Nanophotonic label-free biosensors for environmental monitoring

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Abstract

The field of environmental monitoring has experienced a substantial progress in the last years but still the on-site control of contaminants is an elusive problem. In addition, the growing number of pollutant sources is accompanied by an increasing need of having efficient early warning systems. Several years ago biosensor devices emerged as promising environmental monitoring tools, but their level of miniaturization and their fully operation outside the laboratory prevented their use on-site. In the last period, nanophotonic biosensors based on evanescent sensing have emerged as an outstanding choice for portable point-of-care diagnosis thanks to their capability, among others, of miniaturization, multiplexing, label-free detection and integration in lab-on-chip platforms. This review covers the most relevant nanophotonic biosensors which have been proposed (including interferometric waveguides, grating-couplers, microcavity resonators, photonic crystals and localized surface plasmon resonance sensors) and their recent application for environmental surveillance.

Highlights

- We discuss the performance of different nanophotonic biosensors.
- We discuss the advantages that nanophotonic label-free biosensors confer over standard techniques.
- We present the most recent biosensing results for environmental monitoring.

Introduction

For decades environmental pollution, caused by heedless anthropogenic and industrial activities, has been a crucial concern and has been identified as the origin of the global climate change. Despite the concern at worldwide level and the investment in remediation actions, the slow but steady degradation of water, soil and air quality, continues. Real-time and on-site monitoring of the pollution is vital to managing environmental degradation and protecting their quality for the future of our world. In order to help in the prevention of the environmental degradation, there is an imperative need of innovative monitoring tools and early warning systems.

The conventional analytical techniques, based on chromatographic and spectroscopic technologies, remain the preferred analytical methods for environmental control due to its accuracy and sensitivity. But these methods are limited to centralised laboratories, require expensive instrumentation, are time consuming and need trained personnel. To overcome the high costs and low speed of those analysis, biosensor devices emerged as a promising

alternative tool several years ago[1]. Biosensors can provide the demanded portable analytical tools and early warning systems because they are fast, specific, sensitive, reusable and enable permanent and unattended operation in the field [2].

A biosensor is a device that combines a biological or biomimetic recognition element with a transducer, to convert a specific (bio)chemical interaction into a measurable signal (see Fig. 1). A key component of the biosensor is the selected receptor that affects the specificity, response time, affinity, and lifetime of the biosensor [3]. The main biological receptors are antibodies (Abs) [4], DNA strands, aptamers or enzymes and the biomimetic ones, such as molecular imprinted polymers (MIPs). In addition, the way that the receptor was immobilized or conjugated on the transducer surface is a critical step for the efficient performance of the final biosensor device.

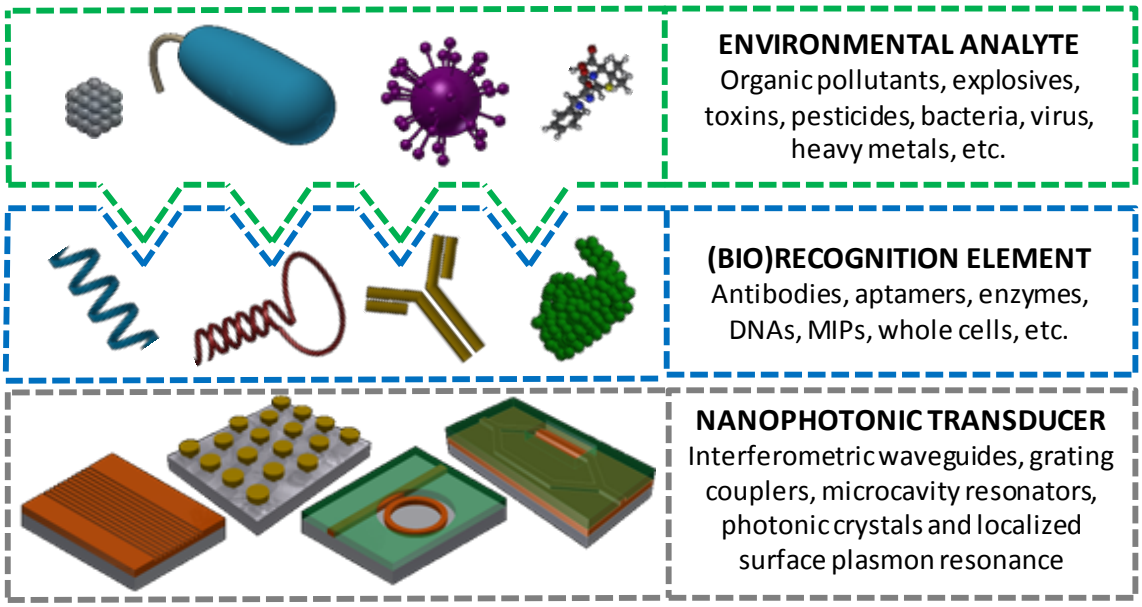

Figure 1. Schematic representation of the nanophotonic transducers and (bio)recognition elements constituting a biosensor device and the main types of environmental analytes to be detected. The (bio)recognition elements represented from left to right are: DNA/RNA strands, aptamer, antibody and enzyme. The nanophotonic transducers represented from left to right are: gratings coupler, Localized Surface Plasmon Resonance nanostructures, microcavity resonator and Mach-Zehnder interferometer.

Regarding the transducer, the most employed are based on electrochemical and optical principles. For those interested in electrochemical biosensors several reviews can be found in [5,6]. Optical transducers offer significant advantages as they can operate in a label-free scheme, avoiding the complicated labelling procedures and using fewer reagents, making the overall detection process shorter and cheaper. In addition, photonic biosensors offers other advantages, as the immunity to electromagnetic interferences, high sensitivity, wide bandwidth, and more importantly, the capacity of miniaturization and portability due to the scalable technologies employed for their fabrication [7].

Photonic biosensors take advantage of the evanescent wave detection, where the biological receptor layer is immobilized onto the core surface of a waveguide. The exposure of the functionalized surface to the complementary analyte and the subsequent biochemical interaction between them induces a local change in the optical properties of the waveguide

transducer (in particular a change in the refractive index). This change is detected via the evanescent field of the guided light, and its amplitude can be correlated to the concentration of the analyte and to the affinity constant of the interaction, yielding a quantitative value of the interaction [7]. Moreover, photonic biosensors are easily integrated in lab-on-a-chip platforms by enabling combination of both fluidic handling and optical analysis onto a single chip.

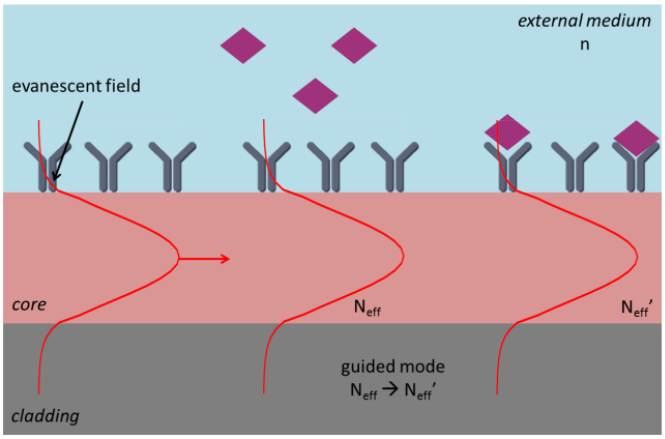

Figure 2. Evanescent wave sensing mechanism: a biomolecular interaction taking places on the surface between the biological receptor and its corresponding specific analyte is monitored through the electromagnetic evanescent field of the light traveling in the waveguide transducer. The (bio)recognition elements are represented as antibodies and the purple rectangles represent the analyte to be sensed which is contained in the sample (external medium). When the interaction takes place between the receptor and the analyte, the evanescent wave of the light traveling inside the core of the optical transducer will be affected by the increase in the refractive index on the sensor surface, therefore modifying its effective refractive index. The change in the effective refractive index can be directly correlated to the concentration of the analyte contained in the sample.

In this review we summarize the last achievements that the technology of nanophotonic label-free biosensors is bringing to the environmental monitoring, with an especial emphasis in the main results from the last two years. We will provide an overview of the main technologies and their working principle, as well their application and performance for environmental analysis of different types of pollutants even in complex matrix samples.

Nanophotonic transducers for label-free environmental monitoring

The most common optical label-free biosensor based on the evanescent wave detection principle is the Surface Plasmon Resonance (SPR) sensor [8,9], in which the variation of the reflectivity on a metallic layer in close contact with a biological media is continuously monitored. The SPR biosensor has been widely developed and commercialize. But this biosensor is difficult to miniaturise and to be converted in a portable tool, and has a limited number of channels to perform multiplexed measurements. During last years the field of optical biosensors has been fueled with more competitive nanophotonic transducers, mainly based on compact waveguide nanostructures, which will be described in the following.

Interferometric waveguide biosensors

The working principle of an interferometric biosensor relies in the creation of an interference pattern, generated by the superposition of two or more light waves [10] in a waveguide. In a

common interferometric device, the incoming light beam is split in two beams of equal intensity that travel through different optical paths (arms) defined in the waveguides. For biosensing applications, one of the arms is used as a reference while the other acts as a sensing one. The most common interferometric waveguide biosensors are the Mach-Zehnder Interferometer (MZI) and the Young Interferometer (YI). In a MZI the two waveguide arms are recombined before arriving at a detector, which collected the interferometric signal. In a YI reference and sensing arms are not recombined before the output. They are out-coupled individually and the interference pattern is generated off-chip. Recently, a new interferometric version, with a common path waveguide, called Bimodal Waveguide Interferometer (BiMW), has been introduced. In this device, the interference pattern at the output is generated by the interference of two transversal modes with the same polarization traveling in the same waveguide, rendering in a more compact and miniaturised device [7]. Interferometric devices are generally fabricated using standard microelectronics technology which allows the fabrication of compact sensors array in a miniaturised format as can be appreciated in Figure3.

MZI biosensors have been applied to the analysis of pollutants and pathogens of environmental relevance. A recent article shows a MZI immunosensor for the detection of *Listeria monocytogenes*, a pathogen found in soil, water and vegetation that can be lethal for humans. The biosensor achieves a limit of detection (LOD) of 10^5 CFU/mL, below the infection dose and shows high specificity over other pathogens [11]. Recent studies report MZI immunosensors for the detection of mycotoxins (*e.g.* aflatoxin M1 or ochratoxin A), secondary toxic metabolites produced by fungus. The detection of these mycotoxins is not only relevant for food monitoring but also for environmental control, because they are contaminants in indoor environments such as water-damaged houses. The company LioniX International (Lionix International; URL: http://www.lionixbv.nl/) has described as a proof-of-concept, an asymmetric MZI (aMZI) immunosensor for the detection of aflatoxin M1, a cancer-causing chemical regulated by the European Commission (EC No. 1881/2006). Although the LOD (3 ng/mL) needs to be improved in order to fit with the European regulation (maximum residue level (MRL) is 50 ng/L in milk) the study proves the great potential of an aMZI device as a miniaturised analytical tool for label-free detection of aflatoxin in milk [13]. Pagkali *et al.* has developed a MZI immunosensor for the detection of Ochratoxin A, one of the most-abundant food-contaminating mycotoxins, with a LOD of 0.25 ng/mL. The biosensor was applied to the analysis of Ochratoxin A directly in beer samples (LOD 2.0 ng/mL) but the same immunosensor can be applied for water quality control. Even though LC/MS–MS methods reach LOD around 0.005–0.1 ng/mL, they employ complex sample preparation procedures that are not required with the MZI sensor [14]. Another company, Optiqua Technologies (Optiqua Technologies, URL: http://www.optiqua.com/), has developed a MZI, the MiniLab™ system, for water quality monitoring. A recent article applies the MiniLab™ system for the detection of bisphenol A, a severe endocrine disruptor, in treated water [15].

One of the earliest applications using a YI immunosensor was herpes virus detection. Its performance was verified in buffer and serum, detecting a concentration of 850 particles/mL [16]. Its sensitivity is comparable to standard methods like PCR. Recently, using MIP as receptor, a proof-of-concept YI sensor was developed for the detection in pure water of melamine, known to cause kidney problems in humans and animals in the presence of cyanuric acid inside the body [17].

Under the European Commission's Seventh Framework Programme (FP7) the project "*Biosensors, Reporters and Algal Autonomous Vessels for Ocean Operation*" (BRAAVOO) is focused on the development of a device that integrates three types of biosensor platforms which, used individually or simultaneously, allow the real-time monitoring of a wide range of chemical pollutants and biological effects. One of these sensing modules are interferometric (BiMW/aMZI) nanoimmunosensors employed for the detection of several chemical pollutants, including Irgarol 1051, a commonly used antifouling paint for marine vessels with endocrine disruptors properties. Results show a LOD of 0.005 µg/L and an IC_{50} value of 0.06 µg/L for the interferometric immunosensor in sea water samples without any sample pre-treatment (Chocarro-Ruiz *et al.*, submitted).

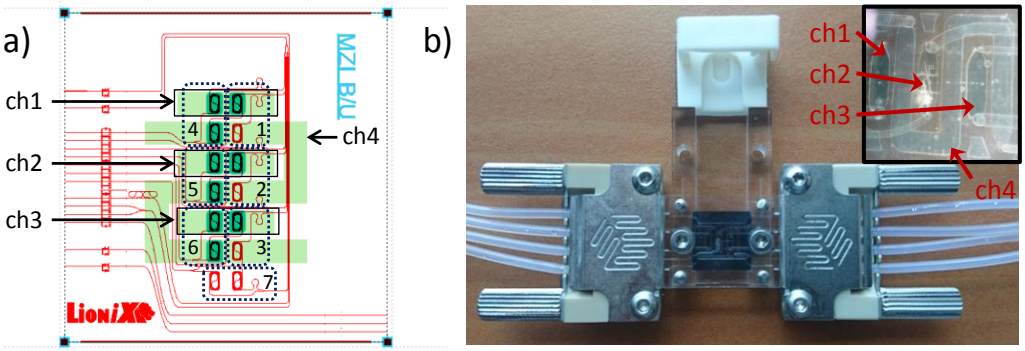

Figure 3. Example of an MZI array chip and its complementary microfluidic cell, designed by LioniX International.a) aMZI biosensor array chip scheme. The chip consists of 7 aMZIs (shown in the Fig. by dotted line regions): three of them (1-3) have a sensing window on top of the sensing arm (dark green regions), other three (4-6) have two sensing windows, one on top of each arm, and the seventh aMZI (7) is left covered by the cladding, to be used as a reference sensor. The fluidic paths in the cartridge are shown by pale green regions (ch1 – ch4); b) aMZI chip placed into a 4-channel microfluidic cell. (*Inlet: detail of the microchannels over the sensing areas*).

Table 1 summarizes the main achievements of interferometric waveguide biosensors for environmental monitoring.

Table 1. Recent results of interferometric waveguide biosensors for environmental monitoring.

Type of sensor	***Recognition element***	***Analyte***	***Matrix***	***LOD***	***Reference***
MZI	Ab	*L. monocytogenes*	Buffer	10^5 CFU/mL	[11], 2014
aMZI	Ab fragment	Aflatoxin M1	Buffer	3 ng/mL	[13], 2016
MZI	Ab	Ochratoxin A	Buffer and spiked beer samples diluted in buffer	2.0 ng/mL	[14], 2016
MZI	Ab	Bisphenol A	Spiked pure/ tap water samples	NA	[15], 2015
YI	Ab	Herpes virus	Buffer and spiked serum samples	<850 particles/mL	[16], 2007
YI	MIP	Melamine	Pure water	NA	[17], 2016

BiMW	Ab	Irgarol 1051	Spiked sea water samples	~ pg/mL	*Submitted*

Grating biosensors

A grating is a system of periodic or corrugated structures which allows the excitation of a light guided mode in a waveguide. When a specific angle of incidence of a light beam is applied on a grating, the incoupling condition is reached and the light is propagating through the waveguide. The incoupling angle is very sensitive to any perturbation in the surface of the waveguide due the interaction through the evanescent field. The company Microvacuum Ltd. (Microvacuum Ltd.; URL: http://www.owls-sensors.com/) commercializes devices based on this technology, measuring the incoupling angle of a polarized laser light, called Optical Waveguide Lightmode Spectroscopy System (OWLS).

Early work showed the performance of an OWLS immunosensor for the detection of the herbicide trifluralin, listed by de European Union as endocrine disrupter. This biosensor improves the LOD (1 fg/mL) in six orders of magnitude as compared to the enzyme-linked immunosorbent assay (ELISA) test [18]. More recent investigations are focused on the sensing of mycotoxins such as deoxynivalenol [19], and aflatoxin B1 and ochratoxin A in flour [20] or zearalenone in ground corn [21]. The biosensors show similar or improved sensitivities than standard ELISA test, and specially, the OWLS biosensor for ochratoxin A detection reaches a better LOD compared with the MZI biosensor previously described [14]. The OWLS biosensors have also been applied for the detection of vitellogenin, an egg yolk protein precursor used as biomarker of endocrine disrupting effects in carp [21,22] and amphibian species [23]. Additionally, a proof-of-concept device has been developed using biosilica expressed in genetically modified *E. coli* for the attachment onto the sensor surface. The whole cell receptor was described for the detection of penicillin G (antibiotic), chloramphenicol (antibiotic), carbofuran (pesticide) and H_2O_2 [24]. Finally, OWLS technology has also been applied for the label-free detection of *Salmonella typhimurium* [25] and *Legionella pneumophila* [26] in water, using antibodies as biorecognition elements.

A summary of the employment of grating biosensors for pollutants detections can be found in Table 2.

Table 2. Recent results of grating biosensors for environmental monitoring.

Recognition element	***Analyte***	***Matrix***	***LOD***	***Reference***
Ab	Trifluralin	Pure water, tap water and field surface water samples	1 fg/mL	[18], 2003
Ab	Deoxynivalenol	Buffer and spiked wheat flour samples (60% acetonitrile)	0.005 ng/mL	[19], 2011
Ab	Aflatoxin B1 and Ochratoxin A	Buffer and spiked barley and wheat	0.5 ng/mL	[20], 2007

		flour samples (60% acetonitrile)		
Ab	Zearalenone	Spiked ground corn samples (60% acetonitrile)	NA	[21], 2009
Ab	Vitellogenin	Carp liver diluted in buffer and serum samples	0.07 ng/mL	[22], 2013
Ab	Vitellogenin	Heart, liver and gonad samples diluted in buffer	0.1 ng/mL	[23], 2015
Whole Cell (modified *E. coli*)	Penicillin G, chloramphenicol; carbofuran and H_2O_2	Buffer	NA	[24], 2013
Ab	*S. typhimurium*	Buffer	1.3×10^3 CFU/mL	[25], 2007
Ab	*L. pneumophila*	Spiked water samples	1.3×10^4 CFU/mL	[26], 2009

Microcavity resonator biosensors

In a microcavity resonator, the light of a specific wavelength is coupled into a circular waveguide through the use of an external coupler. The confined light in the microcavity is propagated in the form of whispering gallery modes (WGM). Any perturbation on the waveguide microcavity surface taking places in the evanescent area induces a shift in the coupled light wavelength, and therefore this structure is an excellent candidate for biosensing applications [27].

The quality Q factor of the microring, which is proportional to the number of times the light circulates within the microcavity, influences notably in the sensitivity of the sensor and, therefore, high Q factors are the preferred ones [28]. This has been confirmed by the single molecule detection of influenza A virions in air [29] or single nucleic acid interactions [30] employing high Q-factors microring biosensors. Moreover, these sensors are fabricated with standard microelectronics technology and can be miniaturised even in an high multiplexed array format for multiple analysis [7].

Recently, WGM biosensors were used for the detection of heavy metals. An aptasensor for mercury (II) ion detection was developed showing high affinity for Hg^{2+} ions over eight common metal ions, reaching close ~1 ng/mL LOD. Drawbacks are some possible unspecific interactions and slower responses when the Hg^{2+} concentration decreases [31]. Another biosensor using glutathione as receptor was developed for Pb(II) detection, showing a LOD of 10 pg/mL, which is significantly lower of what was previously reported, and even in the presence of alkaline and heavy metal interferences [32].

A novel approach for transducer biofunctionalization, as a proof-of-concept for anti-dinitrophenol Ab detection, was developed by Bog *et al.* [33]. Using a polymer (PDMS) stamp, patterned by polymer pen lithography, the sensor surface was functionalized with 2,4-Dinitrophenol, a hazardous pollutant used as pesticide and in the production of sulphur dyes.

This novel functionalization approach allows multiplexing analysis as it shows the possibility to individually functionalize each cavity on a densely packed chip. Yang *et al.* developed a microcavity resonator for the detection of organophosphorus pesticide parathion-methyl, monitoring with enhanced analysis times compared to standard techniques, achieving LOD in line with the admissible levels [34].

Another important research field in environmental monitoring is the detection of biological or chemical warfare agents. A proof-of concept using a microring resonator was developed for ricin and saporin detection [35]. Bonnot et al reported a WGM sensor for the detection of dimethyl methylphosphonate (DMMP), a precursor of Sarin nerve gas in air [36].

Recent works include a WGM aptasensor for the detection of mycotoxin aflatoxin M1 [37]. Ghali *et al.* report a WGM biosensor for the detection of *Staphylococcus aureus* with a phage protein as receptor [38]. An earlier work illustrates a proof-of-concept for *E. coli* detection obtaining a high LOD (10^5 CFU/mL), probably due to a suboptimal Ab functionalization [39]. Microcavity resonator immunosensors have also been employed for virus detection [40,41]. All the above results demonstrate the great potential that this transducer offers for the detection of a wide range of hazards and its suitability using different receptors.

Table 3 summarizes the last advances reported using microcavity resonator biosensors for environmental monitoring.

Table 3. Recent results of microcavity resonator biosensors for environmental monitoring.

Recognition element	***Analyte***	***Matrix***	***LOD***	***Reference***
Aptamer	Hg^{2+}	Buffer	~1 ng/mL	[31], 2016
Glutathione	Pb^{2+}	Pure water	10 pg/mL	[32], 2014
Ab	2,4-Dinitrophenol	Buffer	NA	[33], 2014
AChE enzyme	Parathion-methyl	Buffer	10 pg/mL	[34], 2008
Ab	Ricin and saporin	Buffer	200 pM (~12 ng/mL) (ricin)	[35], 2013
Modified odorant-binding proteins	DMMP (precursor of Sarin)	Spiked gas samples	6.8 ng/mL	[36], 2014
Aptamer	Aflatoxin M1	Buffer	NA	[37], 2015
Phage protein	*S. aureus*	Buffer	5×10^6 CFU/mL	[38], 2016
Ab	*E. coli*	Buffer	10^5 CFU/mL	[39], 2008
Ab	*Bean pod mottle virus*	Buffer and real field complex leaf extracts samples diluted in buffer	10 ng/mL	[40], 2012

Ab	M13 bacteriophage	Buffer	2.3×10^3 PFU/mL	[41], 2008

Photonic crystal biosensors

A photonic crystal (PhC) is a device composed by different refractive index materials disposed as periodic nanostructures. This periodicity affects the propagation of electromagnetic waves and generates a photonic bandgap, *i.e.* range of wavelengths which are not propagated. If a defect is introduced in the nanostructure, the photonic bandgap is disturbed, thus PhC can be used as a sensor.

Recently PhCs have emerged as a promising label-free class of biosensors [42]. While still at an early stage, some articles already report the potential of these nanophotonic sensors for environmental monitoring. One of the first works reports a PhC immunosensor for detecting porcine rotavirus in partially purified water samples with similar sensitivity to ELISA assay [43]. Takahashi *et al.* developed a PhC sensor for the detection of endotoxins in pure water, based on the limulus amebocyte lysate (LAL) reaction. This biosensor could be applied for gram-negative bacteria detection as endotoxins are lipopolysaccharides present in their membrane [44].

Yan *et al.* reported a PhC immunosensor for the detection of gentamicin, an antibiotic used to treat different types of bacterial infections. The sensor exhibits specificity over vancomycin and tobramycin but it has only been tested in buffer conditions (Yan H, *et al.*, abstract in *Proceedings of CLEO (OSA)*, San Jose, CA. May 2015:STu4K.2). The most recent development is a PhC biosensor for cadmium detection, allowing the detection of cadmium-EDTA-BSA conjugate as low as 5 ng/mL (Yan H *et al.*, abstract in *SPIE BiOS,* San Francisco, CA. April 2016:972507–972507).

Table 4 summarizes last advances in PhC biosensor for environmental monitoring applications.

Table 4. Recent results of Photonic Crystal based biosensors for environmental monitoring.

Recognition element	***Analyte***	***Matrix***	***LOD***	***Reference***
Ab	Porcine rotavirus	Partially purified spiked water samples	36 FFU/mL	[43], 2009
LAL	Endotoxin	Pure water	0.0001 EU/mL	[44], 2015
Ab	Gentamicin	Buffer	<0.1ng/mL	2015[a]
Ab	Cadmium-chelate conjugate	NA	5 ng/mL	2016[b]

[a]Yan H *et al.*, abstract in *Proceedings of CLEO (Optical Society of America)*, San Jose, CA. May 2015:STu4K.2

[b]Yan H *et al.*, abstract in *SPIE BiOS* San Francisco, CA. April 2016:972507–972507

Localized surface plasmon resonance biosensors

The interaction of light waves with metal nanostructures, smaller than the incident wavelength, generates a resonance phenomenon called Localized Surface Plasmon Resonance (LSPR). The LSPR biosensors are considered as the next generation of SPR sensing platforms. Also, they show great potential for integration and miniaturization, as well as, high sensitivity and multiplexed capabilities [45,46].

A recent publication illustrates the development of a fibre optic LSPR sensor using MIP as receptor for the detection of 2,4,6-trinitrotoluene (TNT), a nitroaromatic explosive. The performance of this sensor is not optimal since the LOD achieved was 0.16 µg/mL, well above the limit proposed by the Environmental Protection Agency (EPA) (2.0 ng/mL in drinking water). Further improvements should be required to obtain a competitive device for environmental applications but outstands as one of the few biosensors employing MIPs as receptors [47]. Kawaguchi *et al.* developed a LSPR immunosensor showing better LOD (10 pg/mL) for TNT detection [48]. Another MIP LSPR sensor recently was developed for the detection of tetracycline, a common antibiotic used in marine aquaculture. The sensor was fabricated by immobilizing silver nanoparticles over an unclad core of a multimode optical fibre, achieving a LOD of 1 ng/mL in Milli-Q water [49], the lowest compared to other approaches reported in the literature. However, silver has the disadvantage of being chemically unstable and the performance when working with real samples needs to be evaluated [50]. Another common pharmacologically active substance to be found in various environmental waters is stanozolol, a synthetic anabolic steroid. A LSPR immunosensor for stanozolol detection was developed achieving a LOD of 0.7 ng/mL [51]. This one is comparable to a conventional SPR, but above the LOD (0.25 pg/mL) achieved with liquid chromatography tandem mass spectrometry in aqueous matrix (LC-MS/MS) [52]. 5-fluorouracil, an anti-neoplastic drug used in cancer therapy, is an emerging pollutant in aquatic environments [53]. To detect 5-fluorouracil, a LSPR immunosensor was developed reaching a LOD of 10 ng/mL, similar to the ELISA test[54]. LSPR sensors for pesticide detection like paraoxon, using acetylcholinesterase enzyme as receptor [55], or an atrazine immunosensor [56] have also been developed.

Several articles have focused on the development of LSPR sensors for virus detection, including small enveloped RNA viruses (vesicular stomatitis virus (VSV) and pseudo typed Ebola), or large enveloped DNA viruses (vaccinia virus) directly from biological media [57]. A LSPR sensor for the detection of VSV, which can infect cattle or horses, shows a LOD of $<10^5$ PFU/mL from biological media (cell growth medium +7% fetal calf serum). Other immunosensors were developed for the detection of influenza virus [58], dengue virus [59] and orchid viruses [60].

The extracellular adherence protein (EAP), found on the outer surface of the bacterium *S. aureus,* was detected using a LSPR immunosensor, with a LOD of 8 pM (~0.45 ng/mL) [61]. Zhu *et al.* developed a LSPR Au–Ag nanoparticles sensor for the detection of *S. aureus* enterotoxin B using a direct immunoassay. A concentration of 0.1 ng/mL is already too low to be directly detected due to the low amplitude of spectrum [62]. Finally, a LSPR biosensor was developed for the multiplex detection of a range of pathogenic bacteria (*Vibrio vulnificus, Salmonella* spp., *Staphylococcus aureus, Enterococcus faecalis*, *Neisseria gonorrhoea, Staphylococcus epidermidis* and *Klebsiella oxytoca*) using DNA probes as receptors [63].

Table 5 provides some representative LSPR biosensors for pollutants detection.

Table 5. Recent LSPR biosensors for environmental monitoring.

Recognition element	***Analyte***	***Matrix***	***LOD***	***Reference***
MIP	TNT	Pure water	0.16 µg/mL	[47], 2015
Ab	TNT	Buffer	10 pg/mL	[48], 2008
MIP	Tetracycline	Pure water	1 ng/mL	[49], 2015
Ab	Stanozolol	Buffer	0.7 ng/mL	[51], 2008
Ab	5-fluorouracil	NA	10 ng/mL	[54], 2010
Enzyme	Paraoxon	Buffer	0.234 ng/mL	[55], 2006
Ab	Atrazine	Buffer	10 ng/mL	[56], 2014
Ab	VSV, pseudo typed Ebola and vaccinia virus	Buffer and spiked biological media	$<10^5$ PFU/mL	[57], 2010
Ab	Influenza virus	Buffer	1 pg/mL	[58], 2012
Ab	Dengue virus	Buffer	NA	[59], 2013
Ab	*Cymbidium mosaic virus* and *Odontoglossum ringspot virus*	Buffer and real field plant crude saps samples diluted in buffer	48 pg/mL and 42 pg/mL respectively	[60], 2014
Ab	EAP from *S. Aureus*	Buffer	8 pM (~0.45 ng/mL)	[61], 2009
Ab	*S. aureus* enterotoxin B	Buffer	NA	[62], 2009
DNA	Pathogenic bacteria DNA	Pure water	10 fM	[63], 2011

Conclusions and future perspectives

Nanophotonic biosensors based on evanescent wave detection can offer label-free, real-time, sensitive, selective, multiplex, rapid, and inexpensive analyses; the main five technologies discussed in this review (interferometric sensors, grating couplers, microcavity resonators, photonic crystal based sensors and localised surface plasmon resonance) have advantages and disadvantages, and depending on the final application one can be more suitable than another. The main differences between all of them rely in terms of sensitivity and their ability for integration in compact platforms. Sensitivity for label-free detection in real samples is a must and one of the most suitable ones are the interferometric sensors, which have shown outstanding levels of sensitivity. The photonic crystal and the LSPR sensors have shown the most limited sensitivity for a direct and one-step label-free detection. Grating couplers and microcavity sensors exhibit an intermediate level of sensitivity. On the other hand, the fabrication of LSPR and grating couplers sensors is usually less complex and cheaper, while the fabrication of interferometric, microcavity and photonic crystal sensors is more complex, requiring dedicated foundries. However, they have the additional advantage of providing microchip arrays with multiplex sensors of identical performances.

The level of specificity, selectivity and accuracy which can be achieved with the photonic

biosensor technology for environmental applications is mainly related to the bioreceptor employed and the biofunctionalization protocol, rather than due to the transducer itself. Key factors are the ability of getting a complete specific bioreceptor against the pollutant or toxin to be detected, to employ a biofunctionalization protocol which preserves the activity and the functionality of the bioreceptor once attached to the sensor surface, and to ensure antifouling properties of the bioreactor layer for detecting directly in real matrices. Although many of the applications for environmental monitoring have been demonstrated in buffer conditions, the biosensor technology shows great performance in terms of selectivity and sensitivity and some have been successfully applied to spiked samples with excellent performance and accuracy. Taking into account the strong development in antifouling biofunctionalization techniques for biosensor surfaces, there is no doubts that the technology could be employed for real sample evaluation in the near future.

During last years photonic biosensors have shown its capabilities for multiplexing and miniaturisation in compact platforms as laboratory proof-of-concept prototypes; there is no doubt that this novel nanophotonic technology soon will surpass the laboratory stage and will hit the market. This biosensor market was valued at $11.39 Billion in 2013 and is forecast to double by 2020 significantly driven by the growing need for environmental surveillance and the emergence of nanobiosensors (Markets and Markets, URL: http://www.marketsandmarkets.com/Market-Reports/biosensors-market-798.html). Moreover, due the fast growing of mobile technology as tablets and smartphones, probably in the near future a nanophotonic label-free biosensor can be integrated within these gadgets. Thus, such tools for portable analysis and early warning systems in environmental monitoring could be distributed around the world, helping to control the serious problem of the environmental pollution.

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