Surface Plasmon Resonance Biosensors for highly sensitive detection in real samples

B. Sepúlveda, L.G. Carrascosa, D. Regatos, M.A.. Otte, D. Fariña and L. M. Lechuga*
Nanobiosensors and Molecular Nanobiophysics Group
Research Center on Nanoscience and Nanotechnology (CIN2: CSIC-ICN) and CIBER-BBN
Bellaterra, Barcelona

ABSTRACT

In this work we summarize the main results obtained with the portable surface plasmon resonance (SPR) device developed in our group (commercialised by SENSIA, SL, Spain), highlighting its applicability for the real-time detection of extremely low concentrations of toxic pesticides in environmental water samples. In addition, we show applications in clinical diagnosis as, on the one hand, the real-time and label-free detection of DNA hybridization and single point mutations at the gene BRCA-1, related to the predisposition in women to develop an inherited breast cancer and, on the other hand, the analysis of protein biomarkers in biological samples (urine, serum) for early detection of diseases.
Despite the large number of applications already proven, the SPR technology has two main drawbacks: (i) not enough sensitivity for some specific applications (where pM-fM or single-molecule detection are needed) (ii) low multiplexing capabilities. In order solve such drawbacks, we work in several alternative configurations as the Magneto-optical Surface Plasmon Resonance sensor (MOSPR) based on a combination of magnetooptical and ferromagnetic materials, to improve the SPR sensitivity, or the Localized Surface Plasmon Resonance (LSPR) based on nanostructures (nanoparticles, nanoholes,…), for higher multiplexing capabilities.

Keywords: surface plasmon resonance, DNA detection, protein biosensing, magneto-optic SPR, localized surface plasmon resonance

1. INTRODUCTION

Surface Plasmon Resonance (SPR) sensing has become one of the most versatile techniques leading to the most successful label-free and commercially accepted optical biosensor. The SPR is an optical phenomenon generated by charge density oscillations at the interface of a metal and a dielectric. Such collective oscillation of the electron gas of the metal creates very intense and highly confined electromagnetic fields at the interface of both media. The SPR is responsible for the attenuated total reflection in thin metal films, diffraction anomalies in metal gratings or the bright colors of metallic colloids, among other effects. These effects have attracted the attention of scientists in the last decades, leading to a deeper comprehension of the light/metal interaction.

Depending on the propagating or localize nature of the surface plasmon resonance, we can distinguish two different groups of SPR sensors. The first group comprises the sensors based on propagating surface plasmons in thin metallic layers and gratings, so called Surface Plasmon Polaritons (SPPs). The second group includes the sensors that make use of metal nanostructures. Similarly to flat metal films and gratings, metal nanostructures exhibit charge density oscillations giving rise to very intense and confined electromagnetic fields. These excitations are often referred to as Localized Surface Plasmon Resonances (LSPRs).

Biosensing II, edited by Manijeh Razeghi, Hooman Mohseni, Proc. of SPIE Vol. 7397,
73970Y · © 2009 SPIE · CCC code: 0277-786X/09/$18 · doi: 10.1117/12.827062

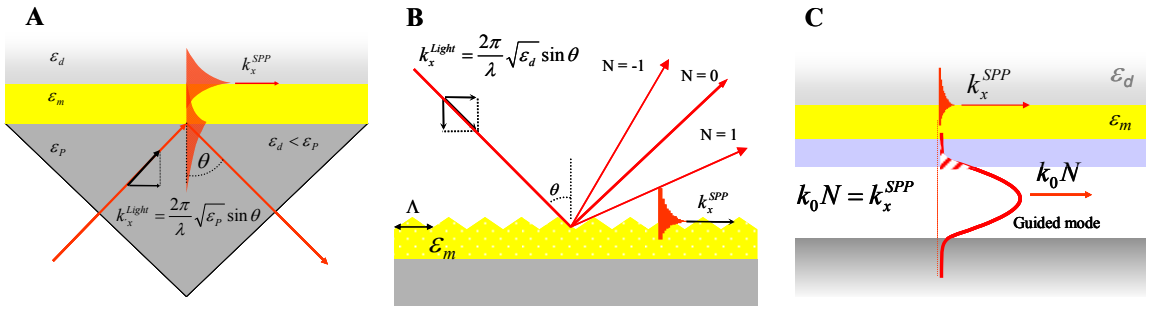

Fig. 1. SPP coupling methods. (A) Prism coupling. (B) Grating coupling. (C) Guided light in optical fibers and waveguides

The surface plasmon polariton is a surface charge wave that propagates at the interface of a metal and a dielectric. This surface wave generates a transversal magnetic electromagnetic field that has maximum amplitude at the interface and an exponential decay in both media. These exponentially decaying fields are also called evanescent fields. The wavevector of the SPP is given by the following expression when the metal and dielectric are semi-infinite:

$$k_{SPP} = \frac{2\pi}{\lambda}\sqrt{\frac{\varepsilon_m(\lambda)\varepsilon_d}{\varepsilon_m(\lambda)+\varepsilon_d}} \tag{1}$$

where λ is the light wavelength in vacuum, and ε_m and ε_d are the dielectric constants of the metal and the dielectric, respectively. The wavevector of the SPPs is always larger than that of free propagating light in the dielectric, which is expressed by:

$$k_{Light} = \frac{2\pi}{\lambda}\sqrt{\varepsilon_d}\sin\theta \tag{2}$$

where θ is the angle of incidence. As a consequence, it is not possible to excite the SPP with light falling directly in the metal for any angle of incidence. The most common and simplest method to excite the SPP is the prism coupling. This method makes use of a prism of high refractive index to excite the SPP mode of a thin metal layer, as Fig. 1A illustrates. In this configuration the following wavevector coupling condition can be satisfied:

$$k_{SPP} = \frac{2\pi}{\lambda}\sqrt{\varepsilon_P}\sin\theta \tag{3}$$

where ε_p is the dielectric constant of the prism. From this equation we can observe that there are two variables to match k_{SPP}, the angle of incidence and the wavelength. Accordingly, the surface plasmon resonance can be detected as a sharp dip in the reflectivity as a function of the angle on incidence (at a fixed wavelength) or in the wavelength spectrum (at a fixed angle of incidence). The wavevector condition can also be matched by patterning a diffraction grating in the metal surface (Fig. 1 B):

$$k_{SPP} = \frac{2\pi}{\lambda}\sqrt{\varepsilon_d}\sin\theta + \mathrm{P}\frac{2\pi}{\Lambda} \tag{4}$$

where Λ is the spatial period of the grating and P the diffraction order. In addition, the SPP can be excited thought the evanescent field of the guided light within an optical waveguide (Fig. 1 C). In this configuration, the SPP is launched when the effective propagation index of the guided mode (N) fits that of the SPP.

The biosensing application of the SPPs is based on: i) the dependence of the SPP wavevector on the refractive index of the dielectric (Eq. 1), ii) the penetration of the evanescent field of the SPP in the dielectric, which is around 100 nm for wavelengths in the visible. Thus, a biochemical interaction at the metal surface induces a local variation of refractive index that changes the wavevector of the SPP. In the prism coupling configuration, the change in k_{SPP} can be detected as a shift of the reflectivity curve. When the refractive index of the dielectric medium increases (decreases) the dip in the reflectivity shifts to higher (lower) angles of incidence or wavelengths. The quantification of the shift provides the way to detect biomolecular interactions.

Using this physical principle, we have developed a fully-automated plasmonic biosensor, as label-free sensing platform (Sensia SL. Spain [1]). The device has two independent flow cells of 300 nL each and integrates the control of pumps and valves for sample injection (see Fig. 2). The system is portable and incorporates specific software for data acquisition and instrument control.

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

2. SPR DETECTION OF POLLUTANT METABOLITES

Our sensor has been the first SPR-based immunosensor applied to the detection and validation of three relevant pesticides (DDT, chlorpyrifos and carbaryl) at the concentration level fixed by the Euroepan Union legislation (maximum concentration 0.1 μg/L) and with higher sensitivity than other immunochemical systems (e.g. ELISA colorimetric formats). For DDT the limit of detection was 0.02 μg/L, for carbaryl 0.9 μg/L and for chlorpyrifos 0.05 μg/L, respectively. The analysis of the pollutants can even be performed in a multi-assay format, via the co-immobilization of the receptors is the same sensing surface, allowing the detection of several pollutant with the same sensor chip [6, 7], keeping similar limits of detection.

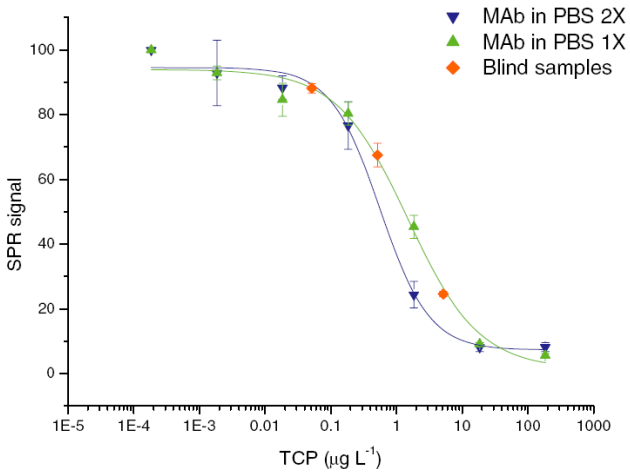

Fig. 3. Calibration curves for TCP spiked urine samples using PBS 1× and PBS 2× as buffer for the antibodies (MAb)

To prove the utility of the SPR technology for human health analysis, we have demonstrated the on-line SPR immunodetection of a pesticide metabolite which can be present in the human body. The metabolite from the pesticide

chlorpyrifos(3,5,6-trichloro-2-Pyridinol (TCP)) has been detected from its primary via of elimination (urine) [2] [3]. The assay is based on the use of a specific monoclonal antibodie which recognize the metabolite using competitive inhibition immunoassays and achieving detection levels in the low $\mu g \cdot L^{-1}$ range (see Fig. 3). Moreover, the methodology allows a highly sensitive detection of TCP directly in human urine without the need of previous clean-up and preparation of the sample. 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 $\mu g \cdot L^{-1}$ was obtained for TCP urinary determinations (see Fig. 3).

Interestingly, the reproducibility and robustness of the protocol was analyzed throughout more than 130 regeneration cycles (see Fig. 4), which allowed the repeated use of the same immunosensor surface without significant variation of the SPR signal. All measurements cycles were done in real-time in only 10 min.

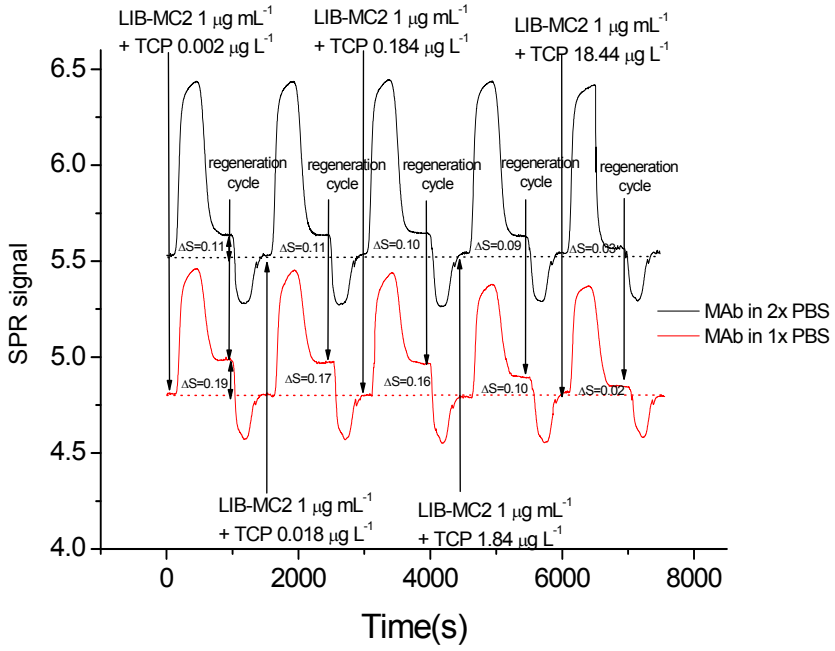

Fig. 4. SPR detection of TCP in human urine. A regeneration cycle is applied after each determination. A limit of detection of 0.1 $\mu g\ L^{-1}$ was obtained.

3. DETECTION OF HORMONES IN HUMAN FLUIDS

A similar format was developed for the direct determination of four pituitary peptide hormones: human thyroid stimulating hormone (hTSH), growth hormone (hGH), follicle stimulating hormone (hFSH), and luteinizing hormone (hLH). The immunoassays were done using a competitive format with the hormone immobilized on the sensor and specific monoclonal antibodies . After achieving an excellent performance under optimized conditions in buffer, the assays were refined to be employed for the testing of human serum and urine samples. We have demonstrated the viability of performing direct measurements of the hormones in those human fluids, without any sample pre-treatment [8, 9] (see Fig. 5). The assays showed sensitivity levels in the range from 1 to 6 $\mu g \cdot L^{-1}$. Moreover, the attachment of the hormones ensured the stability of the SPR signal through repeated use up to 100 consecutive cycles.

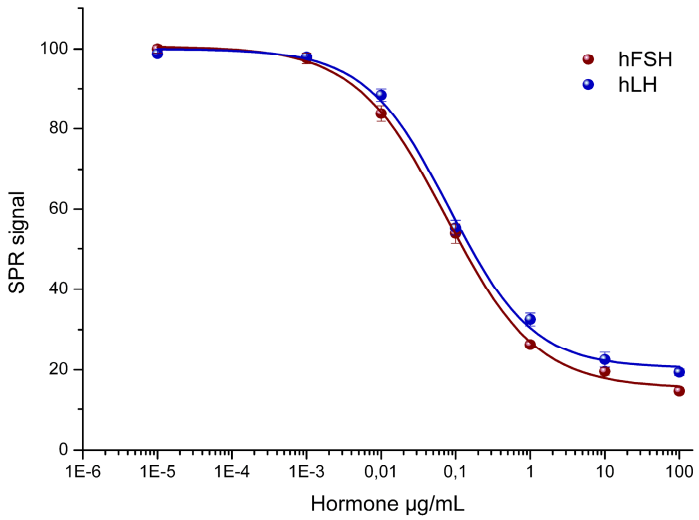

Fig. 5. SPR calibration curves for the detection of FSH and LH hormones in human urine using a multi-analyte detection format

4. DETECTION OF SINGLE-POINT MUTATIONS FOR EARLY CANCER DIAGNOSIS

The SPR technique has been 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 influence of lateral and vertical spacers, as well as several hybridization conditions, were studied to optimize the differentiation between fully complementary and mismatched DNA strands. The use of mercaptohexano (MCH) as a lateral spacer was found to increase sensitivity with short DNA target sequences; however, with longer ones, such as PCR targets, this spacer was not suitable. In this latter case, the use of DNA sequences as lateral spacers was found to be the best option to greatly improve hybridization. The use of a vertical spacer of 15 thymidines was also found to be critical in enhancing hybridization, either for short and PCR sequences. Detection limits of 10 nM were obtained for the detection of 25-mer targets, while for the identification of mutations, the limit of detection was 100 nM. It has been demonstrated that controlling the stringency of the hybridization buffer allowed increasing the selectivity and the differentiation between mutant and wild-type phenotypes. The optimized methodology was employed to detect hybridization on BRCA1-related mutations showing a high sensitivity with detection levels below 50 nM, while presenting an ability to discriminate between perfect and mismatched probes in the range of 80%. These results allocate conventional SPR biosensor methodology as a competitive and complementary tool for DNA analysis.

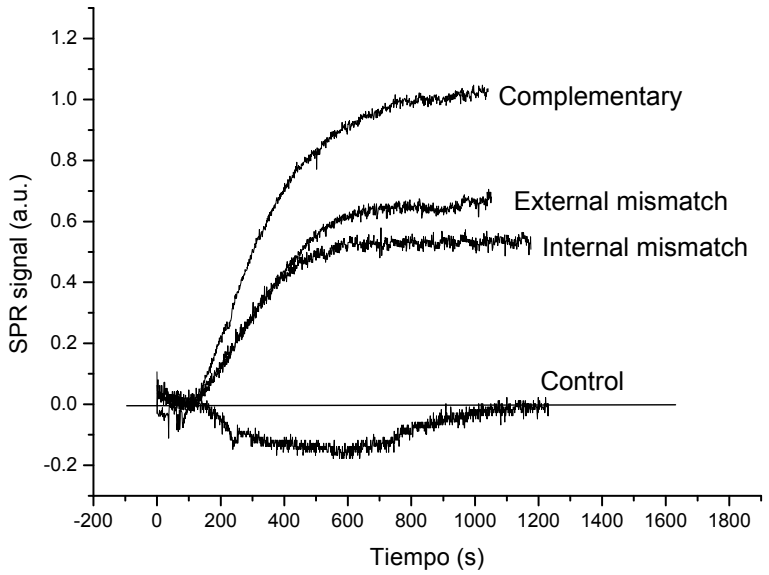

Fig. 6. SPR direct discrimination between fully complementary and mismatches DNA probes. It can be observed: (i) hybridisation of fully complementary DNA strand to the immobilised probe (ii) hybridisation of DNA strand with an internal mismatch and (iii) hybridisation of DNA strand with an external mismatch.

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5. SENSITIVITY IMPROVEMENT

A crucial issue for SPR sensors is the improvement of its limits of detection. With this purpose, several SPP configurations have been described as, for example, the phase sensitive SPR based on a Mach-Zehnder configuration [10], the differential ellipsometric SPR [11], or the optical heterodyne SPR [12].

5.1 Magneto-optic surface plasmon resonance sensor

With a similar aim, we have recently developed a new magneto-plasmonic biosensing concept [13, 14], based on the combination of the magneto-optic activity of ferromagnetic materials and the surface plasmon resonance of metal layers.

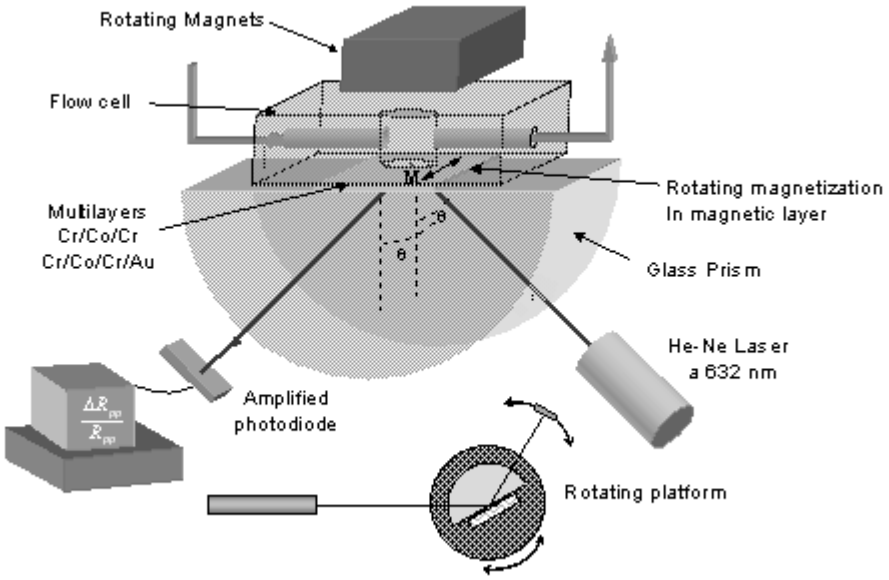

Fig. 7. Schematics of the MOSPR set-up in angular interrogation. A rotating magnet of a

Such a Magneto-Optic Surface Plasmon Resonance (MOSPR) biosensor shows a very sharp magneto-optic resonance, in which the measurements derive from the reflectivity modulation induced by an oscillating magnetization (Fig. 8). In particular, when the magnetization oscillates in the transversal configuration, i.e., parallel to the layer and perpendicular to the incident plane of the incoming light, a relative variation of the reflectivity of the *p*-polarized light is obtained:

$$\frac{\Delta R_{pp}}{R_{pp}} = \frac{R_{pp}(M) - R_{pp}(-M)}{R_{pp}(0)} \tag{5}$$

The combination of the SPR and the MO activity can be found in ferromagnetic metals, like Fe, Co or Ni. In these systems, a large increase of the MO effects can be induced at the SPR condition. However, ferromagnetic materials exhibit also large optical absorption, thus damping the SPR and reducing the biosensing sensitivity. Such sensitivity can be improved with the combination of ferromagnetic metal layers with gold layers typically employed in SPR biosensing. The introduction of Au layers enhances both the slope of the MO curves and their angular displacement when refractive index of the dielectric changes, being responsible for the sensitivity increase (see Figure 8). In addition, this combination offers other advantages as the compatibility with the well-known gold-based immobilization protocols of biomolecules, and the protection of the ferromagnetic layer from oxidation. The experimental demonstration of this sensing concept has shown a 3-fold improvement of the sensitivity as compared to conventional SPR sensors.

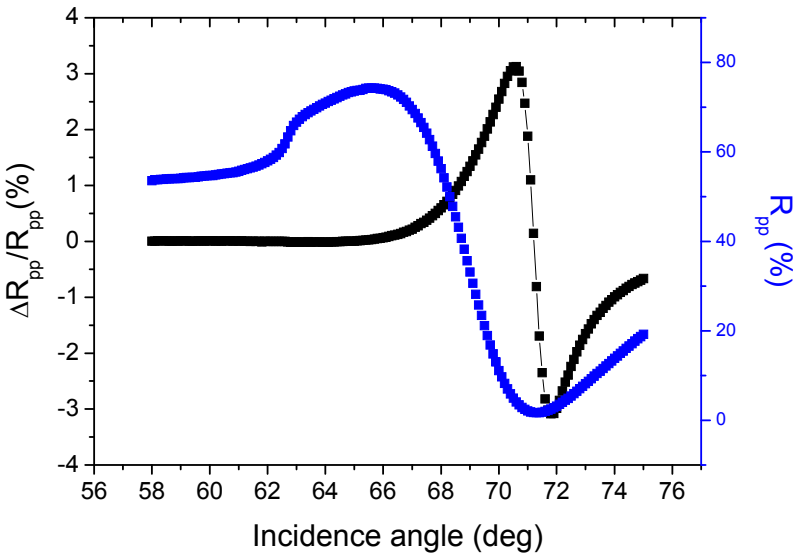

Fig. 8. Example of the experimental optical and magneto-optical angular curves of a multilayer composed Au/Co/Au. The magneto-optic curve exhibits a much sharper feature than the reflectivity

However, optimization of the multilayer transducer and experimental set-up can reduce the signal-to-noise ratio of the measurements and improve the limit of detection of the conventional SPP sensors up to one order of magnitude. The combination of ferromagnetic and plasmonic materials within nanoparticles can also give interesting properties that could find biosensing applications [15].

6. MULTIPLEXING IMPROVEMENTS

In general, SPP sensors based on diffractions gratings [16], integrated optics [17] or fiber optics [18] offer the possibility to design more compact and integrated sensors, although they generally show lower sensitivity than prism coupling devices. Interestingly, the diffraction gratings can be designed to couple the SPP and to disperse the light in a position sensitive detector at the same time. This novel approach allows the wavelength interrogation without the need of external spectrometers [19], which can simplify and reduce the final cost of the device.

But one of the most important challenge for SPP based biosensors is to be multiplexed for high throughput screening. The most simple way of multiplexing can be obtained via surface plasmon imaging (SPI) [20]. In this technique a collimated monochromatic light beam excites the SPP in an extended area via prism coupling. The reflected light suffers an intensity modulation due to the variations of refractive index in different parts of the metallic layer, which can be analyzed in a two dimensional (2D) CCD camera. If various biomolecules are immobilized in different parts of the metal layer, the multianalyte biosensing measurement can be made with the analysis of the 2D reflected intensity pattern. However, this multiplexing technique has several limitations, mainly due to its poor lateral resolution. This drawback comes from to the propagating nature of the SPP, which is around tens of microns in the visible, and increases in the NIR. This effect limits the miniaturization of the sensing channels and creates cross-talk problems between them. In addition, the limit of detection in SPI is generally lower than in conventional SPP sensors with wavelength interrogation. To improve the limit of detection of the SPI systems, a wavelength division multiplexing scheme [21, 22] has been proposed. Such a scheme uses different angles of incidence or dielectric overlayers to spectroscopically discriminate the sensing channels. Using this arrangement the simultaneous measurement of four pairs of channels has been shown [22]. To increase the number of sensing channels, a spatially patterned multilayer with a polarization contrast imaging has been reported [23]. The presented prototype has 108 channels whose size is 400x800 μm^2. The polarization contrast, together with the patterning, increases the resolution and reduces the crosstalk between channels.

However, the most promising plasmonic systems for high throughput applications are plasmonic nanostructures. Unlike the SPR in thin metal films, the optical resonances induced in metal nanostructures are bound to the nanostructure, and are denoted as localized surface plasmon resonances (LSPR). This property, together with the large scattering cross section permits the use of even a single nanoparticle for biosensing purposes, providing huge multiplexing capabilities. Such property, together with their versatility, tunability and sensitivity turn metal nanostructures into very qualified candidates to develop commercial biosensors in the near future [24]. Although the surface sensitivities of SPP and LSPR sensors are similar [25], the tiny volume of the sensing region in metal nanostructures suggests several possible advantages with respect to SPP sensors: much higher possibility of miniaturization, only diffraction limited, superior multiplexing capabilities for high-throughput assays, much lower volume of sample required to achieve the same detection limit, the sensing region can be tailored by choosing the size and shape of the nanostructures or their geometrical distribution to match the dimensions of the biological molecules [26], and finally, they do not required coupling means to excite the LSPR.

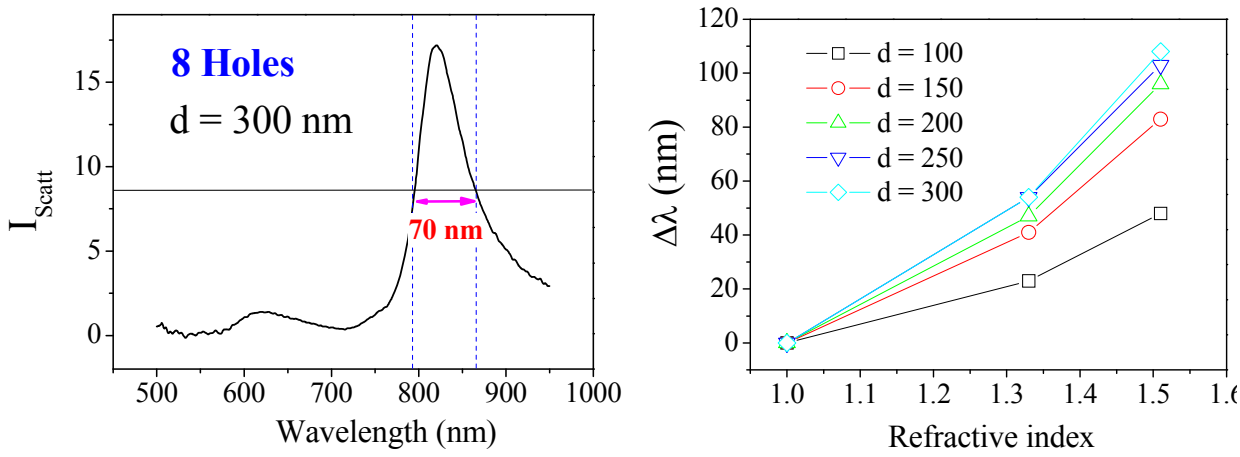

Fig. 9. Example of the LSPR spectrum of a chain of 8 nanoholes when their separation distance is 300 nm (left). Shift of the resonance with the external refractive index is varied, for chain of different separation distances (right).

Within this field, we are working on the optimization of plasmonic nanostructures to develop multiplexed nanosensors. On the one hand, we are developing sensing platforms based on nanoholes arrays in very thin metal films [27-29], where we exploit the combination of the localized and propagating nature of these nanostructures, which can exhibit sharp resonances, even for resonant wavelengths in the near infra-red (see Fig. 9), where the plasmonic resonances generally broaden. We have demonstrated that an improved sensitivity to the bulk and local changes of refractive index can be obtained tailoring the electromagnetic field distribution of the nanostructures with low refractive index substrates [30]. On the other hand, we are analyzing the sensing performance of wavelength interrogated SPR and LSPR sensors with the aim of extracting general features to maximized their sensitivity. This study has shown that there is an optimal sensing region for gold plasmonic sensors, located around a resonance wavelength of 700 nm. In this region, the surface sensitivity of LSPR sensors based on rod-like Au nanoparticles is optimized, being able to outperform the surface sensitivity of SPR sensors.

7. CONCLUSIONS

We have summarized our developments in label-free and real-time applications using our custom-designed SPR biosensor. We have described applications for environmental control and clinical diagnosis, showing the detection in real samples (water, orine and serum) without sample pretreatment. Interestingly, the developed protocols permit the surface regeneration and reutilization of the sensor more than 100 times, and are compatible with multisensing formats within

the same sensing surface. Finally, we have given an outline in our progress towards higher sensitive and multiplexed plasmonic sensing platforms.

8. ACKNOWLEDGEMENTS

Financial support from the M. Botin foundation is gratefully acknowledged.

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