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Bimetallic Layers Increase Sensitivity of Affinity Sensors Based on Surface Plasmon Resonance

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Abstract: Two metals are used in resonant layers for chemical sensors based on surface plasmon resonance (SPR) - gold and silver. Gold displays higher shift of the resonance angle to changes of ambient refraction index and is chemically stable. Silver posses narrower resonance curve thus providing a higher signal/noise ratio of SPR chemical sensors, but has a poor chemical stability. A new structure of resonant metallic film based on bimetallic silver/gold layers (gold as an outer layer) is suggested. It combines advantages of both gold and silver resonant layers. Bimetallic resonant films display so high shift of resonance angle on changes of ambient refraction index as gold films, but show narrower resonance curve, thus providing a higher signal / noise ratio. Additionally, the outer gold layer protects silver against oxidation.

Keywords: Surface plasmon resonance, Biosensor, Sensitivity, Signal / noise ratio

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Introduction

An application of surface plasmon resonance (SPR) for detection of biomolecular interactions is based on the use of either monochromatic [1-3] or white light. In the first case, a devices (Biosuplar from *Analytical μ-Systems*, Germany; Biacore from *Biacore AB*, Sweden) measures an exact position of the plasmon resonance angle [1-3], while in the second case the light wavelength corresponding to plasmon excitation is measured [4,5].

A using of surface plasmon resonance as transducer for affinity sensors allows to get relatively high sensitivity, however detection of small molecules is still problematic. Meanwhile, exactly this limitation hampers a wide application of real time affinity sensors in pharmacological screening and other fields of modern biological and medical science. In this case, even a small increase of sensitivity can open a way to use SPR technology for a new class of substances. Last years, a number of attempts have been made to improve this technique, they include a polarization of the incident light beam [6, 7], a waveguide propagation of light [8-10] or using of porous active phases or even nanocrystals and nanoparticles [11-18]. We suggest here a new way to increase a signal / noise ratio of SPR devices.

Let us to consider here the SPR-devices based on a monochromatic light only. A binding of molecules to the immobilized on the metal surface receptor layer results in increase of the refraction index near metal surface thus providing a shift of the resonance angle. Therefore, we will define here the sensitivity of SPR-transducer as a minimal change in ambience refractive index which can be detected by the SPR- device. A narrower width of SPR curves with silver active layer led to numerous attempts to use this metal [19-24], however it requires a deposition of protective layers of alkylthiols [21], inorganic oxide [24] or zirconium acetate [23]. In the present paper we suggest a new approach to improve the sensitivity of SPR-based biosensors which is based on using of bimetallic layers as an active phase for plasmon excitation.

Experimental

Metallic films were thermally deposited onto polished glass substrates in vacuum of 10^{-3} Pa. A total thickness of metallic layer was 50 nm. Immediately after deposition, the samples were placed in 5 mM solution of 1-hexadecanthiol (*Aldrich*) in ethanol for 48 h, then rinsed shortly with chloroform and dried. A commercial version of SPR-device Biosuplar-2 from *Analytical μ-Systems* (Regensburg, Germany [3]) was used for SPR-measurements. An influence of refraction index on resonance angle was investigated in aqueous solutions of NaCl and ethanol (*Merck*), their refraction indexes were measured independently by an Abbé refractometer IRF-22. As a model biological compound, solutions of bovine serum albumin (*Sigma*) of 10 - 20 μg/ml dissolved in buffer 150 mM KCl, 5 mM phosphate, pH 7.0, were used. The experiments were performed at room temperature (22±2°C). Solutions were prepared with bidistilled water.

Computer simulation

The main value measured by SPR-based devices with monochromatic light is the angle $\varphi_{\min}$, corresponding to the minimal intensity of the reflected light $I(\varphi)$. This angle is determined from the measured angle dependence of the reflected light $I(\varphi)$. The behavior of a smooth function $I(\varphi)$ near its extremum can be approximated by a parabolic function. A signal noise leads to the data scattering around this function. $\varphi_{\min}$ can be calculated by standard mathematical procedures. The bigger is the curvature of the function $I(\varphi)$ near the minimum (i.e., the narrower is its minimum), the higher is the accuracy of $\varphi_{\min}$ determination.

A sensitivity of a SPR technique may be defined as a shift of φ_{min} value ($\Delta\varphi_{min}$) due to change of the ambience refractive index N_s for ΔN_s. Using mathematical equations described in [25], the values $\Delta\varphi_{min}$ and the widths $\Delta\varphi_{0.2}$ of the curves $I(\varphi)$ were calculated for ΔN_s=0.015 RIU for different active layers. The width $\Delta\varphi_{0.2}$ was defined as a width of resonance curve at the level of light intensity corresponding to 0.2 from its maximal value (Fig. 1). The calculations were performed for bimetallic films consisting of silver film and gold film of varied thickness with a constant total thickness of 50 nm. The following values of optical constants for gold and silver were used: $N_{Au} = 0.2 + 3.6i$, $N_{Ag} = 0.12 + 4.2i$. The results of calculations are presented in the Table 1.

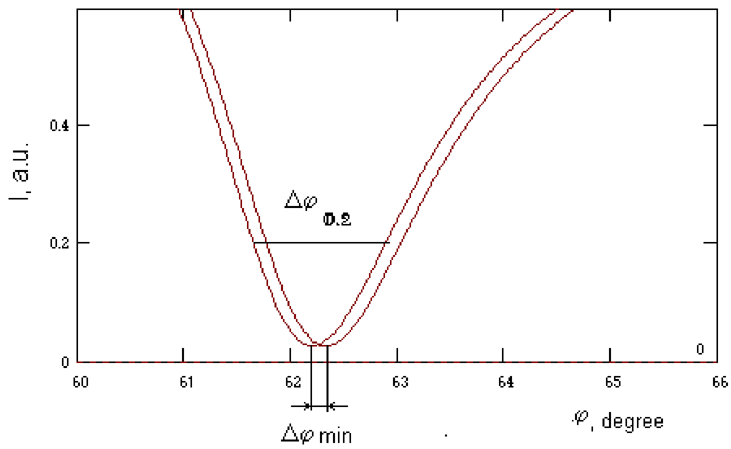

Figure 1. Parameters of SPR curve: the shift of the minimum ($\Delta\varphi_{min}$) and the width measured at the light intensity corresponding 0.2 of the maximal one ($\Delta\varphi_{0.2}$).

The data show that gold films provide the highest shift of the resonance angle ($\Delta\varphi_{min}$). An introduction of a silver sublayer only slightly diminishes the angle shift, but makes the minimum width much narrower, thus providing a higher accuracy of detection of the resonance angle. Such trends were observed for glass prisms with different refraction index. Assuming that the detection accuracy is proportional to $1/\Delta\varphi_{0.2}$, the signal/noise ratio should be proportional to $\Delta\varphi_{min} /\Delta\varphi_{0.2}$. Therefore, the resulting effect of a silver layer on the detection accuracy (signal/noise ratio) can be estimated as $\Delta\varphi_{min} /\Delta\varphi_{0.2}$. The last column in Table 1 demonstrate that the maximal effect is reached for pure silver films. A substitution of silver by gold in the outer part of the metallic layer leads to some decrease of the $\Delta\varphi_{min} /\Delta\varphi_{0.2}$.

Table 1. Results of computer simulation of SPR curves (ΔNs=0.015 RIU).

	N_{glass} / d_{Ag} (nm) / d_{Au} (nm)	$\Delta\varphi_{min}$, degree	$\Delta\varphi_{0.2}$, degree	$\Delta\varphi_{min} /\Delta\varphi_{0.2}$
1	1.61 / 0 / 50	0.14	1.91	0.073
2	1.61 / 25 / 25	0.12	1.43	0.083
3	1.61 / 32 / 18	0.12	1.22	0.097
4	1.61 / 40 / 10	0.12	1.06	0.113
5	1.61 / 50 / 0	0.12	0.75	0.159
6	1.51 / 0 / 50	0.2	2.55	0.078
7	1.51 / 40 / 10	0.18	1.75	0.102

The result obtained is not surprising: indeed, the real part of the dielectric constant for silver is substantially higher than that for gold. The higher sensitivity of SPR-devices with silver layer was probably a reason to use this metal in many chemical and biological sensors. However, the low chemical stability of silver requires a deposition of passive protective layers [22-23], which leads to technological difficulties and to decrease of the signal/noise ratio. We suggest to create this protective layer using a thin gold layer, which works also as an active phase for plasmon exciting; it allows us to protect the silver layer with only a small loss of the signal/noise ratio.

Experimental Results

Theoretical predictions were proved by direct experiments. The influence of refraction index of ambient solution on the shift of resonance angle was measured for different active phases for plasmon excitation: gold, silver and bimetallic gold/silver layers (Fig. 2). In the case of bimetallic layers, the silver layers were placed between gold layers and glass prism. The shifts of the resonance angle measured on gold layers were about 10% higher than on the silver layers, while the bimetallic layers provided about the same shift as the pure gold layers (Fig. 3). It confirms results of the computer simulation presented in Table 1. Therefore, an introduction of a silver layer under the gold layer do not decrease a shift of the resonance angle significantly.

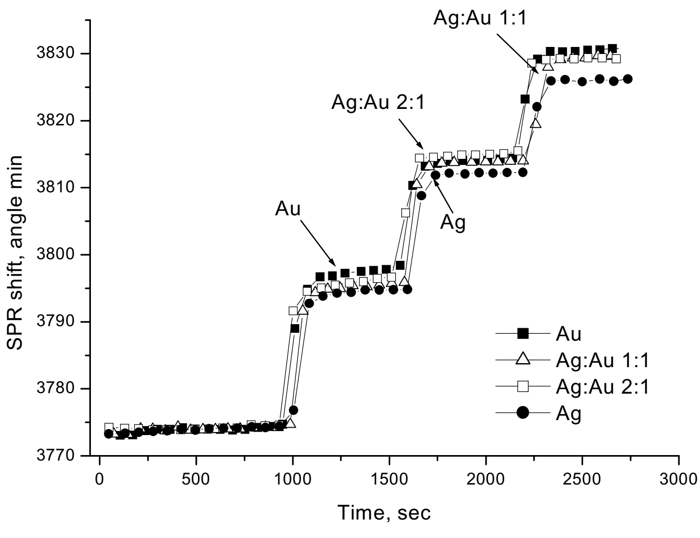

Figure 2. SPR response for various (BI)metallic layers as a result of changes in the refractive index of ambient liquid. Solutions of sodium chloride with increasing concentrations were used, every concentration step resulted in an increase of the refractive index for 0.003.

The resonance curves obtained for different metal films are presented in Fig. 4. The measurements were performed in aqueous solutions with metallic layers consisting of gold (■), silver (●) or bimetallic silver-gold (silver as an inner layer) films (Δ, □, ★). These resonance curves confirm theoretical prediction that an introduction of a silver layer between prism and gold film substantially decreases the curve half-width around resonance angle (Table 1). This improves the accuracy of determination of the curve minimum thus providing a higher signal-to-noise ratio.

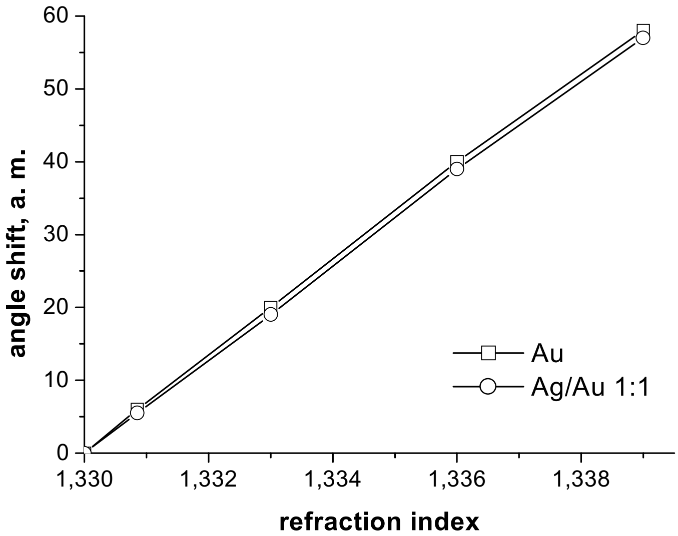

Figure 3. Influence of refractive index of ambient aqueous solution on resonance angle, measured with gold and gold/silver active phases. Refraction index was modified by addition of ethanol. The bimetallic layer consisted of equally thick silver and gold layers, the gold layer was in contact with the solution.

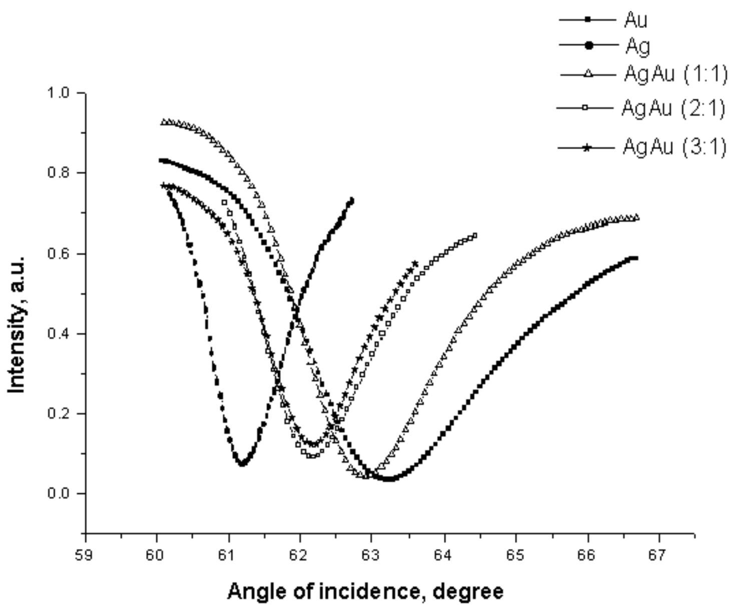

Figure 4. Experimental SPR curves for various (bi)metallic layers.

SPR sensorogramms obtained for adsorption of bovine serum albumin on hexadecanethiol coated gold and bimetallic gold/silver layers are presented in Fig. 5. Both records were measured in identical experimental conditions in tracking mode with the same measurement time, same signal analysis mode and parameters of this analysis. The noise records are magnified and compared in Fig. 6. As one can expect from theoretical analysis, the narrower resonance curve indeed leads to lower noise of the signal and to higher signal/noise ratio.

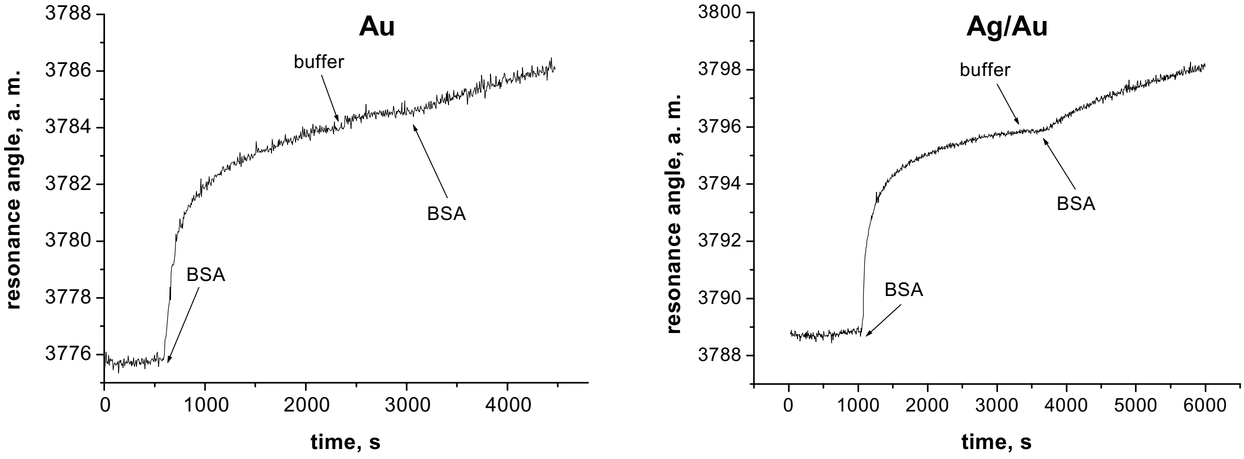

Figure 5. Sensorograms for adsorption of bovine serum albumin onto gold (left) or bimetallic (right) silver/gold (thickness 1:1, silver as inner layer) coated by 1-hexadecanthiol. Buffer: 150 mM KCl, 5 mM phosphate, pH 7.0 (right).

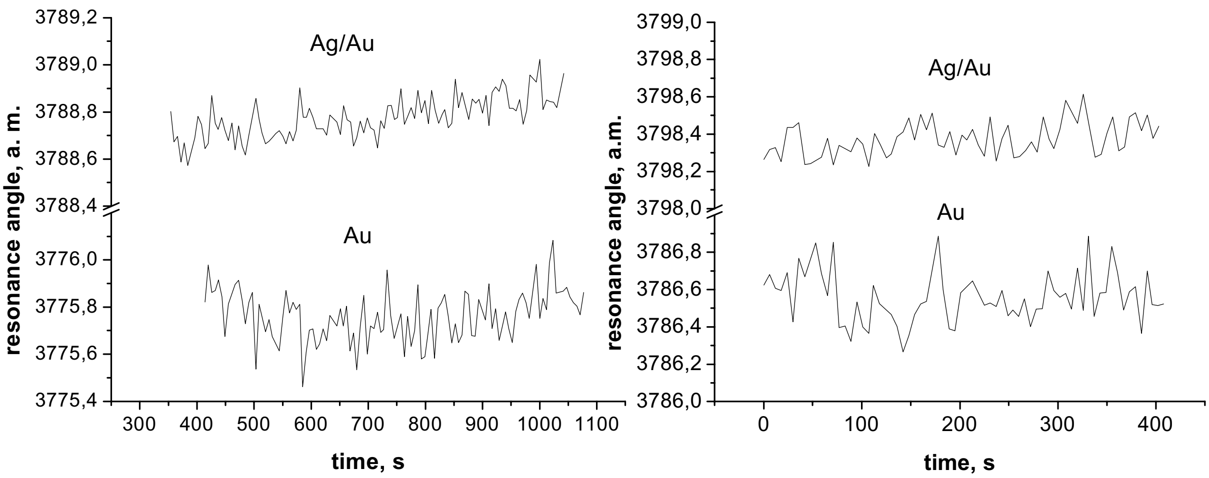

Figure 6. Typical records of noise of resonance angle measured with gold and with bimetallic silver/gold (thickness 1:1, silver as inner layer) in water (left) and after adsorption of bovine serum albumin (20 μg/ml) in 150 mM KC, 5 mM phosphate, pH 7.0 (right).

The signal noise measured in different conditions, was compared quantitatively. The noise was measured as a standard deviation of 200 measurements of the resonance angle. Slow signal changes, caused by temperature fluctuations or/and adsorption of species, were approximated by the cubic polynome and subtracted. The results presented in the Tables 2 and 3. At all conditions studied, the noise of the signal, measured with bimetallic active layers, was less. The noise decrease was about 20% for bimetallic layers of equal thickness and about 40% if the silver layer was 2 times thicker than the gold one.

Table 2. Standard deviation of the resonance angle measured on different resonance phases: an application as a refractometer.

Active layer	**Stand. deviation, a. m. H_2O/NaCl**	**Stand. deviation, a. m. H_2O/ethanol**
Au	0,124457	0,104036
Ag/Au 1:1	0,114879	0,099494
Ag/Au 2:1	0,073425	0,063679
Ag	0,054529	0,052653

Table 3. Standard deviation of the resonance angle measured on different resonance phases: an application for investigation of adsorption or as an affinity biosensor.

Active layer	**Stand. deviation, a. m. 150 mM NaCl, pH 7.0**	**Stand. deviation, a. m. 150 mM NaCl, pH 7.0 + BSA, 20 μg/ml**
Au	0,11	0,10 - 0,14
Ag/Au 1:1	0,09	0,06 - 0,09

A comparison of silver, gold and bimetallic silver/gold layers as active phases for chemical sensors and biosensors based on surface plasmon resonance, has shown that the bimetallic layer (with silver as an inner layer) posses essential advantages. On the one hand, they provide so high shift of the resonance angle to changes of ambient refraction index as gold layers, but have narrower resonance minimum. It leads to an increase of the signal/noise ratio of SPR devices. On the other hand, the outer gold layer, being chemically inert, protect the inner silver layer from oxidation. This new type of resonance layer can be used in all types of chemical and biological sensors based on surface plasmon resonance. An increase of the signal/noise ratio allows user to monitor a binding of smaller molecules, what could be critical for a large number of applications in pharmacology, biology and medicine.

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Sample Availability: Available from the author.