Suitable combination of noble/ferromagnetic metal multilayers for enhanced magneto-plasmonic biosensing

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Abstract: We present a theoretical and experimental study on the biosensing sensitivity of Au/Co/Au multilayers as transducers of the magneto-optic surface-plasmon-resonance (MOSPR) sensor. We demonstrate that the sensing response of these magneto-plasmonic (MP) transducers is a trade-off between the optical absorption and the magneto-optical activity, observing that the MP multilayer with larger MO effect does not provide the best sensing response. We show that it is possible to design highly-sensitive MP transducers able to largely surpass the limit of detection of the conventional surface-plasmon-resonance (SPR) sensor. This was proved comparing the biosensing performance of both sensors for the label-free detection of short DNA chains hybridization. For this purpose, we used and tested a novel label-free biofunctionalization protocol based on polyelectrolytes, which increases the resistance of MP transducers in aqueous environments.

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References and Links


1. Introduction

In the last two decades label-free optical biosensors have turned into essential tools for the real-time detection and identification of chemical and biological species with high sensitivity and selectivity. Surface-plasmon-resonance (SPR) devices are the landmark sensors within photonic biosensing technology and, nowadays, these devices are basic tools for the analysis of biospecific interactions. Different SPR biosensors are commercialized by more than twelve companies around the world, demonstrating remarkable applications in wide areas as proteomics [1], medical diagnostic [2], genomics [3], environmental monitoring [4], food safety and security [5]. These types of sensors are based on the well-known properties of surface plasmon polaritons (SPP) [6], which can be excited by light at the interface of a metal and a dielectric. There are several SPR biosensor configurations [7], depending on the excitation and interrogation layouts. But due to its simplicity, low cost, and high-performance, the intensity-interrogated SPR biosensor is one of the most widely employed, and it is currently an essential analytical tool for real-time and label-free detection. However, its sensitivity is not enough for the label-free detection of low concentrations of small molecular weight analytes. Therefore, the development of any technique which could improve the sensitivity of the SPR biosensor while keeping all its advantages can constitute a very attractive tool for biosensing applications.
For that reason, during last years, different SPR modulation configurations have been proposed to improve the sensitivity of the standard SPR sensors, as for example mechanical [8,9] or phase [10,11] modulated SPR sensors. These configurations use external modulation techniques to improve the signal-to-noise (SNR) ratio of the sensing measurements, thus increasing their limit of detection (LOD). However, the main drawback of these configurations is the high-cost and complexity of the final devices. As an alternative, our group proposed the magneto-optic surface-plasmon-resonance (MOSPR) biosensor [12], based on the non-reciprocal variation of the SPP wave vector with an external magnetic field [13,14]. The so-called magneto-plasmonic (MP) modulation arises from the simultaneous excitation of magneto-optic (MO) effects and the SPP in structures with plasmonic and MO activity. The most suitable structures to generate large MP modulation are multilayers composed of ferromagnetic and noble metals [15,16], since they combine the large MO activity of ferromagnetic materials and the exceptional plasmonic properties of noble metals. Compared to the conventional SPR sensor, the MOSPR only requires a low field electromagnet and a MP transducer instead of the typical gold layer. Although we demonstrated that the MOSPR sensor can surpass the sensitivity of the conventional SPR sensor, its final performance strongly depends on the MP features of the multilayer structures employed as transducer. Therefore, the optimization of the sensor response through the most suitable combination of multilayers becomes essential, not only for the development of MOSPR biosensors, but also for future MP active devices [17].

So far, the MP features of multilayered structures made of noble and ferromagnetic metals have been extensively studied [18–22], but not their sensing properties. For that reason, in this work we present a combined theoretical and experimental analysis of the sensing response of MP multilayered transducers, confirming that the sensing response is a complex balance between optical absorption and MO activity. Thanks to this analysis it is possible to design high-sensitive MP transducers, showing that the MOSPR biosensor with an optimized Au/Co/Au multilayer transducer can exhibit a four-fold improvement of the LOD with respect to conventional SPR biosensors.

### 2. Operating principle of the MOSPR biosensor

In the case of monochromatic p-polarized light in the Kretschmann configuration [23] (or prism-coupling), the excitation of the SPP results in a sharp decrease of the reflected intensity at a specific angle of incident (θ_{spp}). The principle of detection of the conventional SPR sensor [24] is based on the strong dependence of the SPP wave vector (k_{spp}) on the refractive index of the dielectric (n_d) medium. The changes of refractive index in the close proximity of the metal layer, as those generated by biochemical interactions, induce an angular shift of the reflectance resonance dip, which can be employed to quantify the biosensing interactions. In contrast, the MOSPR measurement is based on the simultaneous excitation of the SPP and the transversal magneto-optic Kerr effect (TMOKE), which is generated by applying an external magnetic field parallel to the metal layer but perpendicular to the direction of propagation of the SPP. Such magnetic field induces a non-reciprocal shift of k_{spp}, which is translated into small variations of the SPP excitation angle, Δθ_{spp}, for monochromatic light (Fig. 1(a)) and, subsequently, into variations of the angular reflectance curve:

\[ ΔR_{pp} = R_{pp}(H) - R_{pp}(H = 0) \]  

where R_{pp} is the reflectance of p-polarized light and H the external magnetic field. TMOKE gives access to the derivative of the reflectance resonance dip, given by:

\[ ΔR_{pp}(H) = \frac{∂R_{pp}}{∂θ_{spp}} \times Δθ_{spp}(H) \]  

The operating principle of the MOSPR biosensor is based on the simultaneous excitation of the SPP and the transversal magneto-optic Kerr effect (TMOKE), which is generated by applying an external magnetic field parallel to the metal layer but perpendicular to the direction of propagation of the SPP.
Since $R_{pp}$ has a resonant behavior, the TMOKE will also show a resonant angular dependence, which yields a large enhancement of the MO effect (Fig. 1(b)). As can be deduced from Eq. (2), the MO effect will be highly dependent on the width of the resonant dip ($\Delta R_{pp}/\Delta \theta$) and the intrinsic MO activity of the material ($\Delta \theta_{pp}(H)$).

In order to normalize the MO effect and eliminate the fluctuations of the light source, the MOSPR sensor detects the relative variations of the reflectance:

$$\frac{\Delta R_{pp}}{R_{pp}} = \frac{R_{pp}(H) - R_{pp}(H = 0)}{R_{pp}(H = 0)} \quad (3)$$

Likewise $R_{pp}$ in conventional SPR sensors, the quantity $\Delta R_{pp}/R_{pp}$ also depends on $n_d$. Therefore, the quantification of variations of the resonant TMOKE when the external medium $n_d$ varies provides the sensing principle of the MOSPR sensor (Fig. 1(c)). In this configuration an alternating external magnetic field generates a reflectance modulation, which allows improving the SNR of the biosensing measurements, as a way to reduce the LOD of conventional SPR sensors.

Even though it is possible to generate substantial MO effects in Au nanostructures [25], the TMOKE in the case of Au films is very weak. In contrast, typical ferromagnetic metals (Fe, Co, Ni) show strong MO activity at low magnetic fields, but suffer from large optical absorption, leading to rapid damping of the SPP [18]. Such damping results in much weaker dependence of $k_{pp}$ on the external refractive index and therefore, in a lower biosensing sensitivity. An interesting alternative to combine high MO activity and low damping of the SPP is found in multilayers of plasmonic and ferromagnetic metals, which has been also exploited in the case of nanostructures [26,27]. Then, the optimal configuration of the MOSPR sensor will be located between two extremes: a pure plasmonic layer showing high dependence of $k_{pp}$ on $n_d$ but negligibly weak MP modulation, and a pure ferromagnetic metal with high MP modulation but weak sensitivity of $k_{pp}$ on $n_d$. Our aim is analyzing and
maximizing the response of such MP modulation to the changes of refractive index. We focus our analysis in the dependence of the TMOKE, i.e. $\Delta R_{pp}$, on the refractive index of the external medium $n_d$ for different combinations of plasmonic and ferromagnetic metals. Since we are interested in the biosensing applications, we restrict our study to Au as plasmonic material due to its chemical stability. On the other hand, we select Co as ferromagnetic material owing to its much larger MO activity compared to Fe when it is integrated within Au layers [21].

3. Theoretical analysis of the sensor response of MP multilayer transducers

In any intensity-interrogated sensor as a function of the angle of incidence, the sensitivity can be defined as:

$$\eta = \frac{\partial S}{\partial n_d} = \frac{\partial S}{\partial \theta} \times \frac{\partial \theta_{spp}}{\partial n_d}$$

(4)

where $S$ represents the measured signal (either optic or magneto-optic). Therefore, the maximum sensitivity will be achieved for transducers that are able to combine high slopes in the resonant angular curves and large displacements of the resonance position when the refractive index changes. As a result, we propose the following parameter for the study and optimization of the MOSPR sensor:

$$\eta_{MOSPR} = \frac{\partial \left( \Delta R_{pp} \right)}{\partial n_d}$$

(5)

Another important aspect in the SPP based sensors in Kretschmann configuration is the coupling efficiency of the SPP and, closely related, the intensity of the electromagnetic field at the sensing interface. Both parameters are highly dependent on the thickness of the metal multilayer. The optimal coupling and maximum electromagnetic field at the sensing interface are obtained for multilayers yielding to reflectance values close to 0 at the resonant angle. Therefore, we employed the Transfer Matrix Method [28] to analyze the MO and sensing properties of Au/Co/Au multilayers (Fig. 2(a)) as a function of the thicknesses of the Au top layer ($d_{AuTop}$) and the Co layer ($d_{Co}$), when the thickness of the Au bottom layer ($d_{AuBottom}$) is selected to provide a reflectance $R_{pp}$ at the resonance angle is as close to 0 as possible. Depending on the values of $d_{AuTop}$ and $d_{Co}$, the previous reflectance criterion will be fulfilled for $d_{AuBottom} = 0$ (bilayer structures) or $d_{AuBottom} \neq 0$ (trilayer structures). The thickness of the Au bottom layer that optimizes the coupling efficiency of the SPP can also be determined through the Transfer Matrix Method. In all the calculations, the incident medium is glass ($n_i = 1.516$ RIU) and the external medium is water ($n_d = 1.3323$ RIU). Between glass and the MP multilayer we include a 2 nm thick Ti layer, since it will be required to improve the mechanical adhesion of the experimental multilayers. We assume that the incident light wavelength is 660 nm and, at this wavelength, the dielectric constants of the different materials are [29,30]: $\varepsilon_{xxAu} = -13.7 + 1.04i$, $\varepsilon_{xxCo} = -12.12 + 19.75i$, and $\varepsilon_{xxTi} = -4 + 13.55i$, respectively. The MO constant of cobalt are $\varepsilon_{xzCo} = -0.470 + 0.0010i$. 

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Fig. 2. (color online) (a) Schematic view of the analyzed MP structure in Kretschmann configuration. Contour plots of: (b) the maximum amplitude of the ΔR_{pp} and (c) η_{MOSPR} as a function of the upper gold layer (d_{AuTop}) and cobalt thicknesses (d_{Co}). The value of the gold bottom layer thickness (d_{AuBottom}) was previously optimized to achieve the maximum coupling efficiency of the SPP (see appendix A). The blue line divides the region of bilayer structures (those in which the optimization occurs for d_{AuBottom} = 0) and the region of trilayer structures (where occurs for d_{AuBottom} ≠ 0). Finally, the red numbers of the figure (c) are the combinations of d_{AuTop}-d_{Co} experimentally evaluated in this work.

Once the combination of layers satisfying the reflectance criterion is obtained (see appendix A), we study their TMOKE amplitude. In the theoretical analysis, the maximum thicknesses for the Au top and Co layers were 40 and 20 nm, respectively. Thicker Au top layers are discarded from the analysis since the reflectivity criterion imposes Co layers whose small thickness give rise to a very weak MO effect. Figure 2(b) clearly shows that the maximum amplitude of ΔR_{pp} is obtained for the layers with higher content of Co. In particular, the highest MO effect is exhibited by the pure 20 nm Co layer. According to a previous publication [31], when gold is introduced in the multilayer, the MO effect is always maximized when cobalt is in contact with the external dielectric medium (d_{AuTop} = 0), i.e., in the region where the electromagnetic field associated to the SPP is maximized. However, as we have previously discussed, a larger MO effect does not necessarily mean a higher sensitivity for the biosensing measurements. Indeed, Fig. 2(c), depicting the sensitivity of TMOKE η_{MOSPR} shows a very different scenario. The TMOKE sensitivity presents two clearly defined regions of optimal sensitivity, one in the bilayers region (d_{AuTop} ~40 nm: d_{Co} ~12 nm) and one for trilayers (d_{AuTop} ~25 nm: d_{Co} ~6 nm; d_{AuBottom} ~10 nm). The η_{MOSPR} parameter was numerically determined for a refractive index change of the external medium of 5·10^{-4} RIU.

4. Experimental verification and discussion

To corroborate the theoretical study with experimental results, we fabricated twelve different Au/Co/Au structures on glass substrates, previously coated with a 2 nm thick Ti layer, in order to show the main features of the sensitivity described in Fig. 2(c). The nominal thickness values of the fabricated samples correspond to the twelve points displayed in Fig. 2(c), also gathered in appendix B. The transducers were fabricated by electron-beam
evaporation at room temperature with a base pressure of $10^{-6}$ mbar and the thickness was monitored using a calibrated quartz crystal sensor. Due to the fast oxidation of cobalt in the sensing aqueous environment, we used a minimum of 15 nm as upper gold layer. The magnetization of the Co layer is reversed at a frequency of 64 Hz in the transversal configuration with an alternating magnetic field of 50 Oe. The optic ($R_{pp}$) and MO ($\Delta R_{pp}$) signals are extracted via FFT analysis of the reflected light intensity.

The experimental $\eta_{\text{MOSPR}}$ of each MP multilayer was determined through the real-time variation of $\Delta R_{pp}$ at $\theta = \theta_{\text{spp}}$ produced by an aqueous solution of ethanol (~1%) with respect to deionized water ($\Delta n_{d} = 6 \times 10^{-4}$ RIU). As example of the experimental sensitivity determination, Fig. 3(a) shows the temporal MO signal variation of two different transducers and for three consecutive flow-injections of the same $\Delta n_{d}$ solution. The experimental $\eta_{\text{MOSPR}}$ of this experiment corresponds to the average of $\Delta R_{pp}$ variation of the three flow-injections, and its error is determined by the standard deviation of the average.

**Fig. 3.** (color online) (a) MO signal variation produced by a refractive-index change of $6 \times 10^{-4}$ RIU for No. 3 and No. 6 transducers, evaluated at the resonance angle. (b) Theoretical and experimental $\eta_{\text{MOSPR}}$ for the fabricated transducers. Experimental and theoretical values of $\eta_{\text{MOSPR}}$: (c) different constant values of $d_{\text{AuTop}}$ as a function of $d_{\text{Co}}$ and (d) vice versa. Experimental $\Delta R_{pp}$ and $R_{pp}$ angular curves (e) and $\Delta R_{pp}/R_{pp}$ angular curve (f) for the transducers No. 5 (red dotted-line) and 9 (blue solid-line).

Figure 3(b-d) depicts the good agreement between theoretical and experimental results, confirming the trend of the calculated contour plot of the theoretical $\eta_{\text{MOSPR}}$ parameter (see Fig. 2(c)). The observed differences in $\eta_{\text{MOSPR}}$ can be attributed to the experimental error in
the thickness determination and the roughness of the thinner Au layers. The lower experimental $\eta_{\text{MOSPR}}$ values of the samples with smaller Au top layer (No. 1, 4, 7 and 10) are probably due to the poor protection of this layer from Co partial oxidation. Despite of these subtle deviations, we corroborate the existence of two regions with a maximum value of $\eta_{\text{MOSPR}}$. One corresponds to a bilayer structure (No. 9) with 11 nm of Co located at 37 nm of the SPP surface, and the other to a trilayer structure (No. 5) with 6 nm of Co located at 26 nm of the SPP surface. These results confirm that the most suitable transducer is a trade-off between a structure with high MO signal (high content of magnetic material in the vicinity of the SPP interface) and low damping of the excited SPP (low content of magnetic material and far from the SPP interface).

5. MOSPR vs. SPR as biosensor devices

The final objective of this work is comparing the biosensing performance of the optimized MOSPR and standard SPR sensors for the label-free detection of DNA hybridization of very short DNA chains. The detection of these targets is challenging in standard SPR sensors due to their very low molecular weight. The previous experimental analysis has shown that structure No. 5 exhibits the maximum sensitivity (Fig. 3(b)) and, in addition, the lowest reflectance at the resonance angle (Fig. 3(e)). This combination allows the generation of a larger enhancement of $\Delta R_{pp}/R_{pp}$ (Fig. 3(f)), helping to improve even more the signal-to-noise ratio (SNR) of the biosensing measurement. We used 48 nm Au on 2 nm Ti as transducer of the SPR sensor, since this gold thickness provides the optimal coupling between the incident light ($\lambda = 660$ nm) and the SPP (previous reflectivity criterion). Both transducers were fabricated on the same type of glass substrates and using the same growth conditions (Fig. 4(a)).

The sensitivity comparison must be done taking into account the SNR due to the different nature of the MOSPR and conventional SPR measurements. We defined the system noise as the RMS deviation of the sensing signal acquired during 1000 s with a sampling rate of 1 Hz when $n_d$ is constant. The evaluation of the biosensing capabilities of both sensors was done using the same experimental set-up and biofunctionalization protocols.

![Fig. 4. (color online) (a) Angular detection curves of the optimized MP transducer (solid lines) compared to a standard gold transducer of the SPR biosensor (dotted line). (b) Schematic of the DNA immobilization protocol. The conditions are detailed in appendix C.](image)

To improve the resistance of the MP multilayers in solutions with low pH or high ionic strength we have employed a self-assembled biofunctionalization protocol based on polyelectrolytes (see Fig. 4(b)), as detailed in appendix C. Such protocol allows forming a final PEG monolayer grafted with a homogenous distribution of biotins, ideal for subsequent streptavidin (Sa) based biofunctionalization. This protocol has the advantage of providing binding sites for streptavidin and biotinylated ssDNA with lateral spacing distances that can...
help to maximize the hybridization efficiency. On the other hand, the remaining PEG surface permits the minimization of unspecific adsorptions.

Figure 5(a) and (b) shows that the response of the Sa and biotinylated DNA immobilization on the Au/Co/Au transducer of the MOSPR biosensor are approximately four times larger than that of the standard SPR sensor. Moreover, Fig. 5(c) interestingly demonstrate a similar sensitivity enhancement in the hybridization response of the 11-mer fully complementary target sequence, which allows its unambiguously detection as compared to the barely discernable signal in the standard SPR. Finally, Fig. 5(d) proves that using this novel immobilization protocol it is possible to regenerate the surface of the MP transducer through a formamide solution (35%), enabling more than 10 cycles of hybridization-regeneration of the same MP transducer.

Fig. 5. (color online) Sensor response of the MOSPR and SPR biosensor to (a) Sa and (b) biotinylated DNA immobilization, and (c) DNA-target hybridization. (d) MP transducer regeneration of the sensing surface by a formamide solution.

6. Summary and conclusions

We have carried out a theoretical and experimental analysis of different MP transducers to optimize their biosensing sensitivity. Contrary to what one would expect, the structure with greater enhancement of the MO effect does not provide the best sensing response. We have proved that the design of MP multilayer transducers is a trade-off between the optical absorption and the MO activity, and we have observed that the best MP multilayered transducer for biosensing applications is composed of a trilayer structure, with a 6 nm thick Co layer, separated from the sensing surface by a rather thick Au layer (26 nm). Moreover, the analysis has provided us with relevant information about the role of each layer in the sensing response of the trilayer structure. We have experimentally demonstrated that the signal-to-noise ratio of the MOSPR biosensor with our optimized Au/Co/Au transducer is four times greater than the conventional SPR sensor. Such SNR could be improved even further through the experimental optimization of the multilayer roughness and the thickness of the gold bottom layer, to provide an enhanced coupling of the SPP with longer propagation.
distance due to the reduced scattering. On the other hand, the increase of the modulation frequency using ferrite based electromagnets is another important factor that can contribute to amplify the SNR of the biomeasurements. Finally, we have also proposed and tested a novel label-free biofunctionalization protocol based on polyelectrolytes. This protocol increases the resistance of the MP transducers in aqueous environment thanks to the protective effect of the polyelectrolyte multilayer. Such protective multilayer can be biotinylated, which offers different strategies of further biofunctionalization. The protocol could be used in other types of magnetic or MP transducers, such as magnetic nanoparticles or MP nanostructures.

**Appendices**

A. Complementary theoretical results

![Image of Fig. 6](image1.png)

Fig. 6. (color online) (a) Optimal Au bottom thicknesses (which minimized the value of the $R_{pp}$ at the resonance angle) as function of the upper gold layer ($d_{AuTop}$) and cobalt thicknesses ($d_{Co}$) and, (b) the value of the $R_{pp}$ at the resonance angle of these optimized structures. The blue line divides the region of bilayer structures (those in which the optimization occurs for $d_{AuBottom} = 0$) and the region of trilayer structures (where occurs for $d_{AuBottom} \neq 0$). Finally, the red numbers are the combinations of $d_{AuTop}-d_{Co}$ experimentally evaluated in this work.

![Image of Fig. 7](image2.png)

Fig. 7. (color online) Theoretical $\Delta R_{pp}$ and $R_{pp}$ angular curves (a) and $\Delta R_{pp}/R_{pp}$ angular curves (b) for the transducers No. 5 (red dotted-line) and 9 (blue solid-line).
B. Complementary experimental results

Table 1. Experimental parameters of the fabricated MP multilayers

<table>
<thead>
<tr>
<th>No.</th>
<th>( d_{\text{AuTop}} ) (nm) ± 0.3 nm</th>
<th>( d_{\text{Co}} ) (nm) ± 0.3 nm</th>
<th>( d_{\text{AuBottom}} ) (nm) ± 0.3 nm</th>
<th>( R_{pp}(\theta = \theta_{\text{pp}}) ) (%) ± 0.02%</th>
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C. Polyelectrolyte immobilization protocol

- **Polyelectrolyte multilayer**: a first layer of poly(diallyldimethylammonium chloride) (PDDA), a second layer of poly(sodium 4-styrenesulfonate) (PSS), and finally a poly-L-lysine grafted with a biotinylated poly(ethylene glycol) layer (PLL-PEG)). The deposition was carried out in flux at rate of 20 μl/min using water solutions of PDDA (2 weight percent), PSS (2 weight percent) and a mixture of biotinylated PLL-PEG (0.25 mg/ml) and non-biotinylated PLL-PEG (0.25 mg/ml) on a volume of 300 μl.

- **Streptavidin (Sa) coupling**: The Sa coupling was carried out in flux using 10 μg/ml sample prepared on a volume of 300μl in PBS buffer (10mM phosphate pH 7.4 with 137 mM NaCl, 2.7 mM KCl).

- **DNA immobilization**: we immobilized a biotinylated 26 mer DNA sequence Biotin-TTT TTT TTT TTT AGA ATC CCC AG (Mw 8278.22 g/mol). Only 11 of the 26 mer immobilized are involved in the subsequent hybridization processes. The remaining 15 mer were used as vertical spacers to facilitate the hybridization of the DNA-target sequences. The immobilization of biotinylated DNA was carried out in flux using a 1 μM sample prepared on a volume of 300 μl in 50mM phosphate buffered (PB) solution with 0.5 M NaCl, pH 7.

- **DNA hybridization**: The hybridization to the complementary strand CTG GGG ATT CT (Mw 3363.25 g/mol) was done at room temperature using a 1μM sample target prepared on a volume of 300 μl in 0.075 M sodium citrate (SSC) with 0.75 M NaCl, at neutral pH.

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