Hg and As. The biosensors consist of alginate-immobilized recombinant bacteria emitting light specifically in the presence of bioavailable Hg or As in a dose-dependent manner. The limits of quantification of the developed sensors were 0.0026 mg L⁻¹ for Hg and 0.012 and 0.14 mg L⁻¹ for As(III) and As(V), respectively, which are good enough to allow their use in environmental analysis.

In a different approach, Futra and coworkers [173] propose an approach for monitoring the heavy metal toxicity. The biosensor is based on a recombinant E. coli modified with the green fluorescence protein (GFP) gene, which is directly immobilized on a cellulose nitrate membrane. The fluorescence response of the immobilized GFP-E. coli is monitored by a fiber-optic system, where the sensor membrane has been located in the distal end of the fiber. The response of the biosensor was stable for at least 3 weeks, and the detection limits were of 0.04, 0.32, 0.46, 2.80, 100, 250, 400, 720 and 2600 μg L⁻¹ for Cu(II), Cd(II), Pb(II), Zn(II), Cr(VI), Co(II), Ni(II), Ag(I) and Fe(III), respectively.

4.5. Other receptors in biosensing with FO and PWG

The nature of the receptors is very diverse, thus, we will focus our attention to those used in either optical fiber or planar waveguide platforms. In the last decade, four types of natural receptors have been used as affinity biorecognition elements in FO and PWG platforms combined with fluorescence detection: saccharide and glycoprotein receptors, toxin receptors, antimicrobial peptides, bacteriophages and molecularly imprinted polymers [8,51,174] (Table 4).

4.5.1. Saccharide and glycoprotein-based biosensors

Lectins constitute a broad family of protein receptors involved in numerous biological processes. They are very specific receptors and exhibit strong binding for saccharide moieties via multivalent
interactions. Concanavalin A (ConA) [175] is the most widely used lectin in optical sensors. Several sensing schemes have been applied in the development of optical biosensors based on ConA but competitive and sandwich assays are the most used [176,177]. Besides, the majority are associated to the use of advanced fluorescence techniques such as FRET and fluorescent nanomaterials [178].

Other protein receptors, with no lectin origin, are also used as recognition elements including the rivoflavin binding protein (RBP) [179], the maltose binding proteins (MBPs) [180], or the glucose binding protein [181], etc. A glucose/galactose-binding protein (GBP), labelled with the fluorophore 6-Bromoacetyl-2-Dimethylaminonaphthalene (Badan), was used by Saxl et al. [182] for the development of a glucose sensor. GBP-Badan was attached to the surface of NTA-agarose or polystyrene beads via Histag, and loaded into a porous chamber at the end of an optical fiber. Glucose responses of the immobilized sensor are reversible and stable, showing the best sensor response using Ni–NTA functionalized agarose beads (Kd of 13 mM (agarose) and 20 mM (polystyrene)).

4.5.2. Toxin receptor-based biosensors

Many bacterial toxins and viruses bind specifically to carbohydrate moieties on the cell surfaces to facilitate their invasion. Consequently, sugars and gangliosides have been used as recognition elements due to their specific ability of recognizing pathogens and toxins [183]. One of the most used toxin-receptor biosensor is based on the fluorescently labelled ganglioside GM1 receptor for the detection of cholera toxin [184]. In this case, the sensing scheme is based on a direct assay. The presence of the target molecule, with multiple binding sites, aggregates the labelled GM1 receptors and fluorescence self-quenching is observed.

In a different approach, Sapsford et al. [185] investigated the use of QDs to construct a peptide-FRET probe to detect botulinum neurotoxins (BoNTs), one of the most poisonous protein toxins known. The developed sensor monitors the BoNT serotype A light
chain protease (LcA) activity, using a LcA substrate that combines: a LcA recognition/cleavage moiety, a specific residue to allow labeling a Cy3 acceptor dye, a linker-spacer sequence and a terminal oligohistidine to assemble the peptide on the QDs surface. The proteolytic assay is performed following an indirect pre-exposure of LcA-peptide-Cy3 conjugate to LcA, before the assembly on QDs. In this format, QDs are essentially utilized as a visualization reagent, demonstrating a limit of detection of 17.5 ng mL$^{-1}$ for LcA (Fig. 8).

4.5.3. Antimicrobial peptides - based biosensors

Antimicrobial peptides (AMPs) represent an ancient and efficient innate defense mechanism, which protects species from infection with pathogenic microorganisms [186]. AMPs have the ability to associate with bacterial membranes so they are an alternative to some protein-based receptors for detecting their natural targets such as bacteria, fungi, viruses and also toxins such as botulinum toxins A, B and vaccinia virus [187–189]. The analytical characteristics of AMP-based biosensors were better, in some cases, than those obtained with antibodies as target captures, whereas sensing schemes are similar to those used for immunoassays (section 4.2).

An example of the use of AMPs as affinity recognition elements is described in the study of Taitt’s group [188] for detecting botulinum neurotoxoids A and B in an array biosensor using a sandwich immuno-like assay where the AMP and fluorophore-labelled detection antibody were simultaneously bound to different epitopes on the antigen (target toxoid A and B). Toxoid A could be detected with a limit of detection of 1 ng mL$^{-1}$ in a 75 min assay, whereas toxoid B was detected at 10 ng mL$^{-1}$.

4.5.4. Bacteriophages in biosensing with FO and PWG

Bacteriophages, as well as plant and some animal viruses [190,191], have been used as probes for sensing and imaging [192–194]. Bacteriophages, shortened to phages, can be easily conjugated, they are robust, ubiquitous in nature, harmless to humans and their production is easy and economical. Currently, bacteriophage-based optical biosensors are well established thanks to their easy integration into transduction devices, comparable to antibodies and nucleic acids. They are typically used for the analysis of pathogens in food and water samples, reporting better response than classical microbiological methods [195].

The advances in phage-based biosensors have been impressive in the last few years. In this regard, engineered phages, and their combination with emerging materials and nanostructures, have boosted their analytical applications to the same level as other common biorecognition elements. However, their integration in fluorescence based FO and PWG platforms is still minimal and few examples are available in the literature. For example, Phillips and co-workers [196] describes a device for determining different bacteria, including: *Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa* using recombinant bacteriophages. In this case, the phage is used to deliver, to a targeted bacterial pathogen, either genetic material of a fluorescent detectable biomarker (e.g. GFP) or an operon promoter region and a regulatory gene that encodes the production of an enzyme, such as bacterial luciferase or aequorin. The device consists of a portable system that carries a power source, a fiber-optical detector, a signal processor, and a signaling device for emitting a signal upon detection of the pathogenic bacterium in an environment.

4.5.5. Molecularly imprinted polymers-based biosensors-like

Molecularly imprinted polymers (MIPs) have been proposed as alternatives to antibodies for the development of biomimetic sensors. During the imprinting process recognition sites are formed in the sensing material that enables selective binding of the target molecule even in complex mixtures [197].

Bio(mimetic) optical fiber and planar waveguide fluorescence sensors using MIPs as recognition elements, are usually based on the following sensing schemes [198]: (i) direct, based on the evaluation of the selective binding to the polymer of a naturally or

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**Fig. 7.** Schematic representation of a structure-competitive sensing mechanism of Hg (II) detection. Reproduced with permission from Ref. [161] http://dx.doi.org/10.1016/j.bios.2011.03.022.
| Analyte(s)                  | Material                                      | Sensor platform                         | Scheme | LOD       | Analytical performance          | Ref. |
|----------------------------|-----------------------------------------------|-----------------------------------------|--------|-----------|----------------------------------|------|
| Glucose                    | Natural Receptors                             | • Direct assay format.                  | FLT    | 100 mM    | $K_d = 13$ mM (agarose)          | [182]|
|                            | Glucose/Galactose binding protein-Badan       | • Multimode optical fiber coupled to a porous chamber containing beads. | TC-SP   | $K_d = 20$ mM (polystyrene)      |       |
|                            | Genetically Engineering Whole Cells           | • Imaging microscopy and pulsed laser excitation. |        |           |                                 |      |
|                            | OPH and EGFP co-displaying yeast.             | • Indirect assay format.                | PW     | –         | 0.02–20 mM of paraoxon           | [222]|
|                            |                                               | • Fluorescence Quenching of EGFP by vicinity pH changes related to the OPH activity in the presence of Ops. |        |           | Stability at least a month at 4°C |      |
|                            |                                               | • The optical waveguide core consisted of doped sol-gel (zirconium and silica) at pH 9.0. |        |           |                                 |      |
|                            |                                               | • Multimode optical fiber coupled to a porous chamber containing beads. |        |           |                                 |      |
|                            |                                               | • Imaging microscopy and pulsed laser excitation. |        |           |                                 |      |
|                            |                                               | • Indirect assay format.                | PW     | –         | 0–1 0000 μM of paraoxon          | [223]|
|                            |                                               | • Fluorescence Quenching of EGFP by vicinity pH changes related to the OPH activity in the presence of Ops. |        |           | Stability at least a month at 4°C |      |
|                            |                                               | • The optical waveguide core consisted of doped sol-gel (zirconium and silica) at pH 9.0. |        |           |                                 |      |
|                            |                                               | • Multimode optical fiber coupled to a porous chamber containing beads. |        |           |                                 |      |
|                            |                                               | • Imaging microscopy and pulsed laser excitation. |        |           |                                 |      |
|                            |                                               | • Indirect assay format.                | PW     | –         | 0–1 0000 μM of paraoxon          | [223]|
|                            |                                               | • Fluorescence Quenching of EGFP by vicinity pH changes related to the OPH activity in the presence of Ops. |        |           | Stability at least a month at 4°C |      |
|                            |                                               | • The optical waveguide core consisted of doped sol-gel (zirconium and silica) at pH 9.0. |        |           |                                 |      |
|                            |                                               | • Multimode optical fiber coupled to a porous chamber containing beads. |        |           |                                 |      |
|                            |                                               | • Imaging microscopy and pulsed laser excitation. |        |           |                                 |      |
|                            |                                               | • Indirect assay format.                | PW     | –         | 0–1 0000 μM of paraoxon          | [223]|
|                            |                                               | • Fluorescence Quenching of EGFP by vicinity pH changes related to the OPH activity in the presence of Ops. |        |           | Stability at least a month at 4°C |      |
|                            |                                               | • The optical waveguide core consisted of doped sol-gel (zirconium and silica) at pH 9.0. |        |           |                                 |      |
|                            |                                               | • Multimode optical fiber coupled to a porous chamber containing beads. |        |           |                                 |      |
|                            |                                               | • Imaging microscopy and pulsed laser excitation. |        |           |                                 |      |
|                            |                                               | • Indirect assay format.                | PW     | –         | 0–1 0000 μM of paraoxon          | [223]|
|                            |                                               | • Fluorescence Quenching of EGFP by vicinity pH changes related to the OPH activity in the presence of Ops. |        |           | Stability at least a month at 4°C |      |
|                            |                                               | • The optical waveguide core consisted of doped sol-gel (zirconium and silica) at pH 9.0. |        |           |                                 |      |
|                            |                                               | • Multimode optical fiber coupled to a porous chamber containing beads. |        |           |                                 |      |
|                            |                                               | • Imaging microscopy and pulsed laser excitation. |        |           |                                 |      |
|                            |                                               | • Indirect assay format.                | PW     | –         | 0–1 0000 μM of paraoxon          | [223]|
|                            |                                               | • Fluorescence Quenching of EGFP by vicinity pH changes related to the OPH activity in the presence of Ops. |        |           | Stability at least a month at 4°C |      |
|                            |                                               | • The optical waveguide core consisted of doped sol-gel (zirconium and silica) at pH 9.0. |        |           |                                 |      |
|                            |                                               | • Multimode optical fiber coupled to a porous chamber containing beads. |        |           |                                 |      |
|                            |                                               | • Imaging microscopy and pulsed laser excitation. |        |           |                                 |      |
|                            |                                               | • Indirect assay format.                | PW     | –         | 0–1 0000 μM of paraoxon          | [223]|
|                            |                                               | • Fluorescence Quenching of EGFP by vicinity pH changes related to the OPH activity in the presence of Ops. |        |           | Stability at least a month at 4°C |      |
|                            |                                               | • The optical waveguide core consisted of doped sol-gel (zirconium and silica) at pH 9.0. |        |           |                                 |      |
|                            |                                               | • Multimode optical fiber coupled to a porous chamber containing beads. |        |           |                                 |      |
|                            |                                               | • Imaging microscopy and pulsed laser excitation. |        |           |                                 |      |
|                            |                                               | • Indirect assay format.                | PW     | –         | 0–1 0000 μM of paraoxon          | [223]|
|                            |                                               | • Fluorescence Quenching of EGFP by vicinity pH changes related to the OPH activity in the presence of Ops. |        |           | Stability at least a month at 4°C |      |
|                            |                                               | • The optical waveguide core consisted of doped sol-gel (zirconium and silica) at pH 9.0. |        |           |                                 |      |
|                            |                                               | • Multimode optical fiber coupled to a porous chamber containing beads. |        |           |                                 |      |
|                            |                                               | • Imaging microscopy and pulsed laser excitation. |        |           |                                 |      |
|                            |                                               | • Indirect assay format.                | PW     | –         | 0–1 0000 μM of paraoxon          | [223]|
|                            |                                               | • Fluorescence Quenching of EGFP by vicinity pH changes related to the OPH activity in the presence of Ops. |        |           | Stability at least a month at 4°C |      |
|                            |                                               | • The optical waveguide core consisted of doped sol-gel (zirconium and silica) at pH 9.0. |        |           |                                 |      |

(continued on next page)
| Scheme | Material | Sensor platform | LOD | Dynamic range: | FI | EW |
|-------|----------|----------------|-----|----------------|----|-----|
| FI    | Cellulose nitrate membrane with recombinant E. coli GFP | Fiber optic cable detecting fluorescence by fluorophores facing the probe’s distal end directly to the microbial membrane. | $0.04 \mu g L^{-1}$ | $10 \mu g L^{-1}$ | $0.10 \mu g L^{-1}$ | $0.50 \mu g L^{-1}$ |
| EW    | A layer of Ca-alginate gel formed over the GFP fluorescence excitation at 485 nm. | Sensor response measured at room temperature and 2 min after the initiation of the biochemical reaction. | $0.50 \mu g L^{-1}$ | $1.00 \mu g L^{-1}$ | $1.50 \mu g L^{-1}$ |
| FI    | Cellulose nitrate membrane with recombinant E. coli GFP | Sensor response stable for at least 5 weeks. | $0.32 \mu m$ | $4 \mu m$ | $25 \mu m$ |
| FI    | Cellulose nitrate membrane with recombinant E. coli GFP | Sensor response stable for at least 5 weeks. | $0.32 \mu m$ | $4 \mu m$ | $25 \mu m$ |
| FI    | Cellulose nitrate membrane with recombinant E. coli GFP | Sensor response stable for at least 5 weeks. | $0.32 \mu m$ | $4 \mu m$ | $25 \mu m$ |

5. Conclusions

Optical biosensors are increasingly finding their application in highly diversified areas such as medical and veterinary diagnosis, environmental testing, homeland security or food safety and several commercial devices are already found on the market [5]. This evolution has been the result of the combined efforts of specialists in very different disciplines, as well as the implementation of discoveries in other areas related to biosensor design, such as biochemistry, optics, fluidics or electronics, that in the end allowed the transfer of these devices from laboratory controlled environments, where they were initially applied, to the point of use.

Several factors have been identified that will play an important role in the future of biosensors, including [4,200]: a) the availability of new immobilization strategies to enhance the performance and long-term stability of biomolecules; b) signal amplification schemes allowing higher sensitivities; c) the application of nanotechnology for geometric control of the biochemistry and signal enhancement; d) microfluidics for automation, reaction control and minimization of analysis time; e) the availability of optical elements to facilitate system integration and portability; f) new methodologies that facilitate the development of self-powered devices for the application of biosensors outside the lab walls.

The search of new recognition elements and sensing schemes has always received a great deal of attention. In fact, research in the search of bio and biomimetic selective recognition elements is an area of continuous research. However, although some applications require the use of very specific probes for each target of interest, new efforts are being focused on the application of more generic recognition approaches in combination with chemometric techniques that allow multiplexing capabilities with simpler designs.

Multianalyte sensing is not always a must but for some applications it represents a clear advantage over other detection techniques. DNA arrays and enzyme arrays, especially for clinical analysis of glucose, urea, cholesterol and lactate, are perfectly established. However protein arrays are most complex and still require further development [8].

Nanoparticles, QDs and metallic nanoparticles are being increasingly used for biosensing purposes including in vivo applications, although instrumentation is still a limiting factor in this

labelled fluorescent target derivative. Alternatively, the polymer itself can be labelled and binding can induce a change in the fluorescent properties of the probes incorporated into the polymer backbone; (ii) competitive, as in immunosensors, are based on the competition of the target and a fluorescently labelled derivative for the polymer selective binding sites. Alternatively, the MIP binding sites can be saturated with the fluorescent labelled analogue that will be displaced by the target compound.

One of the main limitations for the development of MIP sensors has been the difficulty of integrating them with the transducer element. To overcome this shortcoming, Carrasco et al. [199] reported a fiber-optic microarray platform prepared by random self-assembly of MIP microspheres into a fiber optic microwell array. The device has been applied to the analysis of enrofloxacin (ENRO), a fluoroquinolone antibiotic in sheep blood serum samples. The sensing mechanism is based on a fluorescent competitive assay between the target antibiotic and a novel highly fluorescent labelled ENRO (Fig. 9). Strong fluorescence is observed in the absence of free analyte. The limit of detection was 40 nM. The selectivity of the assay was evaluated by measuring the cross-reactivity of other fluoroquinolones and non-related antibiotics. Only ciprofloxacin yielded an 8% inhibition of labelled ENRO binding at 10 μM concentration. No response was observed for the other antimicrobials tested.
Coded particles are an alternative as well for multiplexed analysis, but a large effort is being focused on the application of magnetic coded nanoparticles that enable sample treatment and target preconcentration with the corresponding increase in the sensitivity. Easy manipulation is another advantage of the application of such nanoparticles, especially in combination with microfluidic devices. Nevertheless, there is a great concern regarding the environmental toxicity of nanomaterials that must not be overlooked.

Another area that requires future attention is related to the synthesis of new dyes, with long-term stability and brightness, especially near infrared and long lifetime luminophores, as well as materials and surface chemistries that allow higher sensitivities, lower costs and favor mass production [200]. The final goal would be the production of affordable, easy to use, customer driven fully automated biosensors.

In conclusion, although advances in this field have been impressive in the last thirty years, the future of optical biosensors based on fluorescent measurements requires a close collaboration between experts in chemistry, physics, biology, engineers, material scientists and, very important, of end-users to get devices that can facilitate our everyday life.

ConA = Concanavalin A; DES = diethylstilbestrol; EGFP = Enhanced green fluorescence protein, ERE = Estrogen response element (ERE); EW = evanescence wave; FI = fluorescence intensity; FITC = fluorescein; FLT = fluorescence

**Fig. 8.** QD-FRET assay design components. (A) The QD-FRET-based assay format used to investigate LcA enzymatic activity. (B) Peptide sequences of the LcA substrate used in this study. Colors are used to highlight the different functional moieties. Partially reproduced with permission from Ref. [185], http://dx.doi.org/10.1021/nn102997b.

**Fig. 9.** Workflow of the assay protocol. ENRO quantification was based on a competitive assay in which the target analyte competes with the labelled ENRO (BODIFLOXACIN) to bind to specific binding sites on the MIP microspheres. Reproduced with permission from Ref. [199] http://dx.doi.org/10.1039/c5sc00115c - Published by The Royal Society of Chemistry.
lifetime; GFP = Green fluorescence protein; HAI = Hospital acquired infection; MIPS: molecularly imprinted polymers; OPH = Organophosphorus hydrolase; OPs = Organophosphorus compounds; P1ac = Pathogenic bacterium; PEG = polyethylene glycol; PW = Planar Waveguide; QD = Quantum dots; RB = Recombinant bacteriophage; REACH = a non fluorescent YFP mutant (for resonance energy-accepting chromatoprotein); rHs and rHsB = recombinant human estrogen receptor and β; TC-SPC = time correlated single-photon counting; TEV = Tobacco Etch Virus; YFP = yellow fluorescent protein; BPA = bisphenol A.

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