DOI: 10.5604/20830157.1121374

APPLICATION OF A BIOSENSOR BASED ON SPR USING SPECTROSCOPIC ELLIPSOMETER

Katarína Bombarová, Juraj Chlpík, Július Cirák
Faculty of Electrical Engineering and Information Technology, Institute of Nuclear and Physical Engineering, Bratislava

Abstract. *The work is focused on the possibility to use spectroscopic ellipsometer in biosensor application. Operation of the ellipsometer is enhanced by the feature of TIRE (Total Internal Reflection Ellipsometry). The principal goal is to detect analytes in aqueous solutions at low concentrations below 0.1 nmol/l.*

Keywords: SPR, Ellipsometry, TIRE, biosensor

ZASTOSOWANIE CZUJNIKA BIOLOGICZNEGO BAZUJĄCEGO NA POWIERZCHNIOWYM REZONANSIE PLAZMONU (SPR) UŻYWAJĄCEGO ELIPSOMETRII SPEKTROSKOWPOWEJ

Streszczenie. *Praca koncentruje się na możliwości wykorzystania elipsometrii spektroskopowej w aplikacjach czujnika biologicznego. Działanie elipsometru potęguje cechy TIRE (ang. Total Internal Reflection Ellipsometry). Głównym celem jest wykrywanie składników czy substancji chemicznych w roztworze wodnym przy niskich stężeniach poniżej 0,1 nmol / l.*

Słowa kluczowe: SPR, elipsometria, TIRE, biosensor

Introduction

Surface Plasmon Resonance (SPR) is an effect occurring at the metal/dielectric interface. This phenomenon allows toobserve nano-changes in thickness, density fluctuation or molecular adsorption on a metal surface [4]. Surface Plasmon Resonance (SPR) sensors are widely used in biochemistry and pharmacology due to their high sensitivity. They are based on the angular measurement of reflectance of p-polarized light reflected from a metal-dielectric surface.

For a specific wavelength of light and an angle of incidence when the condition of surface plasmon generation is fulfiled, the light energy leaks to the surface plasmon polariton and a rapid decrease in R_p is observed. The resonance condition is highly sensitive to material properties at the metal-dielectrics interface. If any change occurs near the metal surface, e.g. organic molecules are adsorbed onto the metal, the minima in reflectance are shifted to different angles of incidence or wavelengths [5].

Total Internal Reflection Ellipsometry (TIRE) combines two techniques, ellipsometry and SPR, in order to reach better sensitivity. By TIRE, higher sensitivity can be achieved allowing monitoring small molecules binding to the metal substrate. The method can be used to detect affinity interactions on the surface in optical biosensors.

The presented work shows possibility of using ellipsometer Horiba Jobin-Yvon MM-16 enhanced by TIRE as a biosensor based on SPR [3]. Spectroscopic ellipsometer is a device set to investigate properties of thin layers on a solid substrate. The measurement time of spectroscopic ellipsometer is sufficient enough for monitoring slow surface chemical processes. Moreover, SPR enhancement improves sensitivity of the ellipsometer below 1 nm in layer thickness.

1. Ellipsometry and TIRE

Ellipsometry is a nondestrustive optical method used in a wide range of thin layers applications. The basic principle is observation of polarization state changes in the reflected light. After the reflection of light from a layered surface, both the amplitude and the phase of p and s polarization components are changed. Analyzing the data measured, the ellipsometric parameters Δ (phase difference) and ψ (ratio of Fresnel's reflection amplitudes) are extracted using the ellipsometric equation [2]

$$r_p / r_s = \tan\psi\, e^{i\Delta} \tag{1}$$

where r_p, r_s are complex Fresnel's reflection amplitudes [3].

When specific conditions (angle of incidence AOI, wavelength) for SPR generation are fulfilled, the energy of p component of incident light is transformed into the surface plasmon polariton. This resonant process results in light intensity extinction, which could be observed as a considerable minimum in reflectance R_p spectra (Fig. 2 left). TIRE can be applied only when the light passes through optical boundary with the lower index of refraction on the other side and under the total internal reflection condition. If the incident angle is greater than the critical (cut off) angle, the beam does not penetrate into the second medium and the evanescent wave occurs.

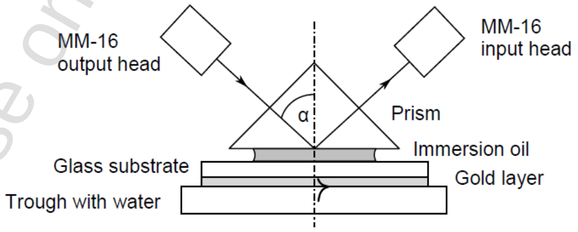

Fig. 1. TIRE optical scheme

2. Results and discussion

Kretschmann's configuration was used in the TIRE experiment. Light beam emitted from the ellipsometer output head passes the optical right-angle BK-7 prism and impinges under the total reflection condition on the glass plate covered with 50 nm gold layer.

The glass plate was optically coupled to the prism via immersion oil. The plate with the gold layer was attached to the Teflon trough of 3 cm^3 (Fig. 1). The spectroscopic polarimeter Horiba Jobin MM-16 with a spectral range of 430 – 850 nm and 2 nm resolution was used. The device is equipped with a motorized goniometer providing the external angle of incidence between 45° and 90° with 0.01° step [1].

Taking into account 2 nm spectral resolution of MM-16 and considering SPR generation as a resonant process, Δ parameter measurement in the vicinity of the SPR point is more sensitive than R_p. Comparing the standard deviation $\sigma_\Delta = 0.62°$ of Δ as given by a long-term measurement and the temperature gradient of water near the room temperature $\partial n/\partial T = -8.0\times10^{-5}\ \mathrm{K}^{-1}$, the standard deviation of the refractive index of water $\sigma_n = 3.3\times10^{-6}$ was obtained.

This number indicates that variations of material parameters can be determined at the level of order of magnitude 10^{-5} using this method. The gradient of rapidly falling Δ in the vicinity of the resonance wavelength (λ_R = 682 nm) was –1.52° per the limiting AOI step (Fig. 2) [2].

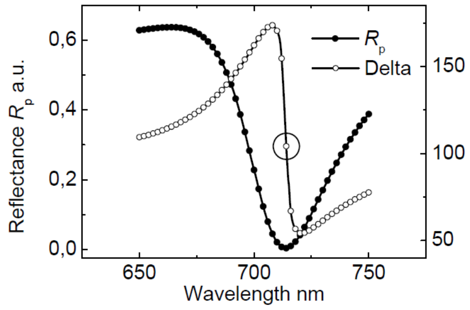

Fig. 2. Reflectance of p-polarized light vs. Δ spectra simulation

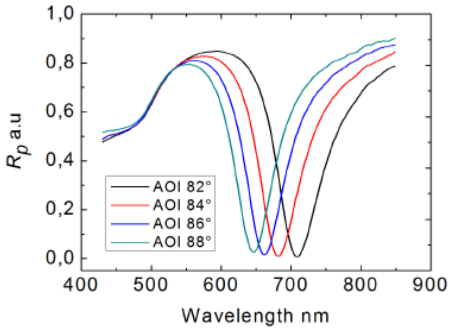

Fig. 3. Shift by the change of different angle of incident

We carried out several measurements to prove how the SPR effect depends on the angle of incidence (Fig. 3). The varying conditions also have an influence on the dependence of Δ on wavelength, as it is obvious from Fig. 4. The slopes of the linear parts are as follows -115, -59.7, -25.9, -15.2, and -8.5 in unit’s°/nm (Fig. 4).

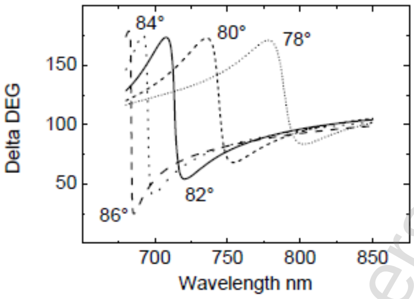

Fig. 4. Comparison of Δ for different angles of incidence

A solid line in Fig. 5 depicts gradient of Δ at different angles of incidence. A dashed line shows a course of SPR wavelengths vs. angle of incidence. The slope of Δ in the linear part is 30.3 DEG/nm and the optimum angle of incidence 82°.

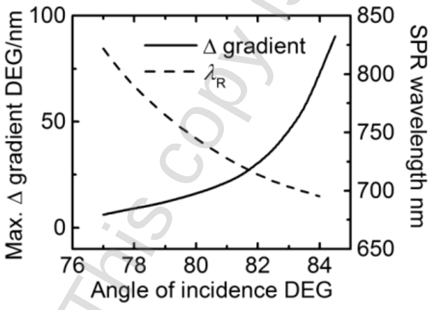

Fig. 5. The dependence of the slope of Δ and SPR wavelengths vs. angle of incidence

Fig. 6 contains the measured dependence of ellipsometric parameter Δ on the angle of incidence (AOI). The measurement documents the ability of spectroscopic ellipsometry to record really subtle changes in SPR generation conditions.

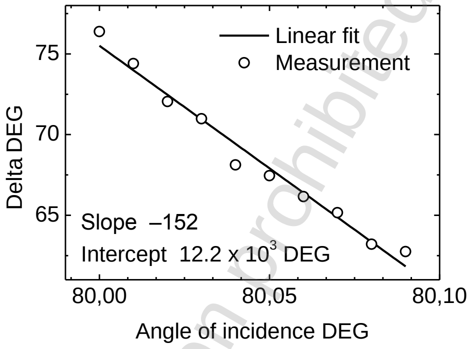

Fig. 6. Limiting angle of incidence step measurement

Some thermal measurements are shown in Fig 7a and 7b. The water was cooled from temperature 35° to room temperature (19°). The temperature of water was measurement by a digital thermometer. The angle of incidence was set to 83° and Δ was measured at 677 nm. Fig. 7a shows a comparison of the measurement and the theoretical model. The reason of the difference between the measurement and the calculation at higher temperature is caused by the fact that the theoretical model includes only temperature and refractive index of water. In the real experiment the increasing temperature is relevant also for the glass substrate and the prism.

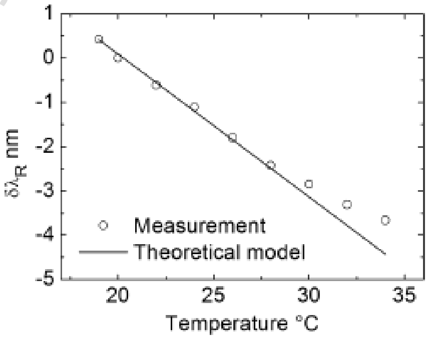

Fig. 7a. Comparison of theoretical model with experiment for temperature measurement

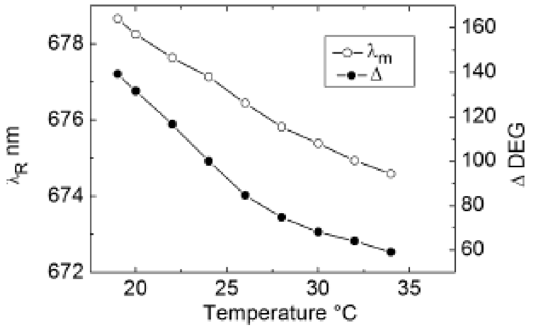

Fig. 7b. Dependence of the resonant wavelength of SPR on the temperature of water

In our theoretical calculations we were able to estimate changes in R_P and Δ attributed to subtle variations in thickness of the organic layer. In Fig. 8 a simulation of increasing thickness of the layer is presented and its effect on the wavelength. The SPR resonant wavelengths are 717.97 nm and 718 nm, for thickness of 2 nm and 2.01 nm, respectively.

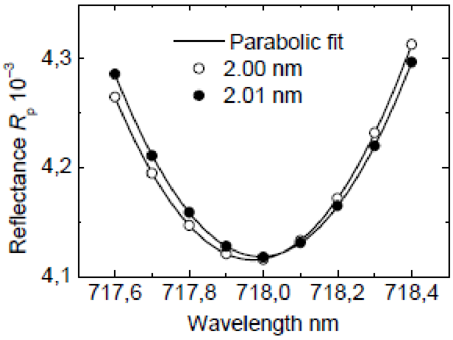

Fig. 8. Simulated R_p *minimum shift after the organic thickness was increase*

It means that the increase in thickness of the organic layer with the index of refraction of 1.45 between 2.00 nm and 2.01 nm caused a SPR resonant wavelength shift by 0.03 nm, which is beyond the MM-16 spectral resolution and this effect, cannot be detected at all. On the other hand, as to the parameter *Δ*, it changes by 0.59° and this shift is measurable. The change of the *Δ* parameter with increasing thickness of the layer on the metal surface with step 0.01 nm is visible from Fig. 9. The working point was chosen at 718 nm.

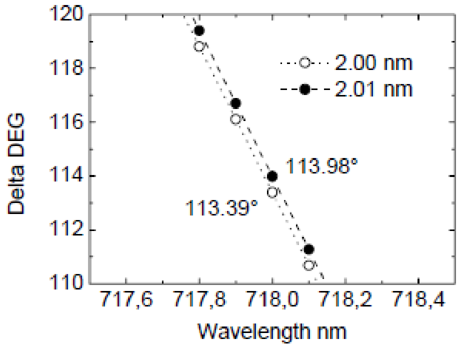

Fig. 9. Change of the Δ parameter with increasing thickness of layer

As biosensor application a monolayer of an aptamer molecule for human thrombin deposited on 50 nm gold layer was used. The exponentially shaped saturation curve of the thrombin adsorption on the aptamer receptor is shown in Fig. 10. The detection limit of thrombin concentration in the buffer solution was less than 0.3 nmol/l.

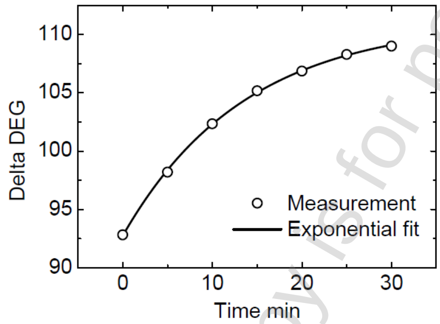

Fig. 10. Experimentally measured thrombin adsorption onto aptamer receptor

3. Remarks and conclusion

The possibility of using spectroscopic ellipsometer Horiba Jobin-Yvon MM-16 enhanced by TIRE as an SPR biosensor was shown. In the vicinity of the SPR resonance λ_R the rapid linear change of *Δ* was exploited to detect the limiting index of refraction of water change $\sigma_n = 3.3\times10^{-6}$ and to reveal the exponentially saturated adsorption process of 0.3 nmol/l concentration of the human thrombin on aptamer biosensor. TIRE approach serves as a sufficient step to achieve considerably enhanced sensitivity of the device in monitoring biomolecules adsorption processes.

Acknowledgements

This work was financially supported by grant of Science and Technology Assistance Agency No's APVV-0262-10 and APVV-0108-11.

Bibliography

[1] Chlpík J., Sohová M., Hianik T., Cirák J.: Total internal reflection ellipsometry for biosensor applications. Proc. of 19th International Conference on Applied Physics of Condensed Matter (APCOM 2013), 2013, pp. 254-257.
[2] Fujiwara H.: Spectroscopic Ellipsometry. Principles and Applications. John Wiley & Sons, 2003.
[3] Nabok A. V., Tsargorodskaya A., Hassan A. K., Starodub N.F.: Total internal reflection ellipsometry and SPR detection of low molecular weight environmental toxins. Applied Surface Science, vol. 246, 4/2005, pp. 381-386.
[4] Wolfbeis O. S., Homola J.: Surface plasmon resonance based sensors. Springer, 2006.
[5] Wood R. W.: Philos. Mag. 1900.

M.Sc. Katarína Bombarová
e-mail: katarina.bombarova@stuba.sk

Katarína Bombarová studied physics at Faculty of mathematics, physics and informatics at Comenius University. She graduated at Department of experimental physics, specialization Optics, laser and optical spectroscopy. She does her Ph.D. at Institute of Nuclear and Physical Engineering at the Faculty of electrical engineering and information technology STU in Bratislava.

RNDr. Juraj Chlpík, Ph.D.
e-mail: juraj.chlpik@stuba.sk

Juraj Chlpík received his Ph.D. degree in Optics and Optoelectronics in 2010 at the Faculty of Mathematics, Physics and Informatics, Commenius University in Bratislava. He is working as a researcher in International Laser Center in Bratislava, and since 2008 as an Assistant Professor at the Faculty of Electrical Engineering and Information Technology, Slovak University of Technology in Bratislava.

Prof. Ing. Július Cirák, C.Sc.
e-mail: julius.cirak@stuba.sk

Július Cirák graduated from the Faculty of Electrical Engineering, Slovak University of Technology, Bratislava, in 1976, and received his C.Sc. (Ph.D.) degree in Experimental physics in 1981. He joined the Department of Physics at the same Faculty, as an Assistant (1982), Associate (1988) and Full Professor (2008). His research experience and interest include: organic molecular thin layer systems and nanostructures; technology, study of electrical and optical properties and applications for organic electronics.

otrzymano/received: 19.06.2014 *przyjęto do druku/accepted: 19.06.2014*