1. Introduction

Surface plasmon resonance (SPR) sensors are widely used in biochemistry and pharmacology due to their high sensitivity. They are based on 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 fulfilled, 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 on the metal-dielectrics interface. If any change occurs near the metal surface, e.g. organic molecules are adsorbed to the metal, the minima in reflectance are shifted to different angles of incidence or wavelengths [1]. Although such a principle is able to detect adsorption of organic molecules, its sensitivity seemed to be insufficient for detection of analytes low concentrations with typical values of 1 pmol/l – 1 nmo/l. It has been shown that spectroscopic ellipsometry of total internal reflection can enhance sensitivity, especially if the surface plasmon resonance phenomena is utilized [2]. The method is usually referred to Surface Plasmon Enhanced Ellipsometry (SPEE) [3] or recently is more commonly known as Total Internal Reflection Ellipsometry (TIRE) [4]. Although spectroscopic ellipsometry is relatively expensive method comparing to SPR, it provides more information about the reflected light. An ellipsometry measurement does not depend on the light intensity, however, the information about the state of light polarization brings a benefit in analyzing phase, which is rapidly changing in the vicinity of surface plasmon resonance.

In this work, the ability of Horiba Jobin-Yvon MM-16 spectroscopic ellipsometer for using in biosensor applications is shown.

2. Numerical analysis of TIRE

One of the difficulties in TIRE experiment consists in placing a measuring cell properly into the ellipsometer optical setup. The position of the SPR resonance peak in the measured spectra strongly depends on the angle of incidence. The sensitivity of the parameter $\Delta$ is also influenced by the tilt of the prism and the glass plate covered with a gold layer. The simulation algorithm based on transfer matrices was developed to analyse the sensitivity of TIRE experiment and its optimization. The software is able to calculate the complex amplitude of reflectance $r_p$ and $r_s$ of both p- and s-polarized light. Using the ellipsometric equation [5]
the ellipsometric parameters $\psi$ and $\Delta$ can be obtained. The result of TIRE simulation with BK7 prism, BK7 glass plate coated by 3 nm thick adhesion chromium layer and 50 nm SPR gold layer, and water at 20 °C are shown in Fig. 1. The angle of incidence is 82°. The complex index of refraction of the materials are taken for BK7, water, chromium from [6] and for gold from [7].

To ensure a high sensitivity of the biosensor, the working point should lie on the linear part of $\Delta$, and, in addition, the slope of the linear part of the spectrum should be as steep as possible. Taking into account the capabilities of the MM-16 ellipsometer, especially its 2 nm spectral resolution, the proper angle of incidence and the $\Delta$ gradient need to be compromised. The linear part of $\Delta$ spectra must contain at least 3 measurement points (see Fig. 1). The dependence of the slope of $\Delta$ is evident from Fig. 2.

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

Fig. 1: Reflectance of p-polarized light vs. $\Delta$ simulation. The circle denotes the working point in the linear part of the $\Delta$ spectrum.

Fig. 2: Comparison of $\Delta$ for different angles of incidence. The slopes of the linear parts are as follows (from left to right): $-115$, $-59.7$, $-25.9$, $-15.2$, and $-8.5$ in units °/nm.

$$ \rho_p / \rho_s = \tan \psi e^{i \Delta} \quad (1) $$

Fig. 3: Simulated $R_p$ minimum shift after the organic layer thickness was increased. The SPR resonant wavelengths are 717.97 nm for 2.00 nm thickness and 718.00 nm for 2.01 nm thickness.

Fig. 4: Change of the $\Delta$ parameter by increasing the organic layer thickness. The working point was chosen as the closest one to the SPR wavelength, 718.00 nm.
Next simulation shows the sensitivity of the method to small changes on the metal surface of the sensor. Usually, in selective biosensor applications a monolayer of specific sensor molecule is deposited on the gold layer and molecules of analyte are diffused in the buffer solution in small concentrations. The analyte molecules are bound at the sensor surface resulting in a slight increase of effective thickness of the organic layer. The typical dimensions of such molecules are of several nanometers. In Fig. 3 and Fig. 4 the simulation of increasing thickness of the layer on the metal by 0.01 nm is shown where $R_p$ minimum shift and $\Delta$ change at the working point are depicted. The increase in thickness from 2.00 nm to 2.01 nm of the organic layer with the index of refraction of 1.45 causes a SPR resonant wavelength shift by 0.03 nm, which is beyond the MM-16 spectral resolution and this effect could not be detected at all. On the other hand, as to the parameter $\Delta$, it changes by 0.59° and this shift is measurable.

3. Experiment and Results

The spectroscopic ellipsometer Horiba Jobin-Yvon MM-16 enhanced by a homemade SPR cell in Kretchman’s configuration was used in TIRE experiments. Light beam emitted from the ellipsometer output head passed the optical right-angle prism and impinged under the total reflection condition upon the glass plate covered with 50 nm gold layer. The plate was optically joined to the prism side using immersion oil. The gold layer was laid on the water or buffer solution surface in the trough of 300 mm$^3$ volume. The spectral range of the ellipsometer is 430 nm – 850 nm with 2 nm resolution. The device is equipped by a motorized goniometer providing the angle of incidence from 45° to 90° with 0.01° step. The optical beam diameter is 1 mm.

![Graph showing the measured dependence of the ellipsometric parameter $\Delta$ on the angle of incidence (AOI). The measurement shows the ability of spectroscopic ellipsometry to record indeed small changes of SPR generation conditions. The limiting 0.01° step of AOI is not detectable in SPR when analysing $R_p$. It corresponds to 0.1 nm in the reflectance minimum shift, which is one order of magnitude below the device spectral resolution. However, the change of $\Delta$ is significant and measurable. Gradient of the dependence is $-1.52°$ per the limiting AOI step, whilst the statistical uncertainty obtained from long-term measurement is $\sigma_\Delta = 0.62°$ [8]. The result should be further improved by optimizing the experimental apparatus in accordance with the numerical analysis presented in this work.

The main goal of this work is to show that the MM-16 ellipsometer is technically capable to serve as a basic platform in construction of a selective biosensor for detecting low concentrations of analytes based on DNA sequences, toxins, etc. The result of biosensor experiment with DNA aptamer as a receptor for human thrombin is shown in Fig. 6. The
exponential saturation shape of thrombin in a buffer solution with concentrations of 0.3 nmol/l demonstrates a potential of the setup. The time constant is highly influenced by the presence of diffusion in the liquid. This effect should be avoided by using flow cell.

Fig. 6: Thrombin adsorption onto aptamer receptor. Concentration of thrombin in buffer solution was less than 0.3 nmol/l. The measured data are fitted by exponential saturation curve $y = y_0 - A \exp(-t/\tau)$, where $y_0 = (111 \pm 0.28)\degree$, $A = (18.4 \pm 0.27)\degree$, and $\tau = (13.6 \pm 0.51)\min$.

4. Conclusion
The possibility of using MM-16 ellipsometer enhanced by total internal reflection for a small concentration of analyte is shown. Since DeltaPsi2 software does not provide TIRE analysis, we developed our own code. Together with a flow cell the device would serve as a complete selective biosensor.

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References