

Highly sensitive biosensor based on UV-imprinted layered polymeric–inorganic composite waveguides

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Abstract: An evanescent field sensor utilizing layered polymeric-inorganic composite waveguide configuration was developed in this work. The composite waveguide structure consists of a UV-imprint patterned polymer inverted rib waveguide with a Ta₂O₅ thin film sputter-deposited on top of the low refractive index polymer layers. The results suggest that the polymer based sensor can achieve a detection limit of 3×10^{-7} RIU for refractive index sensing and corresponding limit of about 100 fg/mm² for molecular adsorption detection. Besides enhancing the sensitivity significantly, the inorganic coating on the polymer layer was found to block water absorption effectively into the waveguide resulting in a stabilized sensor operation. The ability to use the developed sensor in specific molecular detection was confirmed by investigating antibody – antigen binding reactions. The results of this work demonstrate that high performance sensing capability can be obtained with the developed composite waveguide sensor.

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1. Introduction

Optical planar waveguide sensors utilizing evanescent field probing scheme have drawn great interests in biosensing due to its high sensitivity and integration rate for label-free multi-analyte detection. Typical planar waveguide sensors are fabricated with inorganic dielectrics, such as, SOI [1], Si₃N₄ [2], III-V group alloys [3], and Ta₂O₅ [4] with claddings of SiO₂. To reduce sensor manufacture costs alternative materials and sensor configurations suitable for low-cost fabrication process have been investigated. Polymers possessing good optical properties, versatile process-abilities including molding and spin coating techniques can therefore be compatible for low-cost production of optical sensors. Several polymer-based planar waveguide biosensors have been developed recently, such as, ring resonators [5, 6], interferometers [7, 8], and grating couplers [9, 10]. Compared to inorganic materials, the refractive index (RI) of polymers differs only a little leading to a low RI contrast between the core and cladding in a waveguide. Consequently, this limits available configurations of polymer planar waveguide sensors. For example, field localization and high quality resonators are generally difficult to realize. According to our earlier studies on layered inorganic – polymeric composite waveguides [11], the optical field distribution can be manipulated by depositing a thin layer of Ta₂O₅ on top of polymer layers. With the help of the high index ($n = 2.1$) thin film, the guided mode field can be pushed up towards the surface resulting in an enhanced optical field probing at the waveguide surface, where binding interactions of biomolecules take place.

The motivation of this work was to develop a low cost biosensor yet having sensitivity comparable to inorganic based integrated optical (IO) biosensors. With the proposed inverted

rib waveguide structure [12] a simple process consisting of room-temperature UV-imprinting, spin-coating, and sputtering of Ta_2O_5 high index layer is utilized in the sensor fabrication. The influence of water absorption into the polymer is usually neglected in reports on polymer-based waveguide sensors, though the phenomenon of water molecular penetration and absorption exists widely in polymeric materials [13]. In this paper, the effect of water penetration in polymer waveguides is examined and the water blocking effect of the thin Ta_2O_5 layer is investigated. Finally, the behavior of the developed polymeric-inorganic waveguide sensors in bio-measurements is evaluated by investigating the specific binding of CRP (c-reactive protein) antibodies and antigens.

2. Waveguide sensor fabrication and characterization

2.1 Fabrication

Interferometers are considered as one of the most sensitive devices [14] for optical sensing due to the background compensation between the sensing and reference arm against environmental disturbances, such as temperature fluctuations. Figure 1(a) shows a schematic image of the Young interferometer (YI) biosensor configuration utilized in this work. The Young interferometer was chosen as the transducer platform due to its linear correlation between the spatial change of the interference pattern and the induced phase change in the waveguide [15].

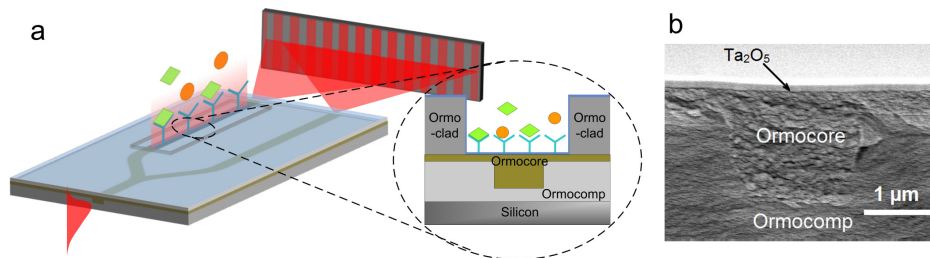


Fig. 1. (a) Schematic image of the integrated YI biosensor based on inverted-rib waveguides; (b) cross-section SEM image of the inverted rib waveguide inside the sensing window.

The materials used for sensor fabrication consist of the UV-curable Ormocer series of hybrid polymers (Micro resist technology GmbH). Ormocomp, Ormocore and Ormoclad having refractive index of 1.520, 1.553 and 1.536 at wavelength of 633 nm are used for the waveguide's under cladding, core and upper cladding respectively. The details of the waveguide fabrication are described in Ref. 12. Fabrication of the polymer inverted-rib waveguide is an etch-free process. Except the final deposition of the high index layer, the whole fabrication process incorporates polymeric materials only.

The waveguide structure was built on a silicon wafer offering mechanical support. Fabrication of the polymer waveguide sensor consists of two main steps: patterning the waveguide under-cladding and filling the patterned grooves with the core material. UV-imprinting used for the rib/groove patterning does not require high pressure during the imprinting process and it is performed at room temperature. When the core material diluted in 1-methoxy-2-propyl acetate is spin coated with a speed of 3000 rpm, a residual layer having a thickness around 400 nm is left above the patterned under cladding. A cross sectional SEM image of the fabricated inverted rib waveguide located inside the sensing window is shown in Fig. 1(b). The groove imprinted in Ormocomp under cladding is filled with Ormocore core material. Ta_2O_5 coating can be seen as a brighter thin layer on top of the polymer layers. The shown waveguide has a dimension of about 2 μm (width) $\times$ 1.6 μm (height) including the slab residual core layer. To create a sensing channel on the other waveguide arm, a thick layer (about 20 μm) of Ormoclad was spin coated above the waveguide structure and a sensing window with a length of 10 mm was formed photo-lithographically. For the under cladding layer Ormocomp material was chosen because of its better compatibility with the diluted

Ormocore core layer. For the upper cladding Ormoclad was used due to lower refractive index contrast with the core material resulting in a single-mode operation. Finally, a thin layer of Ta₂O₅ was reactively sputtered using a 4N pure Ta metal target (Helios XL Pro, Leybold Optics) on top of the fabricated polymer waveguide.

2.2 Waveguide and sensor characterization

In the fabricated integrated YI, the distance between the sensing and referencing waveguide is 50 μm . Part of the YI sensor is shown in the top view microscope image in Fig. 2(a). According to the simulations (Fimmwave, Photon Design Inc.) at 633 nm, the sensing waveguide supports only one TM propagation mode in the sensing window, where DI water serves as its upper cladding with the refractive index of 1.33. The waveguide also operates in single mode in the areas with Ormoclad over cladding layer. The coupling efficiency Γ^2 between the two regions in the sensing waveguide is defined as an overlap integral [16] according to

$$\Gamma^2(x, y) = \frac{\left(\iint E_{clad}(x, y) \cdot E_{Ta_2O_5}(x, y) dx dy \right)^2}{\iint E_{clad}^2(x, y) dx dy \cdot \iint E_{Ta_2O_5}^2(x, y) dx dy} \quad (1)$$

E_{clad} and $E_{Ta_2O_5}$ are the electric field distributions in the Ormoclad covered region and in the Ta₂O₅ coated region, respectively. Theoretical intensity profiles obtained using film-mode-matching (FMM) method are shown in Fig. 2(a) at the indicated locations in the sensing waveguide.

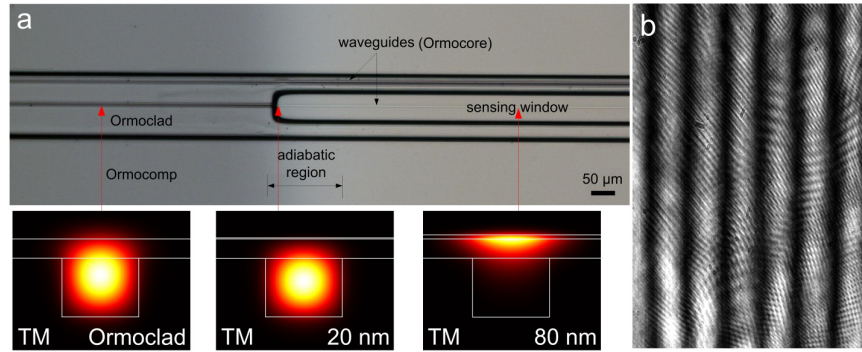


Fig. 2. (a) microscope image showing a part of the YI sensor where the adiabatic transition of the high-index Ta₂O₅ coating takes place. The intensity profiles of the propagating TM- modes are displayed at the location of Ormoclad covered area, the front edge of the sensing window where Ta₂O₅ coating thickness is about 20 nm, and the middle of the sensing window where Ta₂O₅ coating thickness is 80 nm. Inside the sensing window water is considered as the material for the upper cladding; (b) the generated interference pattern at a distance of 2.5 mm from the edge of the sensor chip.

By using the shadow masking technique in the Ta₂O₅ sputtering process, an adiabatic region with gradually increasing Ta₂O₅ thickness was formed between the Ormoclad covered region and the Ta₂O₅ coated region. Without the adiabatic transition the coupling efficiency between the two regions was calculated to be 60%. With the adiabatic transition starting from 20 nm of Ta₂O₅ coating at the front edge of the sensing window, the obtained coupling efficiency was approximately 90%. Assuming that the power loss occurs at the front and the back edge of the sensing window, the overall transmission efficiency was estimated to be around 81%. As evaluated in [17], the intrinsic material loss of a sputtered Ta₂O₅ thin film is about 1 dB/cm. The propagation loss in a composite polymeric waveguide with 100 nm thick Ta₂O₅ layer was estimated to be about 1.6 dB/cm [11]. These attenuation values are small enough to have a good interference fringe visibility at the output of the sensor chip facet. The generated fringe pattern from the referencing waveguide and sensing waveguide with

adiabatic transitions is shown in Fig. 2(b). The clear visibility of the interferogram gives a good resolution when analyzing the fringe pattern phase shifts by two-dimensional fast Fourier transform.

2.3 Theoretical sensitivity evaluation

The IO waveguide biosensors respond to the change of the bulk refractive index of the liquid sample and the surface adsorption of biomolecules. The bulk RI change of the liquid sample is seen as a change in the refractive index of the upper cladding n_c . The adsorption of the biomolecules is translated as a thickness change of the adlayer t_{ad} growing above the sensing waveguide. The induced effective RI change Δn_{eff} of the sensing waveguide can be described by Eq. (2)

$$\Delta n_{eff} = \frac{\partial n_{eff}}{\partial n_c} \Delta n_c + \frac{\partial n_{eff}}{\partial t_{ad}} \Delta t_{ad} \quad (2)$$

where $\partial n_{eff}/\partial n_c$ and $\partial n_{eff}/\partial t_{ad}$ are the sensitivities for the change of bulk RI and thickness of the adlayer, respectively. With the observed physical parameters of the inverted-rib waveguide structure shown Fig. 1(b), the theoretical sensitivities of $\partial n_{eff}/\partial n_c$ and $\partial n_{eff}/\partial t_{ad}$ were examined using FMM method. For the calculation of $\partial n_{eff}/\partial t_{ad}$, $n_{ad} = 1.45$ was used for the RI of adsorbed biomolecule layer. With increasing coating thickness, the optical field distribution of the propagating mode is pushed up towards the waveguide surface resulting in improved sensitivity. As can be seen in Fig. 3(a) and 3(b), the curves for homogeneous refractive index sensing and surface adsorption sensing resemble each other though there is a slight Ta_2O_5 thickness difference for the maximum sensitivity. For TE-polarization state, sensitivity increases with increasing Ta_2O_5 thickness upto about 60 nm for homogenous refractive index sensing and about 70 nm for adsorption sensing after which the further increase in high-index layer thickness does not improve sensitivity. Corresponding thicknesses for TM-polarization state are 120 nm and 140 nm, respectively.

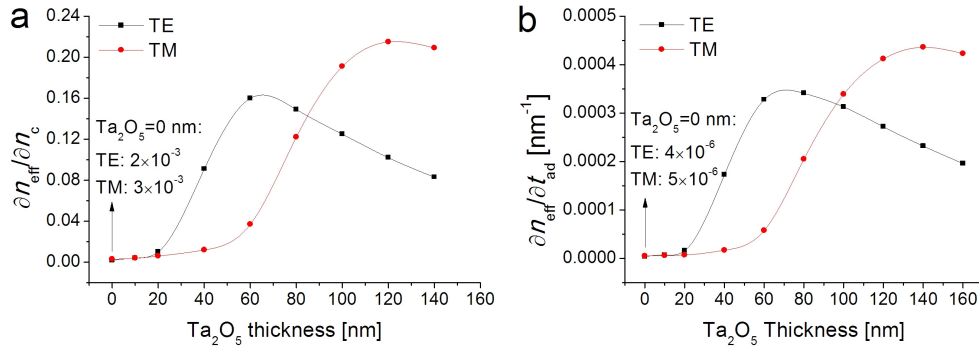


Fig. 3. The sensitivity of (a) homogeneous refractive index sensing and (b) surface sensing of biomolecular adsorption as a function of the thickness of Ta_2O_5 coating for both TE- and TM-coupling modes.

The enhancement in sensitivity is associated with the decreased confinement inside the inverted guiding ridge. Therefore, the composite waveguide structure becomes leaky in the lateral direction, when a much thicker high-index coating is deposited on the polymer layers. To optimize the sensitivity and mode confinement in the sensing region, 80 nm coating and TM-polarization was chosen. According to the simulations the obtained sensitivity of 80 nm Ta_2O_5 coating is 0.12 and $2.1 \times 10^{-4} \text{ nm}^{-1}$ for homogeneous RI and surface molecular adsorption sensing respectively. The sensitivities are enhanced over 40 times when comparing to the polymer waveguide sensor without high index coating while still having 17% power confinement inside the inverted ridge resulting in proper waveguide operation.

3. Experiment and results

A He-Ne (632.8 nm) laser was used as a light source in the measurements. TM-polarization state was adjusted by rotating a polarizer in front of the laser. Microscope objectives were used for coupling light into and out from the sensing chip. The liquid analytes were pumped through a flow cell attached above the sensing element by a continuous flow syringe pump (OPAM Instruments). A CMOS camera (PixeLink) with a resolution of 1280×1024 recorded fringe pattern images at a rate of 1 Hz. For each image two dimensional fast Fourier transform was applied to extract the phase from the fringe pattern.

3.1 Effect of water absorption

When subjected to water absorption the refractive index and volume of a polymer may change [18]. To examine the water absorption in Ormocer waveguide two YI waveguide sensors, one with and the other without Ta_2O_5 coating, were used. The phase responses when injecting water into the sensing window are shown in Fig. 4(a) and 4(b).

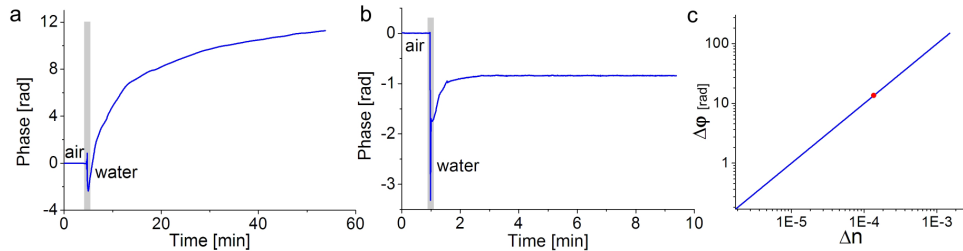


Fig. 4. Sensor responses after applying water to the sensing window (a) of a polymer waveguide interferometer sensor (b) of a composite polymeric-inorganic waveguide interferometer sensor; (c) the induced phase change when the refractive index of the core and cladding are varied by water absorption. Dot indicates the observed phase change.

In the beginning, when water was just introduced into the sensing window, an abrupt change in the phase response was triggered by an immediate change of the refractive index from the value of air ($n = 1$) to the value of water ($n = 1.33$). The swift change resulted in big spikes where a gray area is masked over the sensorgram. Inside these regions the phase responses were not able to be defined explicitly. At the same time, water started to be absorbed in the polymer waveguide sensor without Ta_2O_5 coating resulting in a continuous change in the phase response as shown in Fig. 4(a). The phase converged gradually when the diffusion of the water molecules reached equilibrium. When the polymer waveguides were coated with Ta_2O_5 , the phase response stabilized in a couple of minutes right after the water completely filled the sensing window as shown in Fig. 4(b). These results indicate that 80 nm Ta_2O_5 coating can effectively block the penetration of water into the underneath polymer waveguide. The refractive index change of the used polymers can be estimated by assuming that the water absorption into the polymer waveguide dominates the refractive index change and the amount of change is the same in the core and cladding. Figure 4(c) shows the calculated phase change $\Delta\phi$ when the refractive index of the core and cladding was varied between 10^{-6} and 10^{-3} RIU. The red dot indicates $\Delta\phi$ of 14 rad corresponding to the RI change of 1.4×10^{-4} in the core and cladding. Taking into account the ambiguity in the measurement transient as illustrated with the grey area in Fig. 4(a), the actual RI change in the waveguide core and cladding could be even larger than 10^{-4} . The obtained value of refractive index change of Ormocer is comparable to that of the silicone resin and epoxy resin 10^{-4} [19] and possibly smaller than the polyimide [20] and PMMA 10^{-3} [18] at 100% relative humidity.

3.2 Homogeneous sensing

To evaluate the response against the change of the bulk refractive index, DI-water with varying glucose concentrations (0.005%, 0.02%, 0.04%, 0.05%, 0.1%, 0.2%, 0.6%, 1% w/v)

was applied to the sensing surface. The refractive index change of glucose solution with respect to DI-water was calculated based on the empirical equation [21]

$$\Delta n_c = 0.14713 \times \text{Concentration} \quad (3)$$

The phase responses of the sensor are plotted in Fig. 5. The effective RI change of the sensing waveguide can be extracted from the phase shift of the interference pattern according to the equation $\Delta n_{\text{eff}} = \Delta\phi \lambda / 2\pi L$, where $\Delta\phi$ is the phase shift of the fringe pattern, λ is the wavelength in vacuum and L is the interaction length.

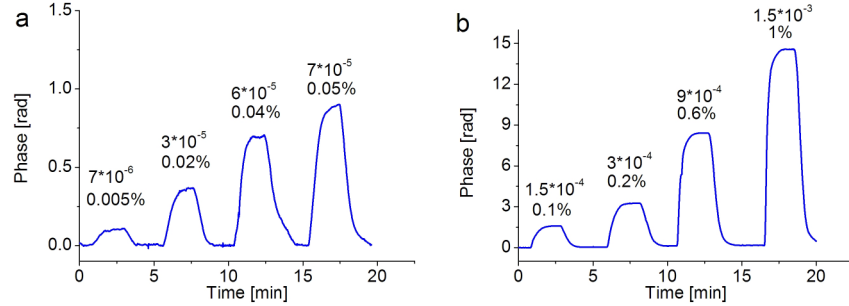


Fig. 5. The phase response of (a) smaller and (b) higher glucose concentrations as a function of time when applying water-glucose-water cycle in the sensor window. The w/v concentration and its corresponding refractive index change Δn_c with respect to the DI water ($n = 1.33$) are marked above each measurement response.

Figure 6 shows the obtained Δn_{eff} values versus Δn_c for all eight glucose solutions. The slope of the linear fit indicates that the actual sensitivity $\partial n_{\text{eff}} / \partial n_c$ equals to 0.11, which is in a good agreement with the simulated sensitivity of 0.12. The noise level, defined as the standard deviation of the baseline, was in the order of 0.003 rad for a 5 minutes measurement. With this value the potential detection limit can be derived to be about 3×10^{-7} RIU for the bulk index changes.

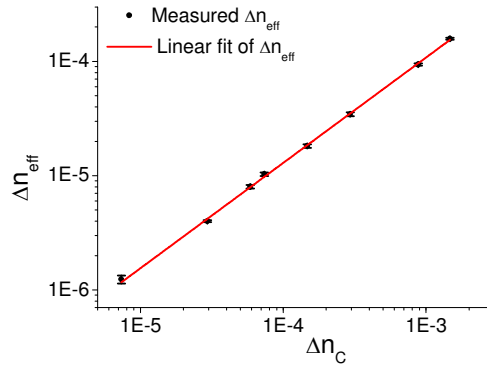


Fig. 6. Changes in the refractive index of the glucose solutions Δn_c versus the induced change in the effective refractive index Δn_{eff} .

Compared to some of the polymer based planar waveguide sensors reported previously [5, 6, 8, 9] the composite waveguide polymeric-inorganic sensor has a detection limit of one order of magnitude better. The obtained detection limit is also comparable or better than some of the inorganic dielectric based biosensors reported recently where the detection limit lies in the range of 10^{-5} – 10^{-7} RIU [1, 2, 22–24] for homogeneous bulk refractive index sensing.

3.3 Surface sensing of protein adsorption

The performance of the developed sensor in biosensing applications was examined by detecting specific molecular binding events between c-reactive protein (CRP antigens) and monoclonal anti-human C-reactive protein (CRP antibodies). Their phase responses are plotted in Fig. 7(a) and corresponding biomolecule bindings are illustrated in Fig. 7(b).

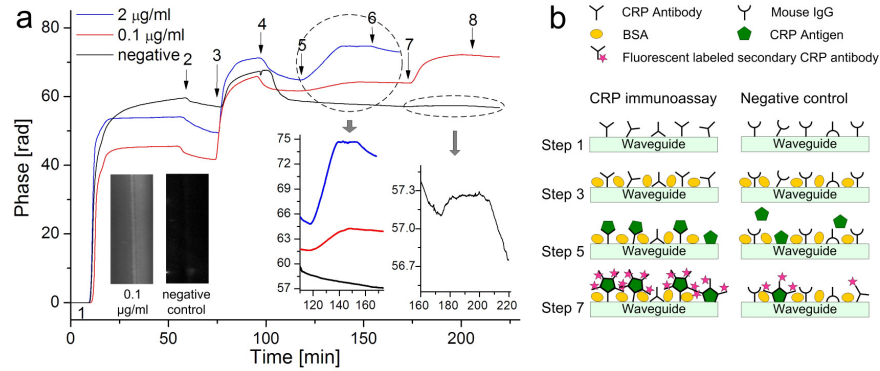


Fig. 7. (a) Phase responses of the molecular binding events between surface attached CRP antibodies and 2 µg/ml (blue line) and 0.1 µg/ml (red line) CRP antigens in buffer solution. The phase response of negative control plotted in black color. Step 1: injection of the CRP antibodies or mouse IgG in negative control; step 3: injection of BSA; step 5: injection of CRP antigens; step 7: injection of secondary CRP antibodies labeled with fluorescent markers; step 2, 4, 6, 8: PBS buffer wash. The images on the lower left corner are the fluorescent microscopic images taken inside the sensing window after the flow of Alexa 546 labeled secondary CRP antibodies; (b) Step by step illustration of biomolecular binding events.

Phosphate buffered saline (PBS, pH 7.4) solution was used as the buffer solution throughout the surface sensing experiments. First, the sensing window was filled with PBS buffer after which 0.5 mg/ml of CPR antibody was pumped continuously through the sensing window with the speed of 16.7 µl/min for 1 hour. CRP antibodies flowing close to the sensing surface were adsorbed nonspecifically to the waveguide surface. Subsequent to a PBS wash, 2 mg/ml bovine serum albumin (BSA) solution was used to block the sensing surface. When the surface was prepared, CRP antigen was pumped through the sensing window. Two different concentrations 2 µg/ml (16 nM) and 0.1 µg/ml (0.8 nM) were used, each on a new sensor chip. In step 1 the slightly different phase responses during the attachment of the primary CRP antibodies might be due to differential adsorption of the biomolecules on the bare polymer waveguide surface. However, after BSA surface blocking procedure (step 3), the phase change of the two different measurements showed rather similar values. As shown in the zoomed-in curves of steps 5 and 6 in Fig. 7(a), the binding events caused a phase change after PBS wash of about 8.2 rad and 2.3 rad for 2 µg/ml and 0.1 µg/ml CRP concentrations, respectively. The phase shifts might be partially suppressed due to a random orientation of nonspecifically adsorbed primary antibodies which prevents binding of the antigen to all antibodies on the surface. Using the theoretical sensitivity of $\partial n_{eff}/\partial a_{ad} = 2.1 \times 10^{-4} \text{ nm}^{-1}$, the growth of the adlayer was determined to be 0.44 nm and 0.12 nm corresponding to the phase changes. The obtained layer thickness does not indicate an exact molecular height but an average thickness taking into account the molecular packing density. The corresponding surface mass density M can be calculated with De Feijter's formula

$$M = \Delta t_{ad} \frac{n_{ad} - n_c}{dn/dc} \quad (4)$$

where dn/dc is the refractive index increment of the protein solution. We used a value of 0.18 cm³/g for dn/dc , a value commonly used for protein adsorption [25]. The calculated surface mass density is 280 pg/mm² for 2 µg/ml and 80 pg/mm² for 0.1 µg/ml CRP antigens. Taking

into account of the noise level of 0.003 rad, the achieved detection limit is estimated to be about 100 fg/mm². The obtained measurement results are much less than a monolayer surface mass coverage of 1.48 ng/mm² [26]. It could be partially due to a random orientation of the CRP antibodies meaning that only part of the CRP antibodies has their binding sites facing out from the waveguide and are available to bind CRP antigens. Moreover, as shown in Fig. 7(a) with the blue and red curves, the signal levels were different for the two sensor chips under investigation after step 1 and step 2. These results indicate that the CRP antibodies in step 1 did not form a dense monolayer leading to a lower surface mass density of the bound CRP antigens. According to previous studies [27, 28], it is not easy to achieve a single but dense monolayer. The surface densities of biomolecules can vary in a broad range being strongly dependent on the surface and the surface modification protocol.

To assure the induced phase responses were not due to nonspecific binding of CRP on the waveguide surface, we performed a negative control test using mouse IgG instead of CRP-antibodies as a receptor. Due to a low binding affinity between the mouse IgG and CRP, no obvious phase response was detected (black curve in Fig. 7(a)). The specific biomolecular interaction was further confirmed by running the secondary CRP antibodies labeled with fluorescence markers through the sensing window. As seen in step 7 of Fig. 7(a), attachment of secondary antibody to primary antibody-antigen complex caused a big phase response (red line) whereas in the negative control experiment (black line) only a very small phase change was detected, possibly representing slight nonspecific binding. A CRP molecule is composed of five identical subunits forming an annular configuration with cyclic pentameric symmetry [29]. Each subunit contains an epitope which is specifically recognized by the antibody. Thus a single CRP molecule can be bound by five different antibody molecules. When a CRP molecule was bound by a CRP antibody on the sensing surface, four available epitopes are left unoccupied. When applying a high concentration (13.2 µg/ml) of secondary antibodies, all the available epitopes were expected to become occupied producing a corresponding about four times increase in phase response as CRP antigens (115 kDa) and CRP antibodies (150 kDa) have a comparable molecular weight. Fluorescence microscopy images in Fig. 7(a) insert show attachment of fluorescent labeled secondary antibodies onto the CRP antibody activated surface but not onto the negative control.

4. Conclusion

In this paper a highly sensitive polymeric-inorganic waveguide sensor was developed. The emphasis was to demonstrate that high-performance sensor configuration is attainable with simplified and potentially low-cost fabrication methods. By depositing 80 nm Ta₂O₅ coating on top of the UV-imprinted polymer YI sensor the sensitivity was improved over 40 times. It was also demonstrated that the deposited thin inorganic layer prevented the water penetration into the polymer waveguides and therefore the sensing response was quickly stabilized after exposure to the aqueous analyte. The fabricated sensor was calibrated with glucose DI-solutions. The actual sensitivity 0.11 was found to have a good correspondence with the theoretical value. An estimated detection limit was about 3×10^{-7} RIU for homogeneous ambient sensing and 100 fg/mm² for surface mass adsorption detection. The biosensing applicability was successfully demonstrated with the immunoassay of CRP.

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