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Magneto-plasmonic biosensor with enhanced analytical response and stability

Sorin David [a], Cristina Polonschii [a], Catalin Luculescu [b], Mihaela Gheorghiu [a], Szilveszter Gáspár [a], Eugen Gheorghiu [a,c,*]

[a] International Centre of Biodynamics, Intrarea Portocalelor 1B, Bucharest 060101, Romania
[b] National Institute for Laser, Plasma and Radiation Physics, Atomistilor 409, Magurele 077125, Romania
[c] University of Bucharest, 4-12 Regina Elisabeta Blvd., Bucharest 030018, Romania

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ABSTRACT

We present novel solutions to surpass current analytic limitations of Magneto-Optical Surface Plasmon Resonance (MOSPR) assays, concerning both the chip structure and the method for data analysis. The structure of the chip is modified to contain a thin layer of Co–Au alloy instead of successive layers of homogeneous metals, as currently used. This alloy presents improved plasmonic and magnetic properties, yet a structural stability similar to Au–SPR chips, allowing for bioaffinity assays in saline solutions. Analyzing the whole reflectivity curve at multiple angles of incidence instead of the reflectivity value at a single incidence angle provides a high signal-to-noise ratio suitable for detection of minute analyte concentrations. Based on assessment of solutions with known refractive indices as well as of a model biomolecular interaction (i.e. IgG–AntiIgG) we demonstrate that the proposed structure of the MOSPR sensing chip and the procedure of data analysis allows for long-time assessment in liquid media with increased sensitivity over standard SPR analyses.

1. Introduction

Surface Plasmon Resonance (SPR)-based methods are currently among the methods of choice to assess bioaffinity interactions at interfaces due to their sensitivity to local changes in the refractive index of adjacent media (Schasfoort and Tudos, 2008). Nevertheless, there is an unmet need to improve the sensitivity of the existing SPR assays. Possible avenues to gain more sensitivity include optimization of the multilayered sensor chip structure, improvement in the sensitivity of photodetectors (Schasfoort and Tudos, 2008) or signal modulation (Regatos et al., 2011; Sepúlveda et al., 2006). Some of the signal modulation approaches are based on the magneto-plasmonic effects (Armelles and González-Díaz, 2008; Belotelov et al., 2013; Temnov et al., 2010). This is accomplished by employing the transverse magneto-optic Kerr effect (TMOKE). The TMOKE effect occurs as a result of applying a magnetic field perpendicular to the propagation plane of the incident p-polarized light and in the plane of a film with magneto-optical properties determining changes in the intensity of the reflected light (Zvezdin and Kotov, 1997). The combination of TMOKE with plasmonic effects (Raether, 1988) lays the foundation of a novel SPR detection configurations, i.e. the magneto-optical SPR (MOSPR) technique (Armelles et al., 2013). Modulation of the reflectivity (i.e. SPR) curve is accomplished by applying an alternative transversal magnetic field, perpendicular to the propagation plane of an incident p-polarized beam of light (Zvezdin and Kotov, 1997), onto a sensor chip exhibiting both magnetic and plasmonic properties (Sepúlveda et al., 2006). When the plasmons are excited, one notices a MOSPR response presenting increased sensitivity to the refractive index changes. This approach yields a reported theoretical improvement in the sensitivity of sensing assays of one (Regatos et al., 2010; Sepúlveda et al., 2006) or even two (Pištora et al., 2010) orders of magnitude.

Usually, the chips for magneto-plasmonic applications comprise metallic multilayers or nanostructures (e.g. nanodiscs, nanocrystals) (Armelles and González-Díaz, 2008; Belotelov et al., 2013; Temnov et al., 2010), whose fabrication could be technologically challenging. For sensing, current MOSPR assays employ multilayered structures; however, these complex structures exhibit lack of stability when exposed to or interrogating (saline) liquid samples. Therefore they are mostly used as gas sensors due to their increased sensitivity as compared to classical SPR assays (Armelles et al., 2013; Manera et al., 2012, 2011). The poor stability in liquids can be due to magnetostrictive effect (Regatos et al.,

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[*] Correspondence to: International Centre of Biodynamics, Intrarea Portocalelor 1B, 060101 Bucharest, Romania. Tel.: +40 21 3104354; fax: +40 21 3104361.
E-mail address: egheorghiu@biodyn.ro (E. Gheorghiu).

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2011), which is the more pronounced, the more heterogeneous the layers in the sensor structure.

We developed and tested a multilayered chip comprising a thin film of amorphous Au–Co alloy, capped with a layer of Au (to allow further functionalization), exhibiting both plasmonic and magnetic properties, and increased stability in (saline) liquid media.

Optimized raw data analysis provides additional routes for sensitivity enhancement. Therefore, both angle-resolved SPR/ MOSPR curves (i.e. the entire angular range of the SPR curve) or the reflectivity values at a single (fixed) angle of incidence, the latter being employed in most current SPR/MOSPR assays (Manera et al., 2012), have been comparatively evaluated.

We demonstrate that the proposed *angle-resolved* MOSPR bioassay based on a Co–Au alloy thin film exhibits both increased sensitivity in comparison with the current SPR/ MOSPR approaches and high stability (similar to the one achieved with classic, gold-only, SPR chips) even when addressing saline liquid samples.

2. Material and methods

2.1. Materials

Bovine serum albumin (BSA), human IgG (HIgG), affinity-isolated anti-human IgG (AHIgG), N-hydroxysuccinimide (NHS), 1-ethyl-3-(dimethylaminopropyl) carbodiimide (EDC) and ethanolamine were purchased from Sigma-Aldrich (Germany). (11-Mercaptoundecyl)hexa(ethylene glycol) acetic acid was purchased from Prochimia Surfaces, Poland. Surfactant P20 was provided by GE Healthcare. Au pellets (99.999%), Co (99.99%) pellets and Ti sputter target (grade 2) were purchased from Kurt J. Lesker USA. N-BK7 glass wafers were purchased from Sydor Optics UK. All the other reagents for buffers were purchased from Sigma.

The buffers used for the experiments are: immobilization buffer, 10 mM acetate buffer pH 5; running buffer: HBS-EP buffer (10 mM HEPES pH 7.4, 150 mM NaCl, 3 mM EDTA, and 0.005% Surfactant P20).

Ultrapure water (Millipore) was used throughout the preparations. All chemical reagents were of analytical grade and were used without further purification.

2.2. Fabrication of SPR and MOSPR chips

Substrates of BK7 glass (0.3 mm × 4 mm × 11 mm) are cleaned by successive sonication in water, acetone and isopropanol and dried in a stream of nitrogen. Further, the substrates are exposed to oxygen plasma and heated to 150 °C for degassing. The pressure inside the evaporation chamber was kept to approximately 2×10^{-6} Torr. Samples were rotated at 10 rot min^{-1} to ensure uniform coating. All the deposition processes were done at room temperature. The thickness of the deposited layers is constantly monitored by a quartz crystal microbalance inside the evaporation chamber. A layer of Ti (4 nm) is first sputtered on the glass substrates for improving the adhesion of subsequent layers. All metals are deposited by thermal evaporation, with the exception of Ti which is sputtered (RF sputtering at 5×10^{-3} Torr Ar pressure and 8 W cm^{-2}). The Au and Co layers are thermally evaporated at rates of 0.5 Å s^{-1} and 3 Å s^{-1} respectively.

Standard SPR chips are fabricated by evaporating 50 nm of Au onto the Ti-modified substrates.

The Au–Co–Au tri-layer consists of a 26 nm Au, 6 nm Co layer, separated from the sensing surface by a 12 nm Au layer. An intermediate layer of 2 nm Ti is inserted between Au and Co to improve adhesion of the two metals.

2.3. The alloy based magneto-plasmonic sensor chip

Currently, different metallic alloys are used for enhancing the plasmonic response (Blaber et al., 2010). Applications of Au–Co composites are mostly related to jewelry to harden the gold and in microelectronic industry as coatings for wear resistant electrical contacts (Goodman, 2002; Okinaka, 1998). Since alloys containing more than ~1% cobalt also exhibit magnetic properties (Kahn, 1992), the Au–Co thin films feature enhanced magneto-plasmonic behavior (Yang et al., 2010).

An important step in the preparation of the sensor chip is the deposition of the composing metal layers. Several techniques have been reported as suitable options for deposition of alloys each with their advantages and pitfalls. Pressure Vapor Deposition (PVD) using the sputtering technique has been used for depositing alloys (Kahn, 1992; Mattox, 2010; Yang et al., 2010); however magnetron sputtering of magnetic materials requires expensive magnetron guns with powerful magnets. Moreover, manufacturing custom sputtering targets comprising gold alloys may have prohibitively high costs. E-beam epitaxy may be also used for growing crystalline films with well controlled magnetic properties, but this is suitable or limited to substrates with crystalline structure (Ferreiro-Vila et al., 2011). Aiming for a simple and cost-effective deposition procedure, we chose the thermal evaporation method in which the metals to form the alloy are mixed and melted from a single source. Despite the different melting points (i.e gold – 1064 °C and cobalt – 1495 °C), the temperatures for reaching a given vapor pressure are similar for the two materials (gold – 947 °C and cobalt – 990 °C for a vapor pressure of 10^{-6} Torr) (Mattox, 2010). Therefore, both metals reach similar evaporation rates at a vacuum pressure of 10^{-6} Torr (Mattox, 2010), the deposited film having a slight depletion of cobalt compared to the composition in the source (as revealed by composition analysis).

The proportion of Au and Co in the alloy should allow for both good plasmonic properties and elevated magnetic characteristics. If there is too much of cobalt in the alloy, there will be an inconvenient dampening in the SPR effect exhibited by gold; conversely, too less cobalt would trigger a limited magnetic effect. Therefore, we have selected an alloy with a volumetric proportion of 10% Co:90% Au, a ratio also providing a good miscibility of the melted substrates (Okamoto et al., 1985) as well as magneto-plasmonic behavior (Yang et al., 2010). A higher proportion of cobalt in the alloy was tested but resulted in a poor SPR signal (data not shown).

Au–Co thin films form nanocomposites comprising clusters of Au and Co with sizes directly proportional to the temperature of the substrates during deposition (Yang et al., 2010). Accordingly, we chose to deposit a thin film of Au–Co alloy at room temperature. Despite yielding a lower magneto-optic (MO) effect, the film obtained under these conditions presents Co clusters of small size (~150 nm) (Yang et al., 2010), potentially leading to smaller magnetostriction and increased stability of the MOSPR chips.

According to Yang et al., the Au–Co mixture, deposited (by sputtering) in thin films, has in-plane anisotropy of the magnetization and a magnetic moment correlated with the amount of Co present. In several studies (e.g. He and McGahan, 1991; Kockar and Meydan, 2002) magnetron-sputtered films and evaporated films of composited materials present similar magneto-optical properties. Hence, we consider that our Co–Au alloy film presents also in-plane anisotropy similar to the Co–Au composite films obtained by magnetron sputtering co-deposition.

The Au–Co alloy chips are fabricated by evaporating a 30 nm layer of Au–Co alloy followed by 15 nm of Au. No intermediate Ti layer is used. The alloy is thermally evaporated at a rate of 0.5 Å/s from a single tungsten boat containing corresponding quantities of