

# Imaging label-free biosensor with microfluidic system

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## ABSTRACT

We present a microfluidic system suitable for parallel label-free detection of several biomarkers utilizing a compact imaging measurement system. The microfluidic system contains a filter unit to separate the plasma from human blood and a functionalized, photonic crystal slab sensor chip. The nanostructure of the photonic crystal slab sensor chip is fabricated by nanoimprint lithography of a period grating surface into a photoresist and subsequent deposition of a  $\text{TiO}_2$  layer. Photonic crystal slabs are slab waveguides supporting quasi-guided modes coupling to far-field radiation, which are sensitive to refractive index changes due to biomarker binding on the functionalized surface. In our imaging read-out system the resulting resonance shift of the quasi-guided mode in the transmission spectrum is converted into an intensity change detectable with a simple camera. By continuously taking photographs of the sensor surface local intensity changes are observed revealing the binding kinetics of the biomarker to its specific target. Data from two distinct measurement fields are used for evaluation. For testing the sensor chip,  $1\ \mu\text{M}$  biotin as well as  $1\ \mu\text{M}$  recombinant human CD40 ligand were immobilized in spots via amin coupling to the sensor surface. Each binding experiment was performed with  $250\ \text{nM}$  streptavidin and  $90\ \text{nM}$  CD40 ligand antibody dissolved in phosphate buffered saline. In the next test series, a functionalized sensor chip was bonded onto a  $15\ \text{mm} \times 15\ \text{mm}$  opening of the  $75\ \text{mm} \times 25\ \text{mm} \times 2\ \text{mm}$  microfluidic system. We demonstrate the functionality of the microfluidic system for filtering human blood such that only blood plasma was transported to the sensor chip. The results of first binding experiments in buffer with this test chip will be presented.

**Keywords:** blood filtration, guided-mode resonances, biosensor, imaging read-out system, microfluidic

## 1. INTRODUCTION

Microfluidic technology with an integrated biosensor is of high interest for the development of point-of-care applications. Such microfluidic systems have the ability to perform biochemical analyses using small reagent volumes and to be adapted to specific customer needs [1]. Especially for blood filtration the small reagent volume needed in microfluidic systems is beneficial compared to conventional diagnostic devices in centralized facilities [2]. To separate plasma from whole blood different concepts were introduced such as special filter materials [3], designed filtration channels [2, 4], or separation via a membrane [5]. Combining microfluidic concepts with a biosensor results in compact, decentralized, and fast operating devices [6, 9]. Biosensors typically consist of a selective biological recognition component (receptor) connected directly to a transducer [7]. The transducer transforms the binding kinetics into a detectable signal. Using photonic crystal slabs (PCS) as the transducer a label-free, compact, and cost-efficient system may be realized [8-10]. PCS are waveguides with a periodic nanostructure in a high refractive index material supporting quasi-guided modes. These modes can be excited in transmission as well as in reflection and are visible as guided-mode resonances (GMRs) in the optical spectrum. They have an evanescent fraction propagating above the PCS surface and are thus sensitive to mass changes at the surface. By functionalizing the surface with, e.g., antibodies or aptamers, protein binding kinetics results in a wavelength shift of the GMR caused by refractive index changes. This can be tracked in real time with an optical read-out system [9-11]. Previously, for other microfluidic devices optical read-out of the resonance spectral position was evaluated with a sensitive spectrometer [9]. With our imaging read-out system the resulting shift of resonances in the transmission spectrum is converted into an intensity change in the specific color channel of a simple CMOS camera aligned to the resonance wavelength [12]. Especially for point-of-care applications a test chip, which is able to detect multiple biomarkers in whole blood, combined with a compact and portable read-out system is of high interest. Here, we present an approach for a disposable test chip to serve all these properties (figure 1). In section 2 the fabrication of the nanostructured transducer surface and the procedure for specific bio-functionalization are described. In section 3 experimental results for imaging measurement of binding kinetics are presented. By continuously taking photographs of the PCS surface locally functionalized with the specific capture molecule local intensity changes are observed. Section 4 discusses the design of the microfluidic chip and the integration with the biosensor. The functionality of the microfluidic system for human blood filtration is demonstrated. Results of first

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binding experiments with 500 nM biotinylated bovine serum albumin dissolved in phosphate buffered saline are presented. Conclusions are drawn in section 5.

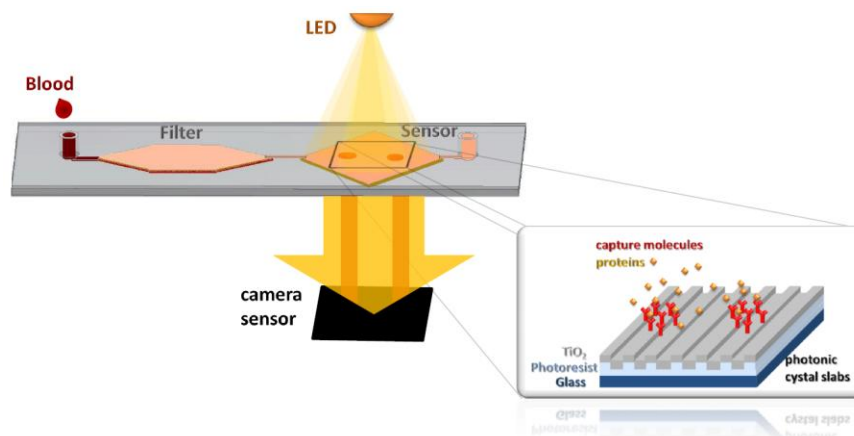


Figure 1: Schematic of microfluidic system for detection of multiple biomarkers with filter unit and bonded sensor chip. Label-free read-out is performed with a compact imaging system and a camera.

## 2. BIOSENSOR FABRICATION

Photonic crystal slabs are fabricated via ultra violet (UV) nanoimprint lithography. First, the nanostructure is transferred from a glass master into a stamp consisting of polydimethylsiloxane (PDMS). Sylgard 184 and curing agent (both from *DOW Corning*) were mixed in a ration of 8:1 for 15 minutes in a mixing tube (*IKA*). The glass master is placed within a teflon adapter und poured over with the PDMS. To remove all bubbles from the PDMS it is evacuated for another 15 minutes inside a vacuum chamber. Afterwards, the PDMS is hardened in an oven at 130°C for 20 minutes. The hardened PDMS is cut out of the teflon adapter and pulled of the glass master.

For sample fabrication 25 x 25 mm<sup>2</sup> glass substrates with a thickness of 1 mm are cleaned with acetone and isopropanol for 15 minutes each in an ultrasonic bath and then are dehydrated on a hotplate at 160°C for 10 minutes. After the glass substrate is cooled down the glass surface is activated with oxygen plasma for 2 minutes (HF power: 200W) to enhance the adhesion of the adhesion promoter and finally of the photoresist. Next 150 µl adhesion promoter (Amoprime from *AMO GmbH*) are spin coated for 30 seconds at 3000 rpm onto the substrate. The substrates are baked on a hotplate for 2 minutes at 115°C and cooled down for another 2 minutes. Now 150 µl of the photoresist Amonil (*AMO GmbH*) are spin coated with the same parameters as used before to generate a 200 nm thick layer. For transferring the nanostructure into the photoresist, the PDMS stamp is pressed carefully into the resist. The substrate including the stamp on top is exposed for 80 seconds with an UV halogen lamp to harden the resist. After the PDMS is removed from the resist, a 70 nm thick high-index layer of TiO<sub>2</sub> is deposited by reactive sputtering (figure 2a). The glass master and thus the fabricated PCS have a period of  $\Lambda = 350$  nm, a structure depth of  $t = 100$  nm, a duty cycle of 40:60, and a size of 25 x 25 mm<sup>2</sup>. For experiments with a fluid cell the PCS is used in this size, for experiments with the microfluidic chip the PCS has to be cut in 15 x 15 mm<sup>2</sup> pieces before functionalization.

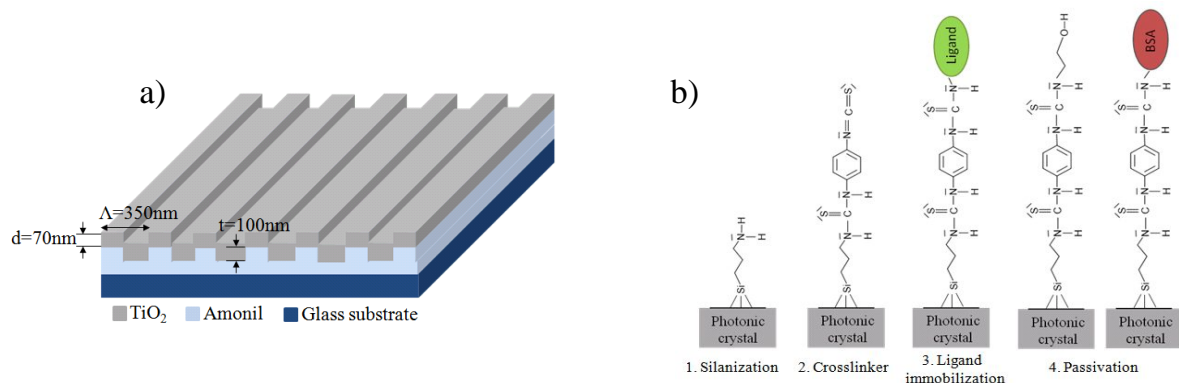


Figure 2: (a) Schematic structure of fabricated photonic crystal slab. (b) Schematic of the resulting molecules for the successive functionalization steps.

To transform the PCS into a biosensor we first activate the surface with oxygen plasma for 5 minutes (HF power: 50W). Afterwards the PCS is transferred into a nitrogen-filled glovebox. It is cleaned in dry ethyl alcohol and in dry methyl alcohol each for 5 minutes. Then the whole  $\text{TiO}_2$  surface is silanated with the molecule (3-aminopropyl)triethoxysilane (APTES) by incubating the sample for 1 hour in a solution of 1% APTES in 25 ml methyl alcohol at room temperature. Subsequently the substrate is washed with plenty methyl alcohol and baked for 20 minutes on a hotplate at  $110^\circ\text{C}$ . To couple the ligand covalently to the APTES molecule on the surface 0.1 g 1,4-phenylene diisothiocyanate (PDC) is dissolved in 0.5 ml pyridine and 4.5 ml N,N-dimethylformamide (DMF) and the substrate is soaked for 2 hours in this solution (all chemicals from *Sigma Aldrich*). PDC acts as a linker between the APTES and the ligand and is reactive with any amino group modified substance. Again the sample is washed with plenty methyl alcohol. For the chemical bonding of the ligand the sample is taken out of the nitrogen atmosphere and placed in a humidity chamber. Now  $1\ \mu\text{M}$  ligand – biotinylated bovine serum albumin (BSA-biotin) or recombinant human CD40 ligand (*PeptoTech GmbH*) – dissolved in sodium acetate buffer (pH 5.5) is placed in 0.5  $\mu\text{l}$  drops on the PCS surface and is incubated over night in the humidity chamber. The next day the non-bound molecules are washed away with Dulbecco's phosphate buffered saline (pH 7.4) (DPBS) (*Lifetechnologies GmbH*). To passivate the remaining functional groups of the PDC first 1 M ethanolamine-HCl (*BIO RAD*) and then 1 mg/ml bovine serum albumin (*Sigma Aldrich*) dissolved in DPBS cover the PCS surface for 30 minutes. After each passivation step the sample is washed with DPBS. In figure 2b schematics of the resulting molecules for each functionalization step are shown.

### 3. PROTEIN DETECTION WITH FLUID CELL

First, we investigated the functionality of our sensor using a fluid cell. An o-ring is squeezed between the  $25 \times 25\ \text{mm}^2$  large biosensor and a glass substrate. For the analyte exchange two butterfly cannulas are pierced through the o-ring and all is fixed with two screwed substrate holders. Figure 3a depicts a photograph of the employed measurement system [12]. The imaging read-out system has an overall size of  $13\ \text{cm} \times 3.5\ \text{cm} \times 4.9\ \text{cm}$  and the whole lens system, two crossed polarizing filters and a light emitting diode (LED) are placed inside the metallic torso. The fluid cell within the sensor is placed between a parallelizing lens for the LED side and a focusing lens for the camera side and between two linear polarizing filters. Because of this arrangement only light interacting with the quasi-guided modes is transmitted through the biosensor to the CMOS sensor of a simple camera. Via the USB interface the LED and the camera are controlled with a computer as well as real-time data processing can be realized for noise minimization during the protein detection. Aligning the guided-mode resonance of the PCS to the falling edge of a colored LED the resonance shift due to binding kinetics of the protein results in an intensity reduction in the transmitted light [13]. This intensity change can be recorded with the camera by continuously taking pictures of the sensor surface during the protein binding process. The applied protein binds to its respective target molecule on the sensor surface and causes corresponding to the local functionalization a local intensity reduction, as is shown in figure 3b.

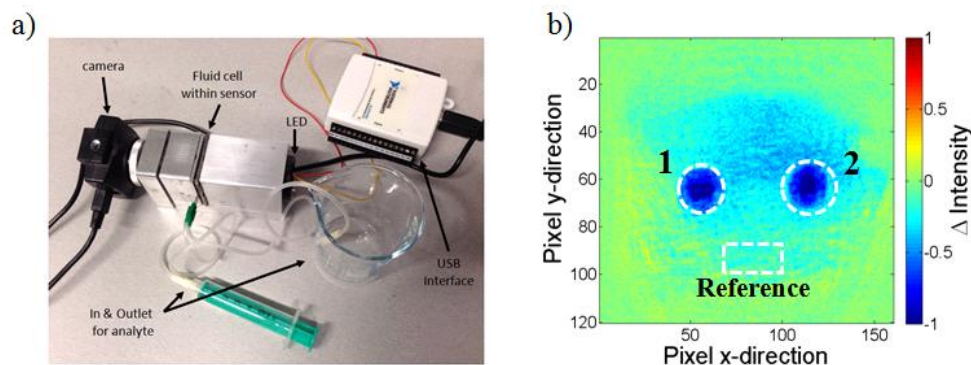


Figure 3: (a) Photograph of the read-out system with fluid cell. (b) Difference image obtained by subtracting the first recorded image from the last image during the protein binding. The white encircled areas '1' and '2' mark the local intensity reduction caused by CD40 ligand antibody association and 'Reference' the reference field.

For figure 4a and b the mean values of the two measuring fields and one reference field are calculated for each taken photograph and the resulting intensity values are plotted against time for each protein detection. To generate a baseline the fluid cell was filled with pure phosphate buffered saline. After seven minutes the pure buffer was replaced by the protein-buffer-solution ((a) 90 nM CD40 ligand antibody and (b) 250 nM streptavidin). Washing again with pure buffer the non-bound protein was washed away and the intensity decreases no longer. For both protein detections the association to the target protein is clearly visible while the reference signal does not show unspecific binding events. But the amplitude measured during the same protein detection results in different intensity reductions. This is caused on the one hand by the low protein concentration (90 nM or 250 nM) compared to the ligand concentration (1  $\mu$ M) functionalized on the PCS surface and on the other hand by the position of the measuring fields. The closer a measuring field is placed to the stream, which is caused by the analyte exchange, the higher is the signal amplitude. Furthermore, different signal amplitudes can be observed comparing the intensity reductions caused by the association of CD40 ligand antibody (reduction of 4%) and streptavidin (reduction of 1.5%). Although the streptavidin concentration is higher than the CD40 ligand antibody concentration, the intensity is decreased much less than one might have expected. We attribute this to the higher molecular weight of the antibody with 150 kDa (streptavidin: 60 kDa) and thus the higher refractive index change on the surface. In summary, for both proteins association kinetics are detectable clearly.

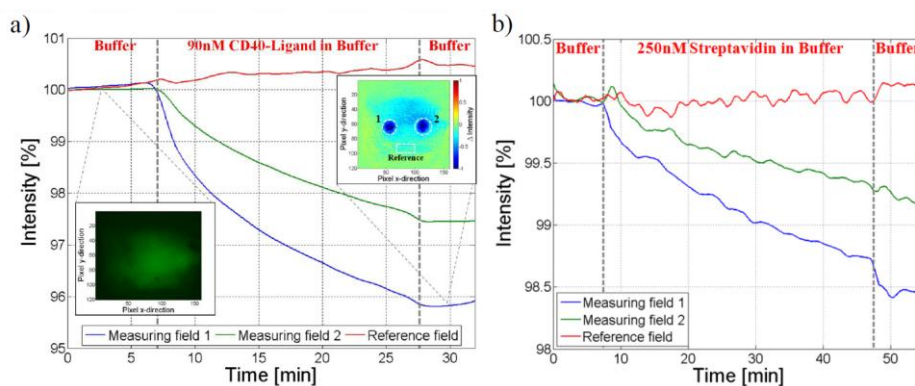


Figure 4: Association of (a) 90 nM anti-CD40 ligand in buffer to a surface functionalized with CD40 ligand and (b) 250 nM streptavidin in buffer to a biotinylated surface causes reduction of intensity over time.

#### 4. PROTEIN DETECTION WITH MICROFLUIDIC TEST CHIP

Next we developed a microfluidic test chip with the size of 75 mm x 25 mm x 2 mm in cooperation with the company *Microfluidic ChipShop GmbH*. As figure 5a shows, the chip consists of two parts. The main chip includes a filler neck for the application of whole blood, capillary channels for blood transport, and a cavity for the sensor. The cover plate includes a filter membrane for blood filtration and an adhesive foil for sensor bonding. After removing the protective foil

from the adhesive foil, the 15 x 15 mm<sup>2</sup> large sensor is pressed in the allocated space. The adhesive foil ensures on the one hand the connection between microfluidic chip and the sensor and on the other hand the imperviousness of the whole system. The blood is spiked with the anticoagulant heparin to inhibit immediately occurring coagulation inside the capillary channels because of surface enlargement. 200 µl blood is aspirated with a pipette and has to be pressed into the capillary channels and through the filter membrane with a pressure of approx. 50 mbar. The cellular remain in the membrane and only the clear blood plasma (approx. 70-100 µl) is transported to the sensor field (figure 5b).

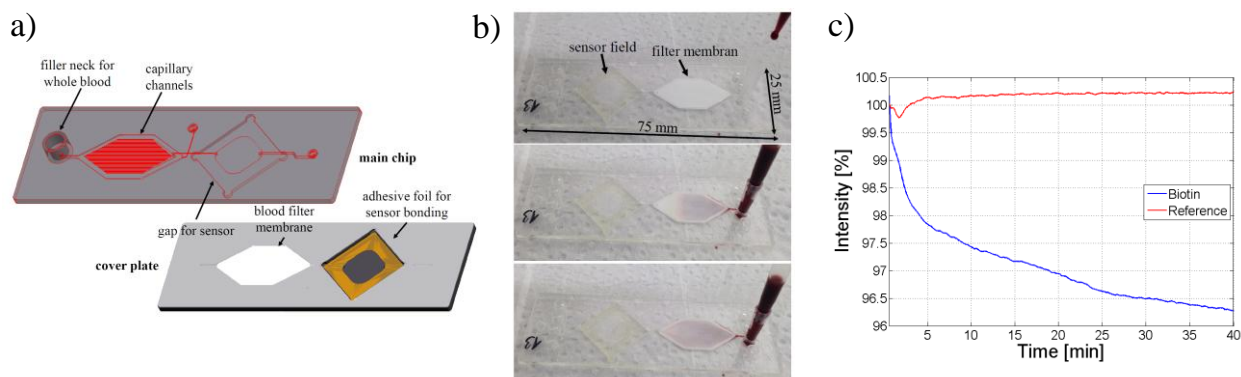


Figure 5: (a) Computer aided-design of microfluidic main chip including filler neck for whole blood, capillary channels and cavity for sensor (top) plus cover plate including blood filter membrane and adhesive foil for sensor bonding (bottom). (b) Layout of microfluidic test chip and human blood injection with pipette through filter. (c) Association of 500 nM BSA-biotin in buffer detected with test chip causes reduction of intensity over time.

To evaluate the functionality of our test chip we performed first binding experiments by immobilizing initially 1 µM BSA-biotin and then 1 µM streptavidin on the sensor surface. To simulate measurement conditions as they would be existent for binding experiments with whole blood 500 nM BSA-biotin dissolved in phosphate buffered saline are filled into the test chip. Immediately the test chip is placed inside our read-out system and pictures are taken continuously during the protein binding process. Figure 5c demonstrates a clear association of BSA-biotin visible in the transmitted light while again the reference does not show any unspecific binding events.

## 5. CONCLUSION

We investigated the functionality of our biosensor and our imaging read-out system by successfully performing detections of 90 nM CD40 ligand antibody as well as of 250 nM streptavidin. The simultaneous tracking of measuring fields and non-functionalized background surface holds particular promise for continuous calibration. Furthermore, we developed and demonstrated a microfluidic chip for blood filtration. First binding experiments of 500 nM BSA-biotin were performed using the test chip with the biosensor and strikingly binding events are visible in the signal sequence. However, measurements with whole blood provided too much background noise so that binding kinetics of biotin could not be identified precisely in the actual setting. The high viscosity of the blood causes a continuous in-flow onto the sensor surface for several minutes. Hence, more optimization effort has to be spent on this point. Nevertheless, this approach is promising for a disposable test chip for multiple protein detection in human blood applicable for point-of-care applications.

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