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Sensitivity and Limit of Detection of biosensors based on ring resonators

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ABSTRACT

In this work, we present a study of the Sensitivity (S) and Limit of Detection (LOD) of microring based photonic biosensors as a function of the waveguide composition and dimensions. The target is Aflatoxin, which is a toxin of major concern for south Europe dairy industry. The sensing device is based on an array of multiple SiON microring resonators, fiber-coupled to both an 850 nm VCSEL and a silicon photodetectors, packaged with a microfluidic circuit. Volumetric sensing with glucose–water solutions of various concentrations yields a best sensitivity of 112 nm/RIU. To link these results to the Limit of Detection of the sensors, we also measured the noise of our experimental readout system and then calculated the LOD of our sensors. We found a best value of LOD of 1.6×10^{-6} RIU (referred to volumetric sensing). Finally, we detected Aflatoxin in solutions of various concentrations (down to 1.58 nM) by functionalized sensors. The functionalization is based on a wet silanization and specific DNA-aptamer binding on the chip. Reproducibility and re-usability of the sensor are investigated by several chemical treatments. Optimum procedure allows multiple sequential measurements with a good endurance. This work was supported by the FP7 EU project "Symphony" (Grant agreement no.: 610580).

1. Introduction

Silicon photonic biosensors are ideal candidate for the development of integrated electro-optic microfluidic chips (Lab on a chip, LOC) devices [1,2]. Their numerous advantages – such as miniaturization, robustness and accuracy – can lead to devices able to perform completely automated biological analysis and clinical diagnostics for pharmaceutical and biotechnology companies. Nowadays, the most widely used optical biosensors are based on Surface Plasmon Resonance (SPR) techniques [3], but nanophotonic devices like ring resonators and optical waveguide gratings are in full expansion [4].

One application in which a silicon photonic biosensor could be a breakthrough innovation is the sensing of toxins in the dairy production. The European Community regulation, in fact, limits the maximum allowable concentration of Aflatoxin in milk products to 50 ng/kg. Presently, the screening procedure involves Enzyme-Linked ImmunoSorbent Assay (ELISA) tests [5], and the suspicious samples need further investigations with High-Performance Liquid Chromatography (HPLC) tests [6], which are costly and time-consuming processes.

Within the European project Symphony, we are developing an optical biosensor to detect Aflatoxin in milk with a fast, cheap and reliable procedure. Since the presence of casein micelles and milk fat globules could affect the performances of our sensor, a pre-purification module is being developed in order to deliver to the photonic sensor a solution cleaned by proteins and contaminants [7]. Therefore, in the following, we will characterize the sensor with model solutions.

In this work, we designed and measured microring-based photonic biosensors. These devices have silicon oxynitride (SiON) as waveguide core material, in order to work in the visible range, and are fabricated by standard CMOS processing. We analyzed their Sensitivity (S) and Limit of Detection (LOD) as a function of the waveguide composition and dimensions. Furthermore, we performed sensing measurements on samples functionalized with biorecognition agents, DNA-aptamers, specific for the detection of Aflatoxin M1 [8,9]. We performed measurements with Aflatoxin M1 in a buffer solution on samples with and without aptamers, in order to observe the binding of Aflatoxin on the surface of the sensor. We repeated and confirmed these measurements regenerating the samples using Glycine solution.

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2. Material and methods

2.1. Fabrication process

For the microring fabrication, SiON films were deposited by plasma-enhanced chemical vapor deposition (PECVD) on five 6-in. 625 μm thick crystalline Si wafers with a 4 μm thick buffer oxide layer. For one wafer, the SiON refractive index was chosen to be 1.8, while, for the others, the SiON refractive index was 1.66. Table 1 reports the thicknesses and refractive indices of all wafers.

The waveguides, ring resonators and directional couplers structures were defined using standard UV-lithography and reactive-ion etching techniques. An annealing step of 1.5 h at 1050 °C was also performed in order to remove hydrogen bonds from the material. Such process should also decrease the losses of the structures. Finally, the processed wafers were covered with a 1 μm TEOS film, used as a cladding layer.

In order to create the sensors, we opened the cladding layer around the resonators through a BHF wet etching. This allows the functionalization of the rings as well as the sensing measurements through their exposure to the ambient and to the molecules to be analyzed.

2.2. Sensor design

A scheme of the photonic sensor architecture is presented in Fig. 1. The input signal is directed to four different microrings. The width of the bus waveguide was fixed to 900 nm, while the resonator waveguide width to 1000 nm. The use of waveguide directional coupler for the splitters allows using a large gap distance between the waveguides (in our case 600 nm) and avoids the use of high lithography resolution. The same directional couplers are used to couple the bus waveguide to the resonator waveguide.

As a resonator structure, we used racetrack shaped ring resonator. The gap between the bus and the ring waveguides was set to 600 nm, while the curvature radius has been imposed to be $R = 100$ μm in order to have negligible bending losses.

2.3. Functionalization process

The functionalization procedure performed on the sensors is based on wet silanization protocol. After a cleaning process (with a Piranha solution) to remove organic contaminations, the samples were immersed in 0.01% v/v of GPTMS (3-glycidoxypropyl methyldiethoxy silane) in anhydrous toluene at 60 °C for 10 min. Then an amino-terminated DNA-aptamer (5′-NH_2-$(CH_2)_6$-GT TGG GCA CGT GTT GTC TCT CTG TGT CTC GTG CCC TTC GCT AGG CCC ACA-3′) at 100 μM in phosphate buffer (50 mM, ionic strength 300 mM, pH 8) was incubated on silanized surfaces for 2 h. The aptameric sequence with a k_D of 10 nM was identified by NeoVentures Biotechnology Inc. [8]. The amino-modified sequence is HPLC purified and was purchased from IDT Integrated DNA Technologies (Leuven, Belgium). Finally, an ethanolamine passivation at 1 mM for 30 min was applied.

2.4. Measurement setup

The sensors were characterized using a single mode tapered fiber placed on a piezoelectric movement as input. For collecting the light at the output of the different resonators simultaneously, we used a fiber array placed on a piezoelectric movement. Glycerol was placed between the sample and the fiber array and acts as index matching gel in order to increase the coupling efficiency between the fibers and the output waveguides. As light source, we used a single mode and polarization VCSEL at 850 nm connected to a single mode fiber. The polarization of the input light was controlled using a 2-paddles polarization controller. For the analysis of the data, we used transimpedance amplified Si-photodetectors and an eight channel USB oscilloscope connected to a computer. A visible camera mounted on top of an optical microscope was also used for the alignment process.

Table 1
Characteristics of the different sensors processed.

Wafer name	SiON refractive index	Deposited thickness (nm)	Shrinkage estimated (%)	After annealing estimated SiON thickness (nm)
L2	1.66	410	15	349
L5	1.8	240	10	216
BS1	1.66	320	8	295
BS2	1.66	350	8	322
BS3	1.66	400	8	368

For sensitivity and sensing measurements, we used a homemade PDMS microfluidic flow cell, with a volume chamber of less than 0.5 μL, connected to a VICI M6 liquid handling pump.

3. Results and discussion

3.1. Sensitivity and Limit of Detection

The spectral sensitivity of the sensor is defined as $S = \Delta\lambda/\Delta n$ where $\Delta\lambda$ represents the shift of the sensor resonance in nm, and Δn the change of the refractive index solution flowing on top of the sensor. In our experiments, we measured the sensitivities of the ring resonators as a function of the SiON refractive index and of the waveguide thickness. The measurement was achieved by exposing the sensors to glucose–water solutions of various concentrations, whose refractive index can be easily estimated [10]. The shift of the resonance of the sensor as a function of the flowing solution allows us to determine the sensitivity of the resonator. The results of these measurements for all wafers are presented in Fig. 2. The highest sensitivity was found in the case of $n_{SiON} = 1.8$ with $S = 112$ nm/RIU. For $n_{SiON} = 1.66$, the best thickness was found in the case of BS2 wafer, with $S = 82$ nm/RIU, and with a very good reproducibility for what concerns similar resonators on the same chip (differences in the order of 1%).

To link these results with the Limit of Detection (*LOD*) of our sensors, we measured the noise level of our experimental setup. The *LOD* is defined as the minimum input quantity that can be distinguished with more than 99% fidelity, and can be calculated as $LOD = 3\in/S$, where $\in$ is the output uncertainty. Using this formula, we measured a LOD of 3×10^{-6} RIU in the case of $n_{SiON} = 1.66$, and 1.6×10^{-6} RIU for $n_{SiON} = 1.8$. According to these results, we decided to perform sensing measurements on BS2 sample, which has the best sensitivity concerning samples with $n_{SiON} = 1.66$.

3.2. Aflatoxin sensing measurements

To perform Aflatoxin-sensing measurements on BS2 samples, we initially filled the microfluidic chamber with a buffer solution. In our case, the buffer solution is mainly based on 2-(N-morpholino)ethanesulfonic acid (MES) with a certain amount of dimethyl sulfoxide (DMSO) that depends on the Aflatoxin. We then injected the solution containing Aflatoxin AFM1 at a known concentration, and monitored the drift in time of the resonance due to the capture of Aflatoxin from the functionalized biosensor. Finally, we injected again the buffer solution. All measurements were done with a flow of 3 μL/min.

Two solutions were investigated, one with AMF1 and one without, in order to see the resonance shift due to the binding of Aflatoxin on the surface of the chip.

It is important to note that after each Aflatoxin injection, two or three Glycine injections were effectuated, in order to detach the toxin