New frontiers in optical biosensing

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Abstract: *The technology of optical biosensors has reached a high degree of maturity and several commercial products are on the market. But problems of stability, sensitivity and size have prevented the general use of optical biosensors for real field applications. Our last developments to solve such drawbacks will be showed, mainly related to the development of portable and highly sensitive photonic sensing platforms.*

Introduction

The progressive demand for the rapid and precise detection of any type of substances has speed-up the development of a large variety of biosensors. For most of the applications it is desirable to have a compact biosensor of high sensitivity, fast response and able to perform real-time analysis. These requirements can be achieved mainly with optical sensors, due to the own nature of the optical measurements that endow a great number of different techniques as emission, absorption, fluorescence, refractometry or polarimetry[1]. Among them, photonic biosensors based on evanescent wave detection have demonstrated its outstanding properties as an extreme high sensitivity method for the direct measurement of biomolecular interactions in real time and in label-free schemes[2].

In the evanescent wave detection, a receptor layer is immobilized onto the waveguide; the exposure of such a surface to the partner analyte molecules produces a biochemical interaction, which induces a change in its optical properties. This change is detected by the evanescent wave. The extent of the optical change will depend on the concentration of the analyte and on the affinity constant of the interaction, obtaining a quantitative signal of the interaction. The evanescent wave decays exponentially as it penetrates the outer medium and, therefore, only detects changes taking place on the surface of the waveguide. For that reason, it is not necessary to carry out a prior separation of non-specific components (as in conventional analysis) because any change in the bulk solution will hardly affect the sensor response. In this way, evanescent wave sensors are selective and sensitive devices for the detection of very low levels of chemicals and biological substances and for the measurement of molecular interactions in-situ and in real time[3].

The advantages of the optical sensing are significantly improved when this approach is used within an integrated optics context[4]. Integrated optics technology allows the integration of passive and active optical components (including fibres, emitters, detectors, waveguides and related devices) onto the same substrate, allowing the flexible development of miniaturised compact sensing devices, with the additional possibility to fabricate multiple sensors on a single chip. The integration offers additional advantages such as miniaturization, robustness, reliability, potential for mass production with consequent reduction of production costs, low energy consumption and simplicity in the alignment of the individual optical elements[4].

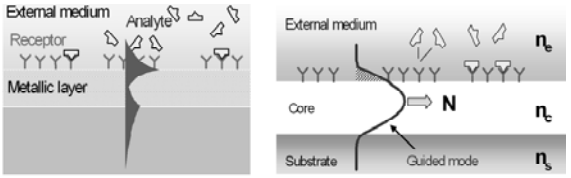

Fig. 1. Evanescent field sensors. Evanescent field detection of biomolecules with (a) SPR sensor and (b) planar waveguides sensors.

Nowadays most of the optical biosensors are based on optical fibers, Surface Plasmon Resonance (SPR) or integrated optics (as interferometers, grating couplers, etc..). Table I details most of the existing optical biosensors.

Table 1. OPTICAL BIOSENSORS

Optical Fibers
Surface Plasmon Resonance Sensors (SPR)
Integrated Interferometers (Mach-Zehnder, Young, Michelson, difference)
Resonant Mirror
Grating Couplers
Ring Resonators, Fabry-Perot Resonators
Photonic Crystals
Localised Surface Plasmon Resonance

With the emergence of nanotechnology new photonic structures has been suggested. A scaling down of the photonic biosensors towards nanometer-size structures could allow a significant increase of their sensitivity[5]. Several transducers as photonic crystals[6], ring resonators[7], porous silicon[8] or nanoparticles for localised surface plasmon resonance (LSPR)[9,10] has been proposed. But the biosensing demonstration using such structures is scarce and their experimental sensitivity is worse than the sensitivity showed by the standard optical biosensors (as surface plasmon resonance or interferometers).

In the following, we will give an overview of the most prominent developments in optical biosensors and the future lines of research which are being investigating in our laboratory.

Plasmonics based biosensors

SPR biosensing has focused a strong effort since the technique was first applied in 1983 and has become one of the most successful label-free and commercially accepted optical biosensor[11]. This technique has been employed in biomolecular engineering, drug design, monoclonal antibody characterization, epitope mapping, phage display libraries, virus-protein interaction, environmental pollutants detection, among other interesting problems[2].

SPR is the predominant optical technology and besides the new developments is SPR, the focus is pointed out in the applications. We will show the applicability of our SPR device in the following fields:

(1) Our sensor is the first SPR-based immunosensor applied to the detection and validation of three relevant pesticides (DDT, chlorpyrifos and carbaryl) with higher sensitivity than other immunochemical systems (e.g. ELISA)[12,13].

Fig 2. Portable SPR platform, including sensor, optics, electronics and flow delivery system.

Measurements of the pollutants are carried out using inhibition binding assays with sensitivity at ppt level. Although the SPR system has only two channels, we have implemented a new procedure for simultaneous multi-analyte determination. Environmental real water samples has been analysed and we have demonstrated that our level of detection accomplishes the EU legislation[14].

(2) The SPR sensor can also be used as an on-line immunoanalytical method for the evaluation of pesticide metabolites which can be present in the human body. We have detected the metabolite from chlorpyrifos pesticide (3,5,6-trichloro-2-Pyridinol (TCP)) from its primary via of elimination (urine). The immunoassay format allows a highly sensitive detection of TCP in human urine without the need of previous clean-up and preparation of samples. The comparison between TCP limits of detection in urine and assay buffer used as control showed similar sensitivity values and a limit of detection of 0.1 μg L^{-1} was obtained for TCP urinary determinations[15].

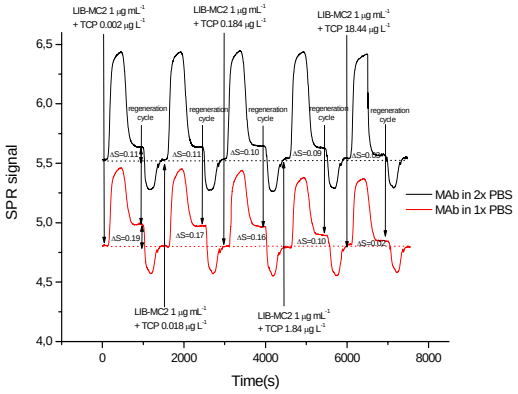

Fig 3. SPR detection of TCP in human urine. A regeneration cycle is applied after each determination. A limit of detection of 0.1 μg L^{-1} was obtained.

(3) The SPR technique has been also applied to the real-time and label-free detection of DNA hybridization and single point mutations. We have studied the mutations at gene BRCA-1 related to the predisposition in women to develop an inherited breast cancer. The SPR sensor has successfully discriminated between normal and mutant sequences with a limit of detection of only 100 nM. The device could be employed for the analysis of point mutations in the DNA samples of patients.

For many applications, improvement in the sensitivity of the SPR sensors is an important issue, and several alternative configurations have been suggested as Localised Surface Plasmon Resonance (LSPR) based on nanoparticles or Magnetooptical Surface Plasmon Resonance sensor (MOSPR).

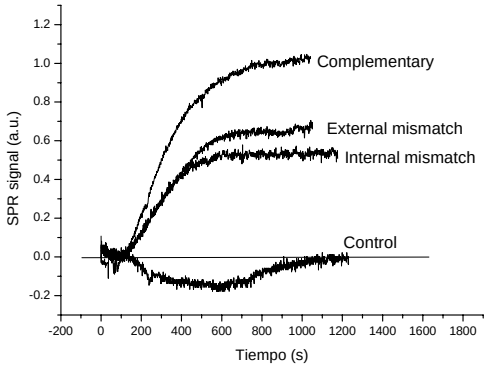

Fig.4. SPR direct discrimination between fully complementary and mismatches DNA probes. It can be observed: (i) hybridisation of fully complementary DNA strand to the immobilised probe (ii) hybridisation of DNA strand with an internal mismatch (iii) hybridisation of DNA strand with an external mismatch (iv) control signal (no hybridisation).

Magnetooptical Surface Plasmon Resonance Sensor

We have recently developed a novel Magnetooptical Surface Plasmon Resonance (MOSPR) Sensor which can improve the sensitivity of the conventional Surface Plasmon Resonance (SPR) sensors[16]. This MOSPR sensor arises from the combination of the Surface Plasmon Resonance in thin metallic layers and the magneto-optic (MO) activity of ferromagnetic metallic materials. Such combination generates a large enhancement of the MO effects closely localized at the surface plasmon resonance.
The sensor uses Co/Au multilayers of nanometric thicknesses as transducers, a prism-coupling configuration and p-polarized light to excite the surface plasmon, rotating magnets or magnetic coils to apply a modulating magnetic field, and detects the magnetooptic effects of the reflected light as a function of the angle of incidence. A large and sharp enhancement of the MO effect is produced at the angle of incidence in which SPR is excited and, likewise the conventional SPR sensors, the enhancement shifts to higher (lower) angles when the refraction index of the adjacent dielectric medium increases (decreases). Therefore, the local variations of refractive index produced within the evanescent field of the surface plasmon can be detected measuring, in real time, the changes of the MO effects of the reflected light.
The experimental characterization of the MOSPR sensor has shown an increase in the limit of detection in a factor of three in changes of refractive index and in the adsorption of biomolecules as compared to the standard SPR sensors. An improvement of the limit of detection up to one order of magnitude could be achieved by an adequate combination of the magnetic-metallic layers and by decreasing the noise of the experimental set-up.
In a further innovation, the thin layer of the magneto-optic transducer could be composed by magnetic nanostructures (2D or 3D) embedded in a noble metal matrix. The incorporation of magneto-optical elements tailored on the nanometer scale into the nanooptical structures can even improve the sensitivity limit due to the localisation of the magnetoplasmonic effect.

Integrated Optics based biosensors

Evanescent wave photonic biosensor devices based on standard microelectronics and related micro/nanotechnologies are providing an integrated technological solution for achieving high sensitive arrays of biosensing devices. But problems of stability, sensitivity and size have prevented the general use of integrated optical biosensors for real field applications.
In order to solve the above drawbacks, we work on the development of ultrasensitive and miniaturised photonic silicon sensors able to be integrated in a "lab-on-a-chip" microsystem platform. As sensors we use integrated Mach-Zehnder interferometer based on TIR waveguides ($Si/SiO_2/Si_3N_4$) of micro/nanodimensions[17] as it is shown on figure 5.

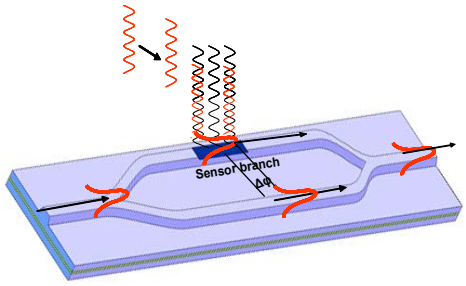

Fig 5. Mach- Zehnder interferometer configuration

For biosensing applications, the optical waveguides of the MZI should have a high surface sensitivity and single mode behaviour. The highest surface sensitivity is obtained in waveguides with a high contrast of refractive index between the core and the substrate [10]. For that reason, we have chosen Si_3N_4 core layers (n_c = 2.00) over a SiO_2 substrate (n_s = 1.46). In this waveguide configuration (and for λ in the visible range), the single mode behaviour is obtained for core thickness below 300 nm, and rib depths below 5 nm, when the rib width is 4 μm. Our modelling shows that the maximum surface sensitivity is obtained for core thicknesses about 150 nm and 75 nm for the TM and TE polarizations, respectively[17]. Figure 6 shows a scheme of the waveguide configuration.

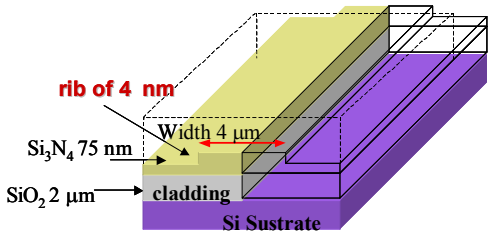

Fig.6. Cross-section scheme of the TIR waveguides employed in the integrated MZI device

The devices are fabricated in our Clean Room facilities. The evaluation was performed in an optical bench where polarized light from a He-Ne laser (λ=0.633 μm) was end-fire coupled to the sensor. The light coming out the interferometer is collected by a single-mode fiber, which is pigtailed to a photodiode. The signal is amplified, digitalized and processed. Precise translation stages are used for the accurate alignment of all the components.
Propagation losses of the waveguides were measured by the Fabry-Perot resonance technique and optical coupling losses were calculated by the cut-back method. Propagation losses vary between 0.13 and 0.15 dB/cm for TE polarization and between 0.27 and 0.30 dB/cm for TM polarization. The fiber-waveguide insertion loss measurements were 5.84 dB

for TM polarization and 3.12 dB for TE polarization and low losses were assured with couplers designed with circular arms of R = 80 mm. The final device has a length of 3 cm and the sensor area is 1.5 cm long and 50 μm wide.

The sensitivity of the sensor for both polarizations was evaluated in the same way as in previous reports[17]. The evaluation was done flowing solutions of water and ethanol of varying concentration (refractive index steps of 10^{-3}), and measuring the output signal in real time. With these measurements, a calibrating curve was obtained. The standard deviations (σ_v) and thermal drifts of the experimental set-up were evaluated in maximum condition of stability and considering the case in which the interferometric devices were located in the quadrature point. Under these conditions, the lowest detection limit in the phase change was calculated assuming the $3\cdot\sigma_v$ criterion, resulting in a value of $\Delta\varphi=1.6x10^{-4}x2\pi$ rads for TM polarization. Taking account the signal-to-noise ratio of this configuration, the lowest detection limit in the variation of the refractive index was found to be (for TM polarization) $\Delta n_{0,\,min} = 1 \cdot 10^{-7}$ ($\Delta N_{eff,\,min} = 6.4 \cdot 10^{-8}$).

As a proof of the utility of MZI technology towards the detection of ultrasensitive biomolecular interaction, we have applied the sensor in the Genomics field. The MZI device has been used for the direct detection of DNA hybridization and for the detection of single nucleotide polymorphisms at the BRCA-1 gene, involved in breast cancer development, without target labeling.

The oligonucleotide probe is immobilized by covalent attachment to the sensor surface through silanization procedures. A silane (3-mercaptopropyltrimethoxysilane) with a thiol group at the free end was employed for the chemical modification of the surface. The thiol-derivatized oligonucleotides (28 mer) used as receptors can bind to the silanized Si_3N_4 surface through a disulphide bond. The DNA probe has also a 15-T tail which is employed as a vertical spacer chain to increase the accessibility to the complementary DNA to the sensor surface.

After DNA immobilization, complementary oligonucleotides (58 mer) were flowing in the sensor for hybridization experiments. The hybridization was performed for different DNA target concentrations from 1 pM to 1 μM. Non-complementary oligonucleotides did not show any significant signal. Regeneration after each hybridization was achieved flowing HCl 3.2 mM.

The calibration curve can be observed in Figure 7. 10 pM of complementary non–labeled DNA in buffer solution was the lowest hybridization limit achieved and means an average DNA growth layer of $1 \cdot 10^{-4}$ nm and an estimation of $2 \cdot 10^{5}$ DNA molecules/cm^2 hybridized on the sensor area of the MZI.

Additionally, we have detected the hybridization of 100 nM DNA target with two mismatching bases corresponding to a mutation of the BRCA-1 gene.

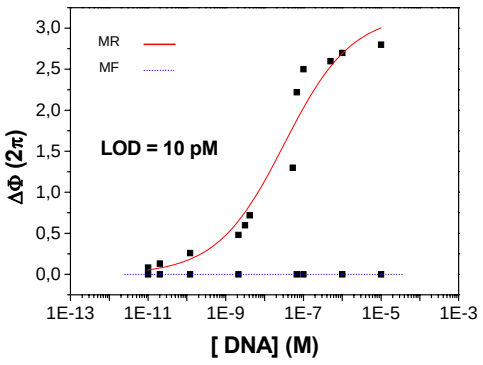

Fig. 7. Calibration curve of the DNA hybridisation evaluated in real-time by the MZI device.

Most of the studies related to the detection of DNA hybridizations in real-time and label free with optical biosensors have been performed using SPR biosensors. The limit of detection of the hybridization with SPR is above 10 nM, three orders of magnitude lower from the MZI hybridization detection limit.

Lab-on-a-chip integration

For the development of a complete lab-on-a-chip microsystem device based on integrated MZI, several units must be incorporated on the same platform: (i) the micro/nanodevices, (ii) the flow cells and the flow delivery system, (iii) a phase modulation system to convert the periodic interferometric signals in direct phase measurements, (iv) integration of the light sources and the photodetectors (v) CMOS processing electronics. For achieving this goal, our first step has been the development of a novel low temperature (100 ºC) CMOS compatible microfluidic technology to create 3D embedded interconnected microfluidic channels made of polymer SU-8[18]. The microfluidic channels have a height from 40 to 60 μm and a width between 100 to 250 μm. In Figure 8 a photograph of the cross section of the channels onto the sensing area of a MZI device can be observed[18].

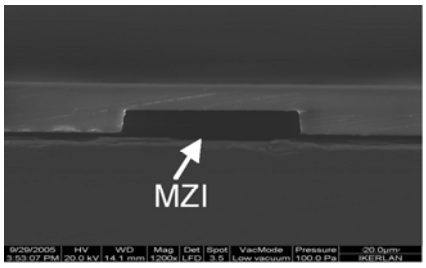

Fig. 8. SEM photograph of the cross section of the polymer microfluidic channel over the MZI sensing area.

Nanomechanical optical biosensors

Microcantilever biosensors came from the microcantilevers used in atomic force microscopy. The principle of their operation is based on the bending induced in the cantilever when a biomolecular interaction takes place on one of its surfaces. In this way, microcantilevers translate the molecular reaction into a nanomechanical motion, which is commonly detected using optical or piezoresistive read-out[19] (see Figure 9). In order to achieve highly integrated microsystem with microcantilever transducers, we have recently introduced a new type of read-out technique. The technique is represented by a new generic sensing probe based on microcantilevers acting as optical waveguides operated in visible range[20,21]. The principle of operation is based on the sensitivity of energy transfer between two butt coupled waveguides to their misalignment with respect to each other as it is represented in Figure 10.

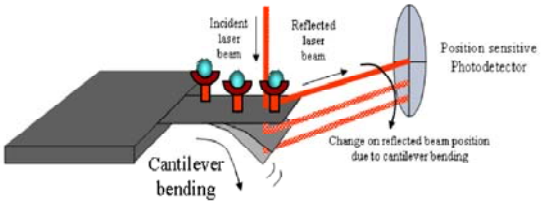

Fig. 9. Principle of working of nanomechanical biosensors with an optical read-out.

This new technique can be considered as an alternative to the known optical methods used for read-out of nanomechanical response of microcantilevers. With the proposed method, sub-nanometer displacement of cantilever free end can be registered with a conventional photodetector. Real-time parallel monitoring of several channels can be realized because no preliminary alignment or adjustment, except for light coupling, is required.

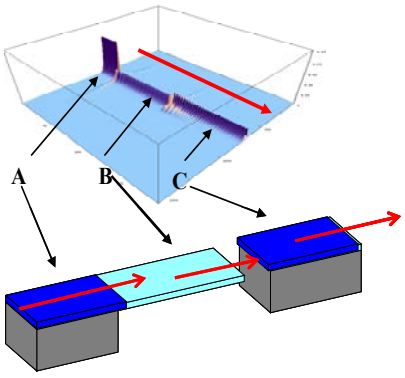

Fig. 10. Principle of working of the waveguided microcantilever sensor and theoretical modelling.

The design of the sensor is shown in Fig. 10 and can be operated both in visible and in infrared ranges. The cantilever is located in a cavity and is a symmetrical waveguide butt coupled with an output waveguide, called receptor, through a short gap.

We have fabricated arrays of 20 optical microcantilevers. Each of them is 200 μm long, 40 μm wide, and 500 nm thick with a spring constant of 0.050 N/m. Fabrication of the sensor was done using standard microelectronic technology. The cantilevers are made of thermal silicon dioxide, transparent in visible range. Input and output waveguides are made of silicon nitride and were 140 nm thick. Coupling of the light in the cantilever is achieved through the evanescent filed of the input waveguide. Both the cantilevers and the waveguides were 40 μm wide. The cantilever has low stress gradient and is practically flat, the misalignment between the output waveguide and the cantilever free end is around 1 μm. Some photographs of the fabricated devices are shown in Figure 11.

The experimental characterization has demonstrated that a subnanometer resolution in the cantilever deflection can be achieved. This new device has shown good performances for biosensing and offers an interesting approach for further integration in lab-on-a-chip microsystems.

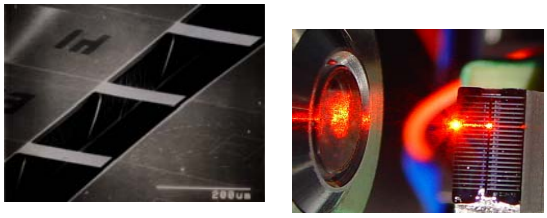

Fig. 11. Photographs of: *(left)* the optical cantilevers and *(right)* coupling of the light inside the device.

Conclusions

The technology of optical biosensors is a powerful one for the high sensitive, label-free and real-time detection of biomolecular interactions. Our last developments in this field, mainly related to the development of portable and highly sensitive photonic sensing platforms, have been shown. Plasmonics and integrated optics approaches look as the most promising ones for achieving devices which could fulfil most of the requirements in order to be transfer in commercial products in the near future.

Acknowledgments

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