

Polarimetric total *internal reflection* biosensing

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Abstract: In this paper, a concept of polarimetric total internal reflection (TIR) biosensor based on the method of temporal phase modulation is presented. Measurements of the phase difference between s- and p-polarized light combined with their amplitudes allow simultaneous detection of the bulk refractive index and thickness of the surface biofilms. Obtained experimental sensitivity is better than 10^{-5} in terms of refractive index unit and 0.5 nm in biolayer thickness. Relatively simple technological implementation of the TIR sensors on the base of inexpensive and transparent substrates opens a number of novel applications in biosensing and microscopy.

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high precision θ -2 θ goniometer (Thorlabs NR360S). In the experiments in air, a 45° prism was used whereas all tests in liquids were performed with a 60° prism connected to the flow injection cell with a 20 μ l working volume. Glass slides with functionalized surface, in immersion contact (Cargile Lab) with the prism, were used for biosensing whereas in the simplified set-up the base of the prism was used for tests.

The final periodic signal obtained by the detector with 45° oriented analyzer is decomposed into harmonics using a lock-in amplifier. Since the time domain signal is periodic and continuous, the harmonics of a frequency spectrum is modeled using a Fourier transform method. Thus, the first two harmonics are given by [10]:

$$F_1 = 2J_1(M)R_p R_s \sin(\alpha + (\phi_p - \phi_s)) \quad (1)$$

$$F_2 = 2J_2(M)R_p R_s \cos(\alpha + (\phi_p - \phi_s)) \quad (2)$$

Here, α is the initial phase retardance introduced by the SBC. R_p , ϕ_p and R_s , ϕ_s are reflection and phase under p- and s- polarization, respectively. J_n indicates a Bessel function of order n and M , the modulation amplitude. Phase retardation $\phi = \phi_p - \phi_s$ and intensity, I , can be derived from Eq. (1) and (2) and are given by [11]:

$$\phi + \alpha = \tan^{-1} \left(\frac{F_1 J_2(M)}{F_2 J_1(M)} \right) \quad \text{and} \quad I = R_p R_s = \sqrt{\left(\frac{F_1}{J_1(M)} \right)^2 + \left(\frac{F_2}{J_2(M)} \right)^2} \quad (3)$$

Using the Fresnel Formalism [12], we have performed theoretical modelling for the angular dependence of the first harmonic with different initial phase retardance α . The most characteristic curves are presented on Fig. 2(a). We obtain for $\alpha = 0$ a behaviour similar to the angular dependence of phase retardation between s- and p- component for TIR [12]. For the surface thin film testing in TIR configuration, application of phase difference as sensing parameter looks very promising [13]. At $\alpha = 90^\circ$, the curve is similar to the TIR angular intensity dependence where critical angle position follows the medium bulk RI. But, in this paper, we propose a method to test bulk RI as well as properties of thin films by measuring angular polarimetric curve (APC) obtained at $\alpha = -\Delta\phi_{\max}$, where $\Delta\phi_{\max}$ is the maximum possible phase difference at total reflection for given prism material. Indeed, this curve is a combination of the two parameters: intensity and phase, where angular position of the peak is sensitive only to the bulk RI and absolute value at the peak follows the dynamic of the surface film deposition (Fig. 2(b)). It is impossible to obtain similar results for SPR or TIR responses.

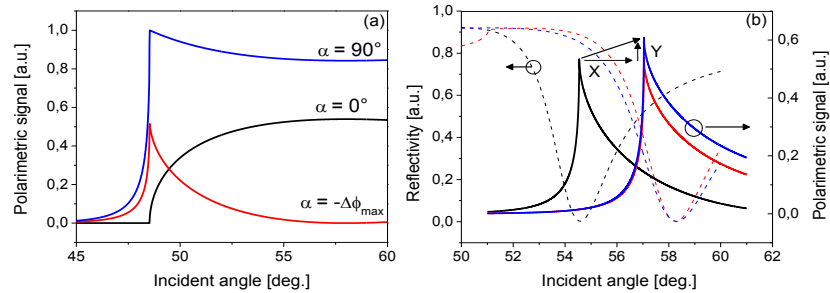


Fig. 2. (a) Theoretical angular polarimetric curve (APC) with different initial retardance α . (b) Comparison of APC ($\alpha = -\Delta\phi_{\max}$ solid lines) with SPR (dashed lines). Black curves are for tests in water and red curves after 10^{-2} RIU bulk changes; blue curves show influence of surface thin film deposition (10 nm, $n=1.45$).

Figure 2(b) presents theoretical results for the biosensing application of the proposed polarimetric TIR geometry (solid curves) in comparison with SPR method (dashed curves). As one can conclude, angular shift of the APC peak is comparable with SPR response for a bulk refractive index change equal to 10^{-2} RIU. Nevertheless, almost identical response could be obtained for SPR sensor after depositing a 10 nm thin film with 1.45 RI (dashed red and

blue line in Fig. 2(b)). This makes very difficult to test independently bulk refractive index changes and formation of thin biofilms on the surface. In contrast, APC preserves angular peak position (after bulk change) and shows only intensity changes after thin film deposition (red and blue solid curves in Fig. 2(b)). Here we propose to use these properties for biosensing, where angular peak position corresponds to the bulk refractive index changes (X displacement) and integral amplitude changes of APC or absolute value at the peak follows the dynamic of surface thin films formation (Y displacement). Note that APC is sharper than SPR resonance curve and, as sensing parameter, we measure the angular position and absolute value at maximum amplitude, as opposed to the SPR method where position of the minimum value of the curve is detected. Finally, TIR method sensitivity could be optimized with different prism materials, where higher prism refractive index provides higher phase changes and correspondingly, higher thin film sensitivity but lower angular shift to the bulk RI changes [12].

3. Experimental set-up optimization and phase sensitivity

In order to estimate the efficiency of the polarimetric experimental set-up for the proposed TIR biosensing methodology, several calibration tests were performed. In the absence of the sensing block, we measured responses of the first and second harmonic to the small phase retardation introduced with a SBC. Optimal signal-to-noise ratio for the phase measurements (Eq. (3)) was obtained at 154° phase modulation depth (M), which provides equal Bessel function $J_1 = J_2$ that after tuning α results in equal harmonics intensities and therefore optimal conditions for the noise elimination (Fig. 3(a) black line). Phase response provides information about surface films and obtained high phase sensitivity in 5.2×10^{-3} deg. confirms efficiency of our polarimetric set-up that also could be used for direct phase measurements at fixed incident angle.

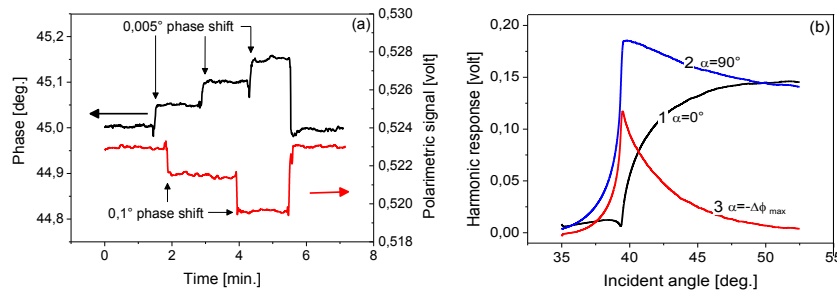


Fig. 3. (a) Phase sensitivity experiment (phase: black line, polarimetric signal: red line) (b) Experimental results for first harmonic with the same retardance as in Fig. 2a.

As follows from Eq. (1) and (2) for independent harmonic measurements, best results were obtained for phase modulation depth providing a maximum detected signal at $M = 105^\circ$ for the first harmonic and at $M = 175^\circ$ for the second. Due to the overall better performance of the electronic equipment at 50 kHz and better phase sensitivity equal to 0.02° for the first harmonic, all further tests were performed at $M = 105^\circ$ (Fig. 3(a) red line). Should be noted that while net phase measurements provides better sensitivity for the thin film testing, more complete biosensing information could be obtained using independent harmonic testing, where combined intensity-phase signal allows simultaneous investigation of bulk RI and surface thin films.

Experimental measurement has been performed to confirm our theoretical consideration shown in Fig. 2(a). The initial retardance $\alpha = \Delta\phi_{\max}$ is a key component to obtain an optimal APC. Experimentally, the angular phase dependence was measured (Eq. (3)) and the system was fixed at an incident angle corresponding to the maximum phase difference between s- and p-polarization (for SF11 prisms: 43° and 68.59° in air and in water, respectively). By adjusting manually the SBC, the amplitude of the first harmonic of the polarimetric signal was

set equal to zero and resulting APC was measured (Fig. 3(b), curve 3). Position of the SBC was then kept for others experiences. Polarimetric curve obtained with phase retardance equal to 0° and 90° are also shown in Fig. 3(b) (curve 1 and 2 respectively).

The sensitivity of the proposed TIR methodology depends on the precision of the APC peak amplitude and angular position determination. Angular resolution is provided by the high precision rotating stages while peak sharpness and half-width depends on angular phase retardance and requires application of a well collimated incident beam. In this work, collimated laser light (collimation test was performed on a distance 20 m with a precision 0.01 deg.) with 2 mm spot was used and corresponding APC is presented on Fig. 4(a). Then, 0.1 deg. convergent or divergent beam was employed and equal decreasing of the APC peak amplitude accompanied with a peak broadening was obtained (Fig. 4(a)). These results show the importance of the proper set-up calibration and could be used as initial beam calibration method: maximum reflected amplitude at fixed angle near the peak corresponds to the condition of well collimated beam on the sensor surface.

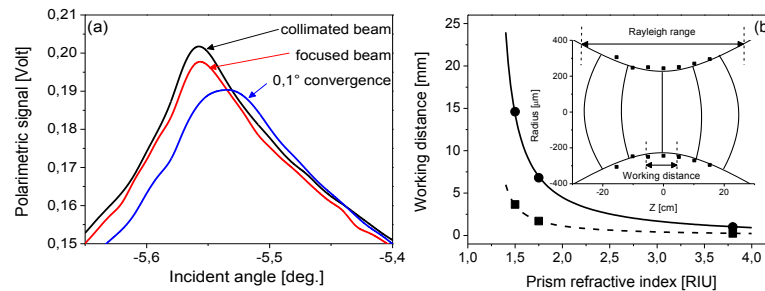


Fig. 4. (a) Angular Polarimetric Curve with different beam propagation geometry. (b) Working distance minimizing the influence of the convergence/divergence near the focal point; Inset: Measured propagation Gaussian beam near the focal point.

Also, we have found that using focused Gaussian beam gives APC close to the optimal (Fig. 4(a) red line). Explanation follows from the fact that Gaussian beam is quasi parallel at the focal point (inset in Fig. 4(b)) [14]. Indeed, in our case we employed a low NA optic ($NA = 9 \times 10^{-4}$) that results in a long Rayleigh range of 285 mm that provides nearly collimated beam with a $400 \mu\text{m}$ spot size and a flat phase at the point of incidence. The inset in Fig. 4(b) shows results of theoretical calculations and experimental data for a beam waist measured with a CCD beam shaper (Ophir-Spiricon). We have calculated the influence of the TIR coupling prism material on the working distance (Fig. 4(b)) where the beam divergence/convergence has an effect on the sensitivity lower or comparable to sensitivity value of 0.005 deg. (dashed line) for the phase and 0.02 deg. (continuous line) for the first harmonic). In this work, a SF11 prism was used that provides a rather long working distance (6.72 mm) whereas TIR system build on the Si will require positioning of the sensor surface in less than 1mm from the beam focal point. Thus, focused beam phase sensitive method is important in the design of portable micro fluidic multi-sensor or imaging systems where spot size provides resolution and sensitivity.

4. Results and discussion

To evaluate the sensing performance of the TIR polarimetric system and to estimate the sensitivity to the bulk refractive index changes, we have performed a series of experiments where aqueous solutions of NaCl with different RI [15] were introduced into flow-injection cell in contact with glass slides cleaned by "piranha" solution. Figure 5(a) presents sensor response as the angular position of the APC peak was measured in real time. For numerical analysis of the obtained APC, polynomial curve fitting method of forth order was used. Taking into account the level of noises (1.6×10^{-3} deg.), the experimental detection limit for the

bulk RI was estimated better than 10^{-5} RIU, which is comparable to SPR sensitivity ($10^{-5} \sim 10^{-6}$ RIU).

To demonstrate the proposed biosensing methodology, we have performed experimental tests where adsorption of the thin biofilms on the surface is accompanied by the bulk RI changes. In order to form a thin biolayer on the sensing surface, different concentrations of Bovine Serum Albumin (BSA) in the phosphate buffered saline (PBS) were prepared and introduced into flow-injection cell. Glass slides with different surface functionalization in immersion contact with the SF11 prism were used as sensing surfaces. Clean glass was first tested and only bulk refractive index change was detected. Indeed, the existence of an electrostatic repulsion between the protein and the clean glass surface [16] prevents BSA layer deposition. Then, experimental results for 20 $\mu\text{g/ml}$ and 50 $\mu\text{g/ml}$ concentrated BSA were obtained on the glass functionalized with 3-(aminopropyl)-triethoxysilane (Sigma-Aldrich) [17], where surface amino-groups promote the non-specific binding of the BSA (Fig. 5(b)). Here the black line represents data for the APC peak maximum value corresponding to the thin biolayer formation and the red line shows the angular position of the peak maximum which is linked to the bulk refractive index of the solution. These information (bulk and thin layer) are completely different in their dynamic (change in the red line are quicker than black line which follows binding dynamic of BSA on amino-group) and in their level (continuous line does not return to the initial baseline due to the adsorption of BSA compared to the red one where solution returns to PBS). We can conclude from this figure that both bulk and thin layer information could be obtained independently.

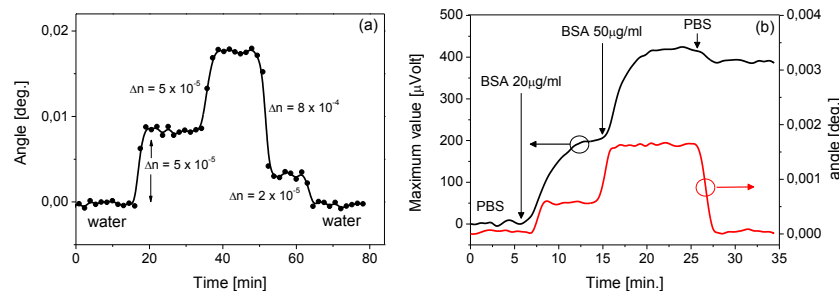


Fig. 5. (a) Angular variation of the peak position for different refractive index solutions. (b) Responses of the system with different concentrations of Bovine Serum Albumin.

The proposed methodology enables to control the thin layer formation and bulk RI changes independently in contrast to the SPR sensor where usually the combination of responses is monitored. Sensitivity of the system could be estimated by considering the value of 1.45 of the BSA monolayer refractive index [18]. Using out theoretical prediction for 0.02 deg. phase resolution, the experimental detection limit is estimated to an average thickness of 0.5 nm biolayer and could be improved for direct phase measurements.

5. Conclusion

In this paper, we demonstrated polarimetric biosensing TIR system based on the method of temporal phase modulation. Measurements of the phase difference between s- and p-polarized light combined with their amplitudes allow to obtain information about medium refractive index changes as well as dynamics and thickness of the deposited thin biofilms. Obtained phase resolution of 0.02 deg. corresponds theoretically to a sensitivity of 0.5 nm in biolayer thickness and tested sensitivity for the bulk refractive index is better than 10^{-5} RIU. The higher refractive index platforms such as high refractive index dielectrics and silicon offer even higher sensitivities for biofilms tests. Microfabrication technology on the glass, optical polymers or silicon enables construction of low-cost integrated biosensing systems.

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