Sensors and Actuators B 207 (2015) 740–747

Contents lists available at ScienceDirect

Sensors and Actuators B: Chemical

journal homepage: www.elsevier.com/locate/snb

Self-referencing SPR-sensor based on integral measurements of light intensity reflected by arbitrarily distributed sensing and referencing spots[☆]

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ARTICLE INFO

Article history:
Received 11 July 2014
Received in revised form 3 October 2014
Accepted 6 October 2014
Available online 12 October 2014

Keywords:
Surface plasmon resonance
SPR
Self-referencing
Patterned surface
Biosensor
Affinity sensor

ABSTRACT

A new approach for self-referencing in SPR biosensors is reported. The method is technologically simple and applicable for a wide range of existing SPR instrumentation with Kretschmann configuration. It is based on the micropatterning of the sensor area with sensing and referencing areas whose shape and distribution can be chosen arbitrarily and their characteristic sizes are larger than the plasmons propagation length. Provided that roughly a half of the area is used as the sensing area while its optical thickness is different from that of the referencing area, an integral measurement of the intensity of the reflected light over such a patterned surface near the summary resonance conditions exhibits self-referencing properties. An over ten-fold suppression of the effect caused by the variation of the bulk refractive index was observed.

1. Introduction

Since the introduction of biosensors based on the surface plasmon resonance [1], they quickly become one of the most popular transducing approaches in affinity sensors [2]. SPR transducers are sensitive to the refractive index of surrounding media. Because an increase in the refractive index near sensing surface is a direct consequence of adsorption, this approach can be applied for different analytes: biopolymers (proteins, DNA, etc.), toxins, drugs, ions, etc. [3]. However, such versatility has also a drawback: the SPR sensors are sensitive to many other effects which also influence the refractive index of media.

In the typical case of implementation of SPR biosensing technologies, a parallel *p*-polarized light beam is reflected from thin metallic layer deposited on the surface of glass (see Fig. 1a). The reflectivity (ratio of reflected and incident light intensities) in such case depends on the coupling condition of incident light to surface plasmons, which is described by well-known relation for the wavenumber k_x:

$$k_x = \frac{2\pi}{\lambda}\sqrt{\frac{\varepsilon_1\varepsilon_2}{\varepsilon_1+\varepsilon_2}} = \frac{2\pi}{\lambda}\sqrt{\varepsilon_0}\sin\theta,$$

where λ is the free-space wavelength of incident light, θ its incidence angle, ε_0, ε_1 and ε_2 are dielectric permittivities of the glass, metal film and aqueous solution correspondingly. A plot of the reflectivity of SPR biosensor versus incidence angle (and/or wavelength) shows a strongly pronounced dip, whose exact position and shape depend also on ε_2 thus providing information on the environment near exposed metal surface (see Fig. 1a). On Fig. 1 such curves are shown for SPR biosensor consisting of 50 nm gold layer with 2 nm Cr adhesion layer deposited on the coupling prism with high refractive index (F2, $n = 1.6$). The gold layer is exposed to water. A deposition of a thin organic layer on the gold surface shifts the curve to the right (see Fig. 1b). This shift characterizes the thickness and the refractive index of the layer. In many cases both incidence angle and wavelength of the incident light are fixed. Such case is shown in Fig. 1c: the light with the wavelength 650 nm is incident at an angle of 64°. In response to increasing thickness of the organic layer a shift of the SPR curve to the right is observed, this leads to an increase in the intensity of the reflected light. Usually the point with the highest slope on the descending part of SPR curve is taken (like shown at 64°). This is not a mandatory requirement, moreover,

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[☆] The topic was presented at the 7th European conference on Optical Sensors and Biosensors, Athens, Greece, 13–16 April 2014 only as a generally written abstract without disclosure of the principle.

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http://dx.doi.org/10.1016/j.snb.2014.10.022

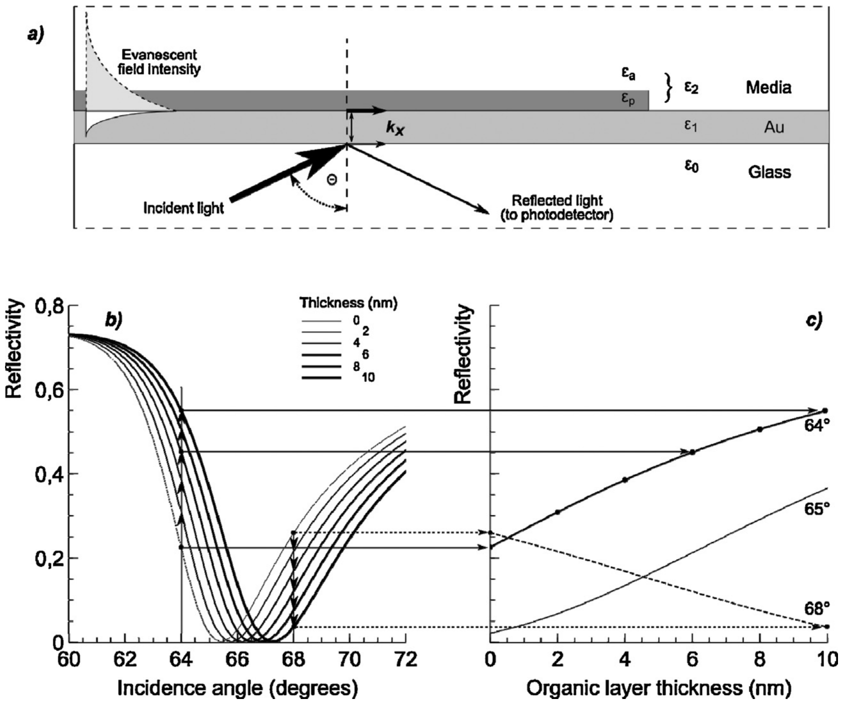

Fig. 1. Kretschmann configuration for SPR measurement (a), SPR curves calculated for different thickness of organic layer on the metallic surface (b) and corresponding reflectivity changes measured at fixed incidence angle (64, 65 and 68°). Parameters for the calculation: metallic layer consists from 2 nm Cr and 50 nm Au layer (refractive indices: Cr: 3.1045 + 3.3273i, Au: 0.1678 + 3.1377i); glass – F2 (refractive index 1.6154); wavelength – 650 nm; organic layer with refractive index of 1.4882 and thickness of 0, 2, 4, 6, 8 or 10 nm is contacting with water at 22 °C (refractive index of water: 1.3317).

fixing of the incidence angle closer to the SPR minimum (e.g. at 65°) improves signal to noise ratio despite the loss of the absolute magnitude in the light intensity changes [4]. If the incidence angle is larger than the resonance angle (Fig. 1c, 68° curve), the sensor response becomes negative: an increase in the thickness of the organic layer results in the decrease in the reflected light intensity.

However, SPR curves are shifted not only because of the changes of layer thickness or its composition: a contribution of the bulk solution behind this layer might be even more important. The SPR-sensor measures the change of the effective refractive index near metal surface [5]:

$$n_{eff} = \frac{2}{z_2} \int n_2(z) \times \exp(-2z/z_2) dz$$

where $n_2(z)$ is the distribution of refractive index in aqueous media defined by dielectric permittivity of adsorbed layer (ε_p) and bulk aqueous solution (ε_a), z_2 is the penetration depth of the evanescent wave. For the described sensor layout the penetration depth is about 190 nm, therefore the contribution of a few nanometers thick organic layer is much smaller than the contribution of the bulk solution. It leads to cross-sensitivity of SPR sensor to variations of solute concentration or temperature. This is especially critical in many biosensing applications: a change of temperature for 0.1 °C would lead to changes of the refractive index for 8×10^{-6} [6], which is higher than the effect due to adsorption of analytes with low molecular weight. That is why the referencing in SPR biosensors is very important, otherwise the useful signal can be masked by changes of the refractive index in the bulk.

A number of referencing approaches for SPR sensors were suggested. A common approach is based on introduction of a dedicated referencing channel, spatially separated from the sensing one and coated by a material with a decreased affinity to the analyte of interest. Subtracting the signal of the referencing channel from the signal of the sensing channel, possible changes of the volume refractive index [7,8] can be compensated. The overall efficacy of such spatial referencing is increased by scaling down mean distance between the sensing and referencing spots into microscopic range while maintaining macroscopic value of total area of the sensing and referencing spots; this approach named as distributed referencing was suggested in [9]. The idea of having separate reference and sensing area in the same flow cell was extended into an array of microfluidic cells [10]. Separate measurements of SPR phase shifts in the referencing and sensing spots can be used for self-referencing as well [11]. A diffraction grating formed by the sensing and referencing strip shaped spots was used for inherent self-referencing [12]. Also other realizations of self-referencing SPR transducing based on the patterning of sensing and referencing spots were suggested. In [13–15] the reference channel was implemented using a 12–18 nm thick dielectric (Ta_2O_5) layer. Alternatively, a 35 nm thick plasma polymerized hexamethyldisiloxane layer was used [16]. Two distinct spectral bands, associated with SPR in the sensing and referencing spots, were measured by spectrophotometer and quantified simultaneously.

The spectral readout is poor compatible with SPR imaging mode which is of high importance for sensor arrays and high-throughput techniques. This was a motivation for the development of self-referencing techniques based on SPR imaging at single wavelength. In [17] two kinds of multilayer spots were used in pairs: differing by Ti sublayer of either 4 or 12 nm, on the top of which a 200 nm aluminum oxide layer and a 40 nm gold layer were deposited. SPR response in each pair of these elements showed an increased SPR sensitivity. In [18,19] a microwell array in the gold layer or in the glass substrate was described, bottom of wells and their surroundings serve as sensing or referencing areas.

Spatial separation of sensing and referencing spots results in a loss of precious sensor surface. Another frequent drawback is a requirement of these techniques for a pre- or post-determination of the positions of sensing and referencing spots. This was a motivation for the development of other self-referencing techniques