

Low-cost label-free biosensors using photonic crystals embedded between crossed polarizers

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Abstract: There is a strong need for low-cost biosensors to enable rapid, on-site analysis of biological, biomedical, or chemical substances. We propose a platform for label-free optical biosensors based on applying the analyte onto a surface-functionalized photonic crystal slab and performing a transmission measurement with two crossed polarization filters. This dark-field approach allows for efficient background suppression as only the photonic crystal guided-mode resonances interacting with the functionalized surface experience significant polarization rotation. We present a compact biosensor demonstrator using a low-cost light emitting diode and a simple photodiode capable of detecting the binding kinetics of a 2.5 nM solution of the protein streptavidin on a biotin-functionalized photonic crystal surface.

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1. Introduction

Biosensors are devices that use biological materials to detect other biological, biomedical, or chemical substances [1-8]. In particular, these devices are used to measure protein-protein interactions, binding affinities, and kinetic processes. These biosensors are applied for the determination of active concentrations, for screening, and for the characterization of biological substances. All these tests are critical processes for, e.g., biological research and drug discovery. Today's established optical label-free methods are based on surface plasmon resonances (SPR) [1] or optical modes in resonator structures with high quality factors [5-8]. To detect a given analyte by these methods the respective resonance is spectrally analyzed; a procedure that often requires spectrometers and computational resources. Due to the high acquisition costs of such devices their application is limited. On the other hand, compact biosensors are promising tools for medical diagnostics. For instance, detection of biomarkers has much improved the diagnostics of diseases such as cancer [9]. In this work we present a low-cost and compact technology platform for label-free biosensors based on surface-functionalized photonic crystal slabs and efficient background suppression.

Photonic crystal slabs (PCSs) are optical thin-films with a periodically nanostructured surface. They exhibit discrete photonic modes, which are confined in the slab, but show an evanescent fraction in adjacency to the surface that can interact with biological material. One type of photonic modes in such structures are guided-mode resonances (GMRs). These modes lie above the light line and couple to far field radiation [10]. When performing transmission experiments in the direction normal to the surface of the PCS, these modes appear as Fano resonances superimposed on the spectrum of the light source. Changes in geometrical parameters in the vicinity of the photonic crystal's surface, such as a change in the refractive index of the surrounding medium, or the presence of a layer on the surface of the PCS cause a shift in spectral position. Therefore, PCSs may be applied as transducers in label-free biosensing. While previous work relied on a spectral evaluation of the mode distribution [6,7], we demonstrate the advantages of observing only GMRs by effectively suppressing background light with two orthogonally oriented polarization filters, placed before and after the PCS (Fig. 1(a)). Since only the GMRs undergo a significant polarization rotation in this configuration, the resulting spectrum allows direct evaluation of these distinctive resonances [11,12]. This polarization rotation has its origin in the projection of the polarizer direction into the grating and the projection of the grating into the analyzer direction [13]. In best case, which is for $\theta = 45^\circ$, 1/8 of the GMR's intensity is transmitted. In Fig. 1(b) the transmission spectrum through the PCS without polarization filters is compared to the transmission spectrum (magnified $\times 10$) using crossed polarization filters.

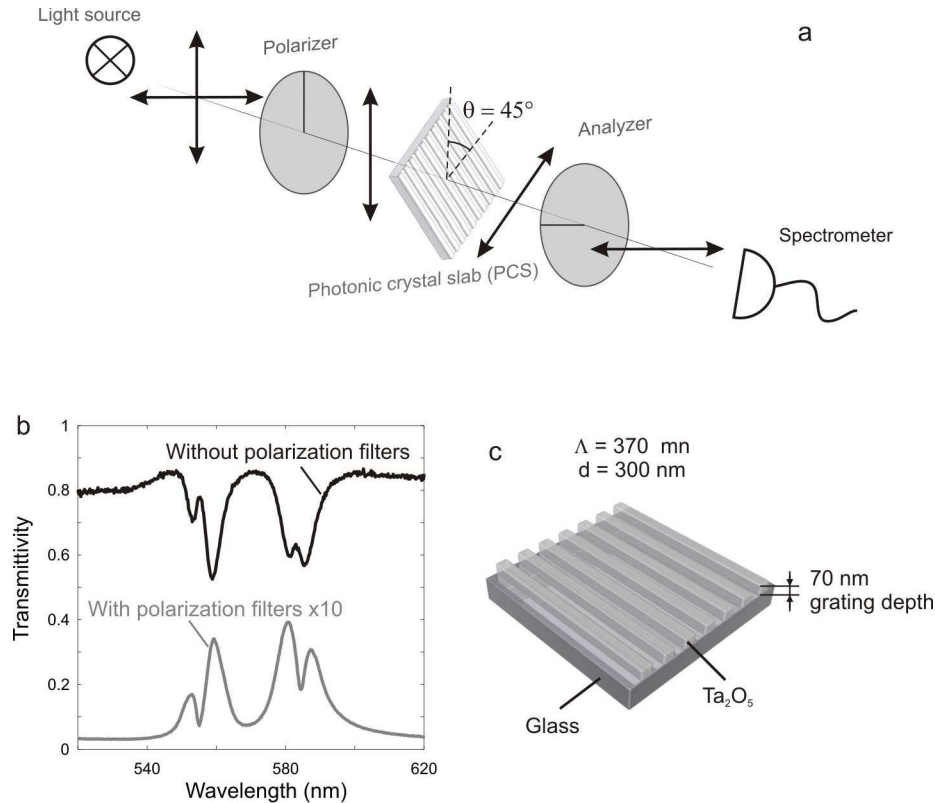


Fig. 1. Background suppression for transmission measurements through photonic crystal slabs (PCS). (a) Setup for background suppression. The PCS is placed between two crossed polarization filters. Due to polarization rotation only light interacting with the PCS can pass this configuration. (b) Transmission measurements with and without crossed polarization filters. Using polarization filters only the GMRs are transmitted, which are sensitive to the environmental condition of the PCS. (c) Schematic representation of the PCS used in this paper. It is composed of a Ta_2O_5 layer ($d = 300 \text{ nm}$) on a glass substrate with a linear periodic nanostructure. The periodicity of the PCS was chosen to be $\Lambda = 370 \text{ nm}$.

2. Guided-mode resonance shift determination via intensity measurements

Using a PCS as transducer for biosensor applications, a key process is the determination of the spectral position shift of GMRs. To solve this issue in a cost-efficient and compact way, we propose to use a light source possessing a rising or a falling edge in the spectral region of the GMRs. The convolution of the shifted GMR with the spectrum of the light source results in a function of GMR's spectral shift versus the intensity of the transmission. This function shows a pure positive slope, when GMRs overlap with the rising edge of the light source, and a negative slope for GMRs overlapping with the sloping edge of the light source. Therefore, a simple intensity measurement can replace spectral analysis of GMRs.

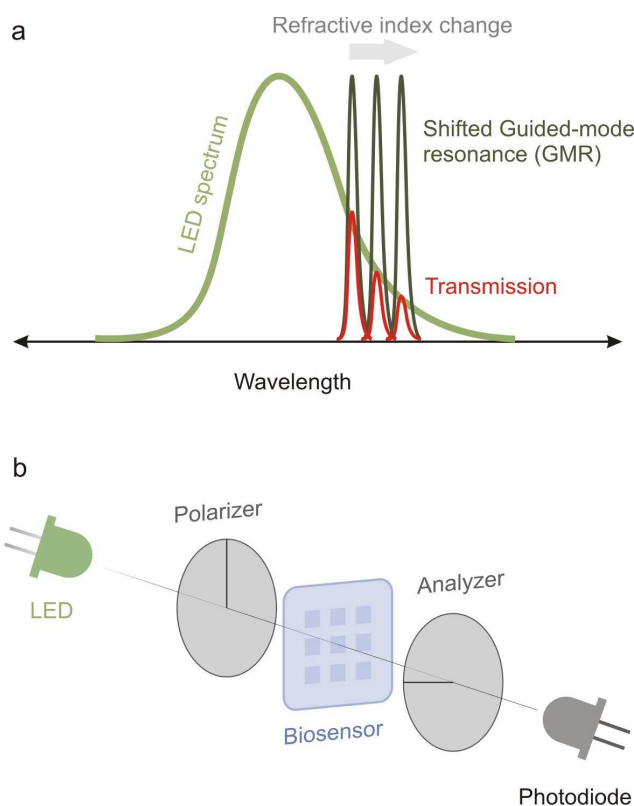


Fig. 2. (a) Basic concept of the conversion of GMR shift into intensity change using an LED as the light source. The convolution of the LED spectrum with the GMR results in a function of the spectral shift of GMR versus the intensity of the transmission. (b) Schematic of a compact and low-cost biosensor for affinity measurements in real time. Here, an LED is applied as the light source, while a photo diode is used for the intensity detection.

These considerations are summarized in an example depicted in Fig. 2(a). Here the spectrally limited light source is chosen to be a standard LED, for which the GMR overlaps only with its falling edge. A refractive index change on the surface of the PCS would result in a shift of the GMR. The intensity of the light source is reduced at the new spectral position of the GMR and therefore the transmitted intensity is decreased. A simple setup, as shown in Fig. 2(b) contains all required elements to realize the desired device. Here, solely a photodiode is utilized to detect the intensity of the transmission.

3. Bulk refractive index measurements

To prove the principle of this measurement method, we perform bulk refractive index measurements. In a bulk refractive index measurement the refractive index of the liquid surrounding the PCS is tuned, while the GMR's shift is recorded and related to the refractive index of the liquid. To yield more information about the intensity modulation for each GMR, we initially analyze the spectrum for these experiments with a spectrometer.

Throughout this paper we used PCSs composed of a 300 nm thick tantalum pentoxide (Ta_2O_5) layer on a glass substrate with a 70 nm deep linear grating (Fig. 1(c)). The PCSs were fabricated by laser interference lithography in a photo resist and transferred into the Ta_2O_5 layer by a plasma etching process [14]. The PCSs cover the whole glass substrate, which had lateral dimensions of 2.5 cm x 2.5 cm. The transmission spectra of this PCS with and without crossed polarization filters are shown in Fig. 1(b). With a periodicity of $\Lambda = 370$ nm of the grating and surrounded by water, this PCS shows four GMRs, all of which lie within the falling edge of the spectrum of a selected green LED. The LED's central wavelength is at

518 nm. All four resonances shift similarly and contribute to the sensitivity of the sensor. We determined the sensitivity of the PCS as transducer in a previous study. For the four measured resonances, the average sensitivity was $\Delta\lambda/\Delta n = \text{ca. } 25 \text{ nm/RIU}$ (nm per refractive index unit). The PCS used in this paper was a linear grating in a high index layer. PCSs with higher order symmetry, however, can be applied as the transducer for this technology platform, too. Shi et al. [15] have shown a triangular PCS with a sensitivity of 327 nm/RIU for an individual mode operating at 600 nm. Using such a PCS would result in biosensors with higher sensitivities than presented here.

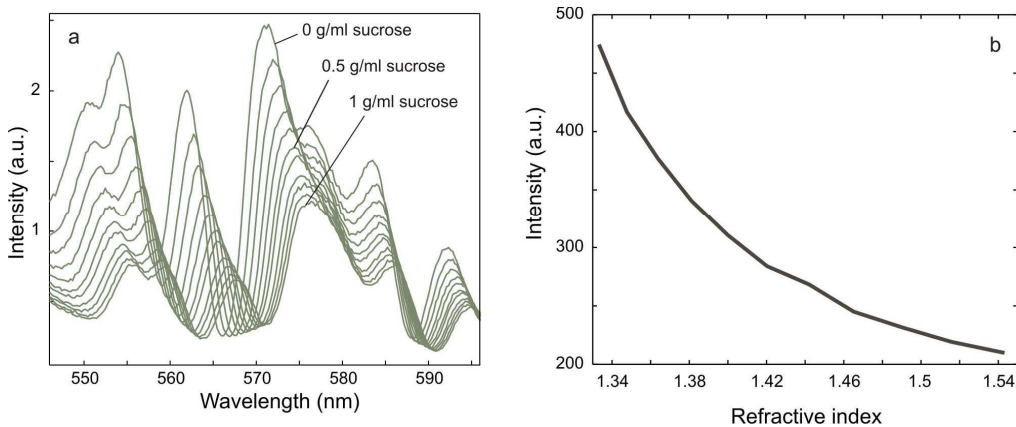


Fig. 3. (a) Transmission measurements with tuned refractive index and an LED as the light source. An intensity drop of up to 75% is observed, which stands in relation to the GMR shift. (b) Integrated intensity as deduced from spectra versus refractive index of the surrounded liquid obtained by integrating spectra obtained in Fig. 3(a). The decreasing intensity is due to the GMR shift, which is a function of the refractive index. As expected the intensity drop follows the sloping edge of the LED spectrum.

A series of water-sucrose ($\text{C}_{12}\text{H}_{22}\text{O}_{11}$) dilutions is prepared, which is used to cover the PCS surface. In Fig. 3(a) 11 measurements with sucrose concentrations from 0 g/ml to 1 g/ml with 0.1 g/ml steps are shown. Comparing these transmission measurements with results obtained in Fig. 1(b), we observe differences in mode intensity and spectral position. This is a result of the limited LED spectrum and its radiation characteristics. The short interaction length of the light with the solution and the low sucrose concentrations allow us to neglect the specific rotation of sucrose, which is a polarization rotation of light passing the solution.

Due to the tuned refractive index, GMRs show an average spectral shift of about 10 nm. Moreover, we observe as expected an intensity drop for all modes and a maximum change of 75% for individual modes.

The integration of the spectra over all GMRs yields intensity values comparable with those expected for a photodiode measurement. In Fig. 3(b) this intensity curve is plotted versus the associated refractive index. As expected, the intensity of transmitted GMRs follows the sloping edge of the LED spectrum and drops by more than 45%.

4. Compact demonstrator

To demonstrate the potential of this method to allow for compact and cost-efficient systems, we designed and realized a demonstrator. The setup of the demonstrator is as simple as depicted in Fig. 2(b). To detect the transmitted intensity, which is a function of the GMR shift, we use a silicon photodiode. The LED and the photodiode exhibit integrated epoxy optics. On the LED side this optics partly parallelizes the emitted light and directs it towards the detector. On the detector side the epoxy optic focuses the transmitted light into the detecting area.

The flow-cell (Fig. 4(a)) consists of an elastic o-ring squeezed between a glass substrate and the PCS, which is fabricated on a glass substrate as well, using fold back clips. A liquid supply containing the analyte is realized using a butterfly cannula, which is pierced into the

o-ring. Similarly for the outflow also a butterfly cannula is used. The pumping of the liquid is performed by manual operation of a syringe through the supply cannula, while the outflow cannula is opened. The liquid capacity of the flow-cell depends on the diameter and the thickness of the o-ring. In the present case the liquid volume was approximately 200 μl . For bulk refractive index measurements a complete liquid exchange of the flow-cell is of high impact. For this flow-cell 3 ml fluid sufficed for this purpose.

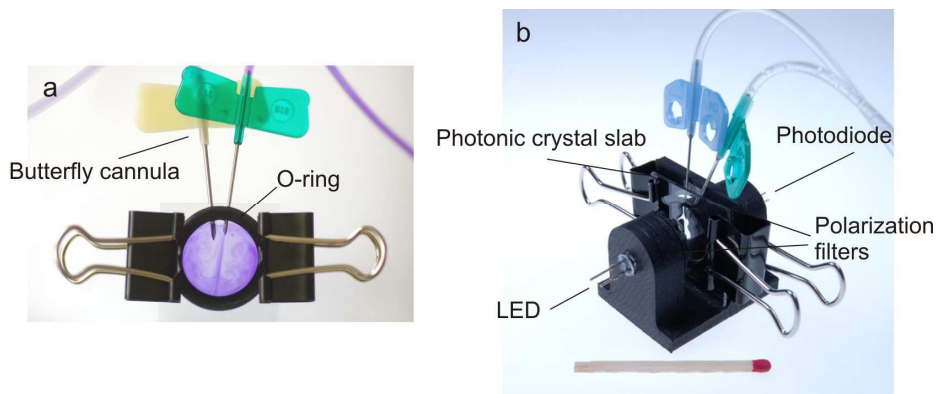


Fig. 4. (a) Photograph of the flow-cell during volume change with an ink-water dilution. The flow-cell consists of an o-ring squeezed between two glass substrates (one with the PCS on its surface). The liquid supply is realized using a butterfly cannula, which is pierced into the o-ring. The capacity of the flow-cell depends on the diameter and the thickness of the o-ring and was in this case approximately 200 μl . (b) Photograph of the assembled biosensor demonstrator.

To combine all components of the demonstrator in a compact way and guarantee correct adjustment, a polymer frame was designed by CAD software and fabricated using a 3D plotter. The 3D plotter uses a polymeric material for the fabrication and provides a resolution of about 0.1 mm. A photograph of the assembled biosensor demonstrator is shown in Fig. 4(b).

As the resonance shift determination is converted into an intensity measurement, a stable luminous flux of the light source is an essential issue. For this purpose we use a current source, which delivers a constant current independent of the LED voltage. The photodiode, on the other hand, is driven in a short circuit configuration with a current-to-voltage converter. The output voltage is analyzed and recorded using a data acquisition device, which was connected to a computer.

Again a bulk refractive index study is performed to estimate the detection limit of the demonstrator. We prepared three solutions with refractive indices close to each other, using water-isopropanol dilutions. With an isopropanol concentration of 0%, 0.5% and 1%, we achieved tuning of the refractive index in three steps with a $\Delta n = 2.25 \cdot 10^{-4}$ (with a refractive index of 1.333 @20°C for water and 1.378 @20° C for isopropanol).

We pumped these solutions into the flow-cell in two experiments. First, we alternate between pure water and 1% isopropanol dilution and exchange the whole volume of the flow-cell in periods with duration of 60 s. In a second experiment we alternate between water and 0.5% isopropanol. The injection into the flow-cell is performed with a syringe with a capacity of 3 ml and has a duration of about 10 s. Both results are plotted in Fig. 5, which is the output signal in voltage as a function of time. We observed three signal level, namely for pure water, for 0.5% and 1% isopropanol dilution. These signal levels are equally spaced with a voltage relative difference of about 0.13%.

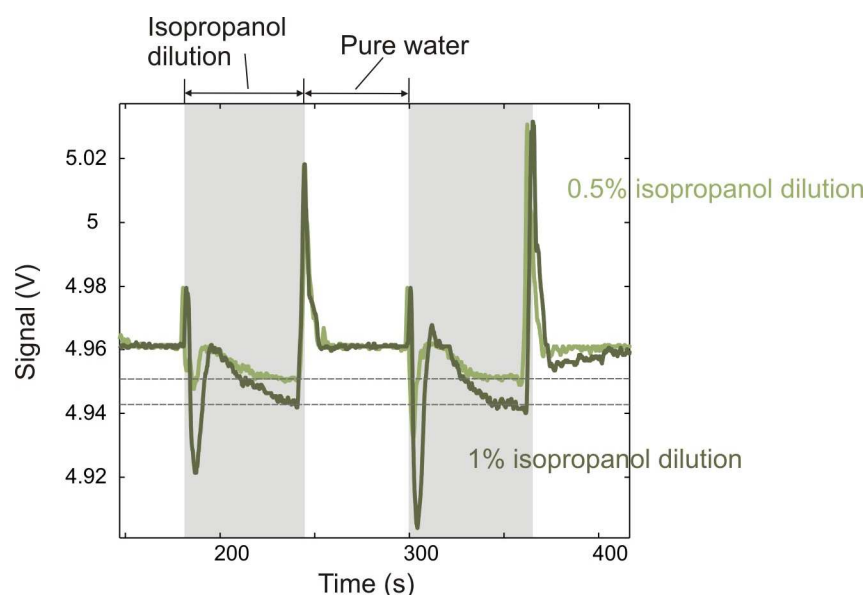


Fig. 5. Determination of the detection limit of the demonstrator, utilizing bulk refractive index tests. A 0.5% isopropanol-water dilution could be clearly differentiated from pure water. Hence, a bulk refractive index detection limit of $\Delta n = 2.25 \cdot 10^{-4}$ is obtained.

In addition, we observe signal peaks at every injection procedure (Fig. 5). We believe that this behaviour is caused due to the overpressure in the flow-cell during the injection procedure. These peaks are either due to refractive index changes caused by changed pressure or, more likely, because of displacement of the PCS relative to the light source.

5. Real-time label-free detection of streptavidin binding to biotin

To validate the optoelectronic sensor application as a biomolecule detector, we studied a key-lock system composed of streptavidin and biotin, two molecules that have great affinities to each other [16]. The surface of the PCS was functionalized with biotin, using a compound of biotinylated phospholipid 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine-N-(cap biotinyl) (N-Biotinyl Cap-PE) mixed in different mol% ratios with phospholipid 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC) in order to control the amount of the functional biotin-headgroup lipids deposited on the surface. We chose spin-coating as the method to apply biotinylated phospholipids on the PCS surface due to its simplicity of application and possibility to control the coating conditions. We used the flow-cell in order to apply streptavidin solution in phosphate buffered saline (PBS) (concentrations varying from 50 nMol to 2.5 nMol) onto the biotin-functionalized PCS surface. Figure 6 shows the time-dependent transmission analyzed as a control experiment with a spectrometer using an LED as light source. In this experiment, we injected 50 nMol of streptavidin into the flow-cell and characterized the second resonance of the PCS over a total time of 1,000 seconds. We observed a resonance shift of $\Delta\lambda = 0.5$ nm as well as an intensity decrease of the transmission, which follows the falling edge of the LED spectrum.

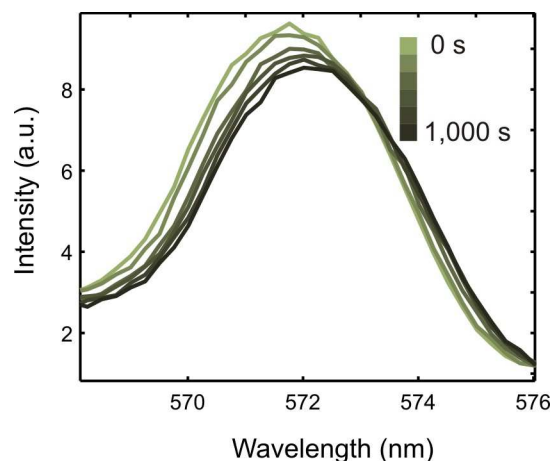


Fig. 6. Spectral analysis of the second resonance with a spectrometer and an LED as light source during the streptavidin coupling process. We observe a resonance shift of 0.5 nm as well as an intensity decrease of the transmission following the falling edge of the LED spectrum.

We have applied the presented demonstrator and have performed affinity measurements in real time. Figure 7(a) shows the effect of the ratio of biotin-DOPC mixtures on the signal. The green and the red curves show the relative signal response to 25 nMol streptavidin for the surface functionalized with 10 mol% and 4 mol% biotinylated DOPC, respectively. As expected, the streptavidin-biotin binding process was accelerated at a higher concentration of biotin. Furthermore, we observe saturation of the signal after 1,000 s. for the surface functionalized with 10 mol% biotinylated DOPC. This indicates that all the streptavidin molecules in the flow-cell are bound to the surface. The inhomogeneous rise of the signal is manifested by the non-homogeneous functionalization, which might be caused by imperfect spin-coating. Using 10 mol% biotinylated DOPC as functionalization, we reduced the concentration of streptavidin to 2.5 nMol and still observe a relative signal reduction of about 0.4% (Fig. 7(b)).

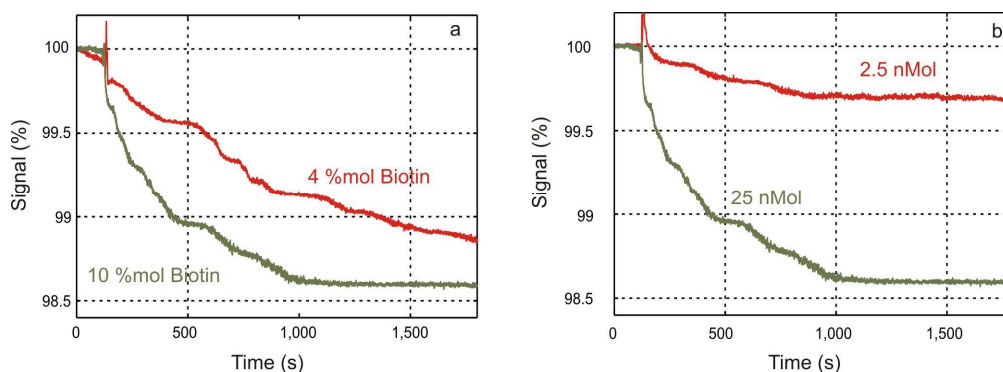


Fig. 7. (a) Relative voltage reduction using demonstrator of (Fig. 4a) as a function of time for two different composition ratios of N-Biotinyl Cap-PE admixed in DOPC. Signal saturation is reached faster with a 10 mol% of N-Biotinyl Cap-PE content in DOPC. (b) The influence of streptavidin concentration on the signal. Lowering the streptavidin concentration, the saturation level decreases. 2.5nMol streptavidin binding to N-Biotinyl Cap-PE functionalized surface was clearly detectable.

6. Conclusions

In conclusion, we have introduced a novel easy-to-implement technology platform for biosensors based on functionalized PCS in combination with crossed polarization filters.

Based on the proposed platform, we presented a biosensor demonstrator that uses an LED as light source and a photodiode as detector to perform label-free molecular affinity measurements in real time. We detected a 2.5 nMol streptavidin solution, which is a relevant concentration in life sciences.

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