

On-Glass Optoelectronic Platform for On-Chip Detection of DNA [†]

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Abstract: Lab-on-chip are analytical systems which, compared to traditional methods, offer significant reduction of sample, reagent, energy consumption and waste production. Within this framework, we report on the development and testing of an optoelectronic platform suitable for the on-chip detection of fluorescent molecules. The platform combines on a single glass substrate hydrogenated amorphous silicon photosensors and a long pass interferential filter. The design of the optoelectronic components has been carried out taking into account the spectral properties of the selected fluorescent molecule. We have chosen the $[\text{Ru}(\text{phen})_2(\text{dppz})]^{2+}$ which exhibits a high fluorescence when it is complexed with nucleic acids in double helix. The on-glass optoelectronic platform, coupled with a microfluidic network, has been tested in detection of double-stranded DNA (dsDNA) reaching a detection limit as low as 10 ng/ μL .

Keywords: lab-on-chip; amorphous silicon; photosensors; thin film device; fluorescence; interferential filter; DNA detection

1. Introduction

Detection of deoxyribonucleic acid (DNA) has always received a lot of attention in different and important fields such as genetic research [1], food safety [2], forensic [3] and so on. At the same time, in the last years, lab-on-chip systems have known a great development due to the significant reduction of sample, reagent, energy consumption and waste production that they offer with respect to the traditional methods. Detection systems based on electrochemical [4] mechanical [5] and optical [6] methods have been developed. Among the optical techniques, mostly of those used for DNA detection are based on the detection of fluorescence. This is usually determined by fluorophores/fluorochromes intercalated into the target DNA molecules [7].

In this paper, we report the technological development of an on-glass optoelectronic platform based on thin film technologies for fluorescence detection. The proposed platform is a very compact system, because reduces to a few hundred of microns the distance between the fluorescence source and the photosensor. This characteristic leads to a strong decrease of optical losses due to light diffusion and to an improvement of limit of detection and sensitivity of the system.

2. Materials and Methods

The optoelectronic devices have been fabricated through standard microelectronic thin film technologies such as photolithography, metal, semiconductor and oxide deposition and etching. The platform assembly foresees at first the photosensor fabrication and subsequently the filter deposition.

2.1. a-Si:H Photosensor Fabrication

The a-Si:H sensors are n-type doped/intrinsic/p-type doped stacked structures fabricated through a five-mask procedure described in [8]. In particular, the photodiodes have been deposited by Plasma Enhanced Chemical Vapor Deposition at temperature below 200 °C. The thickness of the n, i, and p regions are 50, 350 and 10 nm, respectively.

2.2. Filter Fabrication

The filter is a long-pass interferential constituted by alternating layers of zinc sulfide (ZnS) and magnesium fluoride (MgF₂) deposited directly over the a-Si:H diodes. Its structure is reported in Figure 1a. In order to avoid degradation of the optoelectronic performances of the previously deposited a-Si:H photodiodes, the filter has been deposited at room temperature by Electron Beam Physical Vapor Deposition without any patterning process.

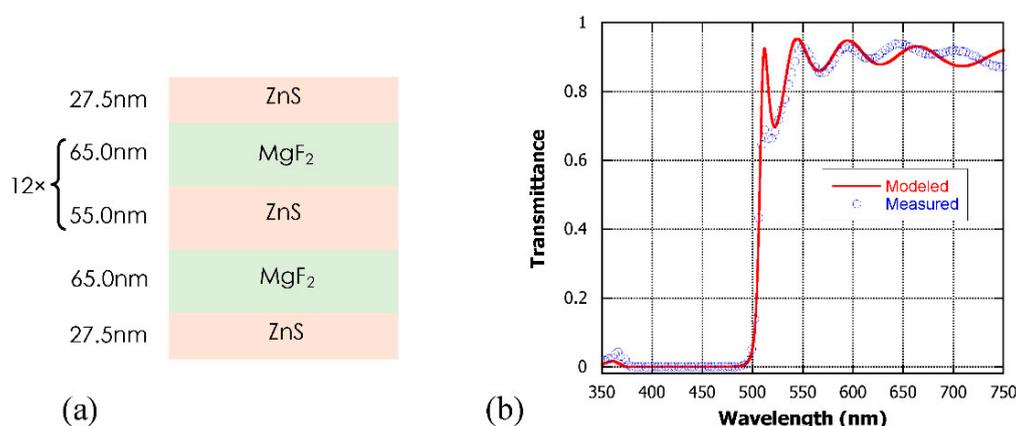


Figure 1. (a) Cross section of the interferential filter structure. (b) Modeled and measured transmittance of the filter deposited on a plain glass substrate.

2.3. Microfluidic Network Fabrication

The microfluidic network, constituted by a 40 mm-long, 1 mm-wide and 50 μm-high linear channel, is made in polydimethylsiloxane (PDMS). The one used in this work is the Sylgard 184 (Dow Corning, Midland, MI, USA), constituted by a base and a curing agent. The microfluidic chip has been fabricated by pouring a mixture of the PDMS linear polymer and the crosslinker over a mold deposited on a silicon wafer. After curing (at 60 °C for 2 h 30 min), the PDMS has been peeled from the mold and bonded to the glass substrate using the transfer bonding technique with PDMS as adhesive [9].

3. Results and Discussion

As reported in Figure 2, the platform combines on a single glass substrate a-Si:H photosensors [10] and a long pass interferential filter [11]. The operation principle of the system is based on the excitation of a fluorescent dye complexed with the double-stranded DNA (dsDNA) molecules through a suitable radiation source. The filter reflects the excitation radiation and transmit only the re-emitted light, which is detected by the photosensors and monitored as output current signal.

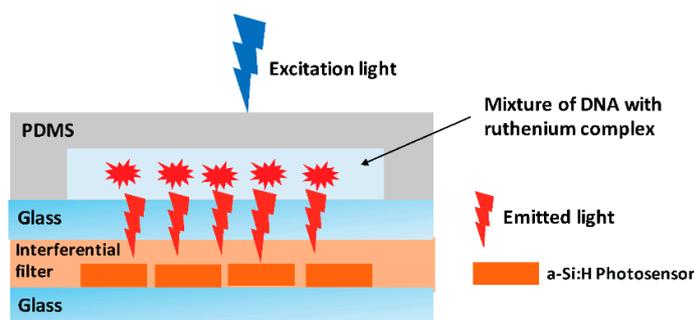


Figure 2. Schematic cross section of the structure for the on-chip detection of DNA. The thicknesses of the interferential filter is a few tens of microns. The thickness of each glass is 1 mm.

The design of both photosensors and filter depends on the optical properties of the selected fluorescent dye. In our work, we have chosen the ruthenium complex $[Ru(phen)_2(dppz)]^{2+}$ which exhibits a high fluorescence when it is complexed with nucleic acids in double helix. Specifically, the absorption spectrum of the $[Ru(phen)_2(dppz)]^{2+}$ -dsDNA complex is centered at 400 nm, while its fluorescence shows a strong emission with a peak around 610 nm [12]. Taking into account these characteristics, the filter has been designed as an interferential structure by using XOP [13].

We have preliminarily measured the performances of the interferential filter and the photosensors. The experimental data of transmittance of the filter deposited on a plain glass substrate are reported in Figure 1b. We observe a long-pass behavior with a cut-off wavelength (50% of transmittance) at 510 nm and a very good agreement between theory and experiment. Furthermore, from the raw data we derive that the ratio between the transmittance at 610 nm and 400 nm is equal to 10^5 indication of a very good rejection of the excitation source.

From the experimental characterization of the photosensors before and after the filter deposition, we have found that while the spectral response remains almost unchanged for wavelength longer than 550 nm, it presents a sharp decrease at wavelengths below 530 nm. Specifically, we found that:

$$\frac{QY_B(400\text{ nm})}{QY_A(450\text{ nm})} = 400, \tag{1}$$

where QY_B and QY_A are the quantum efficiency before and after the filter deposition, respectively.

Testing of the system in detection has been performed by coupling the optoelectronic platform to the microfluidic network. The micro-channels have been aligned with the a-Si:H photosensors and filled with solutions at different dsDNA concentrations and a fixed concentration of ruthenium complex equal to 6×10^{-5} M. Results are reported in Figure 3. Data demonstrate the successful detection of dsDNA with a detection limit of 10 ng/ μ L.

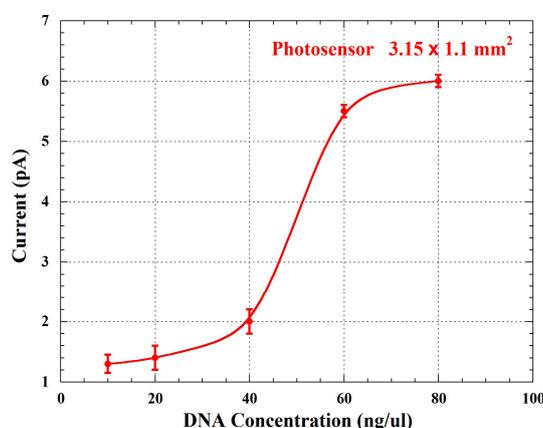


Figure 3. Sensor photocurrents as a function of the DNA concentration in the solution inside the microfluidic channel. Concentration of ruthenium complex is 6×10^{-5} M. Symbols and error bars represent the averaged values and standard deviations over three independent measurements, respectively.

4. Conclusions

We have presented an integrated optoelectronic platform designed to detect fluorescent molecule. In particular, a-Si:H photosensors and a thin film interferential filter have been fabricated on a single glass substrate, by using thin film microelectronic technologies. The system has been tested by coupling the optoelectronic platform to a PDMS microfluidic network, filled with solutions at different dsDNA concentrations and a fixed concentration of $[\text{Ru}(\text{phen})_2(\text{dppz})]^{2+}$. A detection limit of 10 ng/ μL has been achieved, suggesting that the proposed system is effective for application in the real-time monitoring of DNA amplification.

Author Contributions: D.C., A.N., G.C. designed the optoelectronic platform, F.C. conceived and designed the DNA experiments; M.N., D.C. and G.C. and fabricated the optoelectronic platform, F.C. and N.L. performed the experiments on DNA; all authors analyzed the data; D.C. wrote the paper.

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Conflicts of Interest: The authors declare no conflict of interest.

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