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Invited Paper

Biosensing microsystem platform based on the integration of Si Mach-Zehnder interferometer, microfluidics and grating couplers

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ABSTRACT

We have achieved the design, fabrication and packaging of microfluidic networks with photonic sensors for novel lab-on-chip platforms which incorporate the on-chip biosensing detection. As sensors, we used an integrated Mach-Zehnder interferometer (MZI) based on TIR waveguides ($Si/SiO_2/Si_3N_4$) of micro/nanodimensions for evanescent field detection of biomolecular interactions onto the sensing areas. For the lab-on-a-chip development, the biosensors have been integrated with a 3D microfluidic polymer network. In addition, we have developed a novel hybrid device for coupling light into the waveguides of the Mach-Zehnder sensor. The coupling hybrid device is composed of a thin film of diffraction gratings fixed to a polydimethylsiloxane film. The optical characterization has shown the excellent compatibility and integration between fabrication technologies of silicon MZI, SU-8 polymer microfluidic network and mountable grating couplers.

Keywords: Photonic Biosensor, Mach-Zender, Interferometer, Microfluidic, lab-on-a-chip.

1. INTRODUCTION

Most clinical diagnostic tests are based on time-consuming, expensive, and sophisticated techniques performed by specialized technicians in laboratory environments. These techniques typically require labeling of the samples or reagents with fluorescent or radioactive markers. The ideal situation would be to have diagnostic methods that can provide a fast, simple, direct and cost-effective testing. Advances in micro- and nanobiosensors technology is offering the implementation of diagnostic tools with increased sensitivity, specificity, and reliability for *in vivo* and *in vitro* applications. These sensors could also done simultaneous measurements in parallel of hundreds of substances. The best option would be the integration of several analytical steps, from sample preparation to detection, into a single miniaturized device (i.e., lab-on-a-chip or LOC)[1,2]. Such a device could contain enough hard wired intelligence and robustness to be used by the patient and deliver a multitude of data to the practitioner or the central database of a hospital. In the future, these devices will have a wide influence in patient management, in improving patient's quality of life and in lowering mortality rates. It is clear that the application of a portable, easy-to-use and highly sensitive biosensor LOC for real-time clinical diagnosis could offer significant advantages over current methods.

Two main factors play a role in the choice of the detection method for LOC application: sensitivity and scalability to smaller dimensions. Initial developments in LOC-based analysis employed optical fluorescence detection and it still is a preferred detection mode nowadays, due to its superior sensitivity[2]. However, this type of detection is

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bulky and off-chip. The key point in the development of a LOC with on-chip detection is the integration of the detection unit with the microfluidics. In the literature, there are a few examples of such integration and it is still a challenge, especially if label-free sensors are employed as detection units. One way could be the employment of integrated photonic biosensors, as the integration of planar waveguide technologies with microfluidic devices is an excellent solution towards minituarization. Some few work deaed with the integration of microfluidic technologies with photonic label-free biosensor devices but with poor results[3]: microfluidic structures were attached manually to the optical devices by using adhesive epoxies. This manual method was not practical for the alignment of complex (up to several cm lengths and a few microns width) microfluidic structures onto microphotonic sensor arrays.

But even with the advantages of the miniaturization, problems of sensitivity, stability and packaging have prevented the development of a LOC with on-chip optical detection. The complexity of the design, fabrication and optical adjustments is the major drawback[2]. However, the utilization of silicon micro/nanotechnology for the hybrid integration of the different components of a LOC device is opening a way to do it, as with this technology is possible to have a better control of the light path by the use of optical waveguides, mechanical stability, higher sensitivity, miniaturisation and the possibility of mass-production.

Our main aim is to develop a highly sensitive LOC platform based on label-free biosensing detection and integrated in a microsystem instrument fabricated with low-cost technologies of polymer and silicon, and meeting the requirements of cost, disposability and portability. Although label-free biosensing techniques are well-known, they are typically only used at the research level and their full potential for commercial devices has not been exploited so far. The biosensor LOC microsystem is being assembled by integrating the following parts: (i) micro/nano sensors fabricated with standard silicon technology, (ii) a polymer microfluidic cartridge monolithically integrated with the sensor and with the corresponding tube connections, (iii) novel grating coupler devices for the in and out coupling of the light in all the photonic channels for multisensing (iv) robust immobilisation and regeneration protocols for the biological receptor (iv) CMOS photodetectors, electronic & software control (v) final integration and packaging. In the following, we will show our last developments in the sensor, microfludics and grating couplers steps and the integration between them.

2. MACH-ZENHDER INTERFEROMETRIC BIOSENSOR

The key element of the microsystem was an array of photonic microsensors based on Mach–Zehnder interferometer devices of high sensitivity. The interferometer devices were fabricated on silicon technology and constructed from rib waveguides of nanometer dimensions (and wavelength in the visible range). In the MZI configuration (see Figure 1), the coupled guided light is splitted into two arms, and after a certain distance, are recombined again in an output optical waveguide. In one of the arms a sensor area of length L is opened to bring the waveguide and the environment into contact. When the evanescent wave of the light circulating in the sensor area interacts with the receptor biomolecules on the waveguide surface, there is a change in the properties of the light. It is possible to measure, quantify and to establish the relationship between such change and the concentration of the adsorbed molecules. For the array of sensors, each sensing arm can be functionalised with a different biological receptor specific to the substance to be detected. In this way, we can obtain a device for the real-time detection of substances at the pico/femtomolar level without using fluorescent o radioactive labels. The high sensitivity of the analysis is guaranteed by the design of the photonic structures in which the evanescent wave interaction is maximized.

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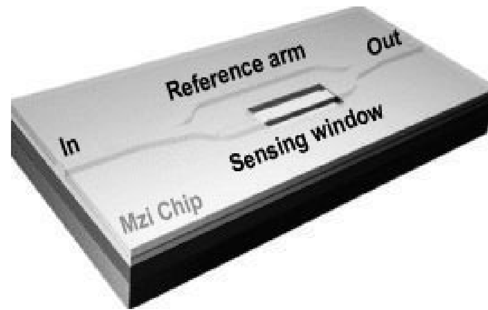

Figure 1. A scheme of the Mach-Zehnder Interferometer device, showing the reference and the sensing windows of the device.

For biosensing applications, the optical waveguides of the MZI must have high surface sensitivity and single mode behaviour. For that, we employed Si_3N_4 core layers (n_c =2.00) with thickness of hundreds of nms (150 to 300 nm) over a SiO_2 cladding (n_s = 1.46 and thickness 2 µm)[4]. In order to provide single mode operation in the lateral direction, rib structures (see Figure 2) of several nanometers high (below 3 nm) and rib widths of 4 µm were fabricated.

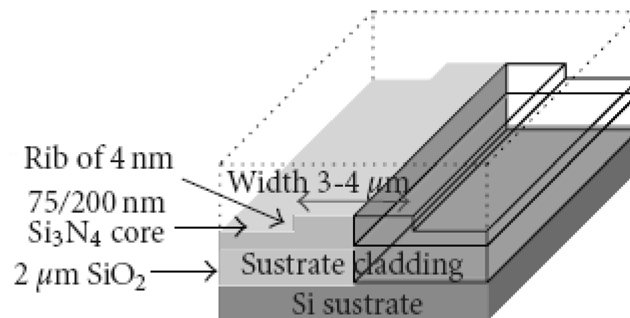

Figure 2. Parameters of the optical waveguides used in the MZI chips

In the MZI, Y-shape divisors with circular arms of R = 80 mm were designed to direct light in the two branches of the MZI with 3 dB split ratio. To protect the device from temperature fluctuations, the waveguide branches were placed very close to each other (100 µm,). The final devices have a length of 3 cm, the sensor area is 1.5 cm long and 50 µm wide. The experimental evaluation of the device was performed using solutions with varying refractive indexes[4]. Taking into account the signal-to-noise ratio of our system, the lowest detection limit[4] in the variation of the refractive index (n_0) for TM polarization was found to be $\Delta n_{0,\,min} = 1 \cdot 10^{-7}$.

In order to prove the high sensitivity of the MZI devices prior any further integration with the mcirofluidics and/or the grating couplers, biosensing experiments were done using a macroflow cell. For biosensing testing-as described before[4]- a oligonucleotide probe of 28 mer was immobilized by covalent attachment to the sensor surface through a silanization procedure. A silane (3-mercaptopropyltrimethoxysilane) with a thiol group at the free end was employed for the chemical modification of the surface. The thiol-derivatized oligonucleotides used as receptors can bind to the silanized Si_3N_4 surface through a disulphide bond. The DNA probe had also a 15-tiamine tail which was employed as a vertical spacer to increase the accessibility to the complementary oligonucleotide to the sensor surface. After DNA immobilization, complementary oligonucleotides (58 mer) were flowed in the sensing area for hybridization experiments. A range of DNA concentrations from 1 pM to 1 µM were analyzed. Regeneration of the receptor probe after hybridization was achieved flowing deionized water and HCl 3.2 mM. The MZI-based sensor demonstrated a limit of detection in the picomolar range for fully hybridized DNA[5]. In addition, the device successfully discriminated between normal and mutant sequences with a limit of detection in the nanomolar range for single point mutations[5].

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3. THE INTEGRATED MICROFLUIDIC NETWORK

One key issue in the development of a LOC with on-chip biosensor detection is the way to bring the sample in contact with the sensing area. The volume of the sample and the flow rate are critical parameters, especially for clinical testing, where it is extremely important to reduce the sample volume as much as possible. The size of the channels in the fluidic network, their design, fabrication, connection and alignment to the sensor area are critical steps.

We have performed the integration of the MZI biosensor with a microfluidic polymeric network at the wafer level[6]. The microfabrication technique developed to generate the array of the microchannel paths onto the MZI devices was based on an adhesive bonding process and releasing steps of two photolithographic patterned SU-8 polymeric resist layers on separate wafers: the bottom substrate (the wafer containing the MZI devices) and a temporary top substrate (Kapton™ on borosilicate glass wafer). Once the two-dimensional SU-8 layers were generated, the patterned wafers were aligned and bonded together to form a three-dimensional (3D) microchannel structure between them. Due to the transparency of the SU-8 film on borosilicate glass, alignment was straightforward regardless of the MZI wafer. The bonding recipe adapted from[6] was carried out. Briefly, both wafers were brought into contact using a substrate bonder at 0.1Pa, pressed together with 300 kPa and heated to 100ºC for 20 min. As a result the two polymer layers were irreversibly bonded together. After the bonding, the top glass wafer and the Kapton™ film were detached from the SU-8 stack and bottom substrate. First, the bonded wafers were rinsed in an IPA ultrasonic bath for 10 min and the glass wafer was separated. Next, the Kapton™ film was easily peeled off from the SU-8 stack. The SU-8 surface remained undamaged after the peeling process as a consequence of the poor adhesion between the two materials[7].

We have observed that a main issue after the SU-8 photoresist development was the remaining SU-8 scum layers on top of the MZI sensing areas. Scum layers are a common problem in SU-8 processing. These SU-8 impurities should be completely removed; otherwise the evanescent field could not interact with the outside medium. Oxygen plasmas were first used to remove this scum layer, but this procedure was not always reproducible. We have developed a second approach: an aluminium layer was sputtered prior to SU-8 processing as a protective sacrificial layer. The first step before starting to build the microfluidic network was to define the protective aluminium layer on top of the biosensors by standard photolithography. After that, the process described in[8] was performed but with an additional step for the etching of the sacrificial aluminium layer. The scum layer was released with the aluminium layer, so the biosensor surface was completely free of any residual polymer.

Finally, the generated microfluidic channels had a height close to 50 µm and 100 µm width. The microchannel is homogeneous and it had the same heiht along the 15 mm length of the MZI sensor area. The successful results are showed in Figure 3 with a top overview of the microfluidic showing one microfluidic channel over a sensor window with a perfectly coupled mode propagating and exposed to the channel environment (in these case air). A cross section view of the microfluidic channels in the middle of the 15 mm length can be seen on the right in Figure 3.

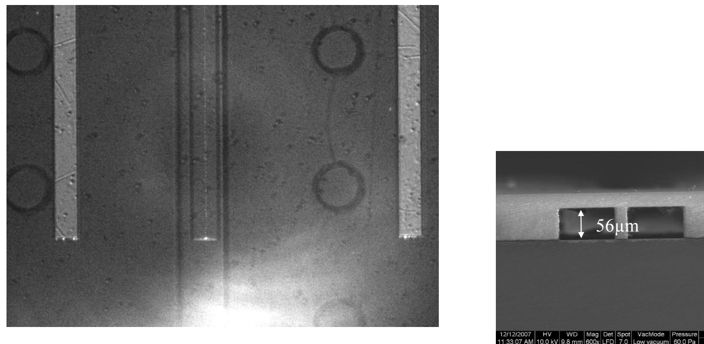

Figure 3. Light propagating in a sensor window integrated with a 56 µm microfluidic channel. In the botton corner a transversal SEM view of two 56 µm high microchannels.

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For connection to the macro world we employed an acrylic housing with eight macrofluidic channels with corresponding ends on the same position than the microfluidic microchannel outputs. Due to different size of the micro and the macro channels the housing has "O" rings to ensure a hermetic channel connection. The complete platform can be seeing in Figure 4. It can operate at pressure drops up to 1000 kPa under steady-state flow rates ranging from 1 to 1000μl/min in the laminar flow regime.

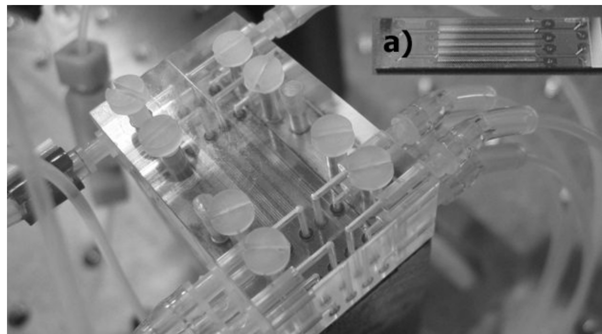

Figure 4. Acrylic housing for connection to the macroworld. (a) top view of the integrated SU-8 photoresist microflow cell with the sensor chip.

4. THE DIFFRACTION GRATING COUPLER (DGC) INTEGRATION

The method of excitation light into a TIR waveguides is one of the aspects which should be properly addressed for lab-on-a-chip biosensing devices. Efficient coupling increases the power of the output signal which defines the maximum sensitivity of the device via the signal-to-noise ratio. Coupling technique defines the complexity of the system and the reproducibility of the experiments.

There are three methods commonly used for light coupling: prism-coupling, end-fired and grating coupling. End-fired is the most common one, partially because of its simplicity. But for end-fired coupling it is needed a very precise alignment and a high mechanical stability of the whole optical system. Prism couplers, despite their reliability and efficiency, are bulky. One prism can be used to work with many chips in sequence, but integration of several prisms on each chip of small size for batch fabrication is impracticable.

Light coupling in/out of the waveguides can be realized by using diffraction gratings (DG) with sub-micron period[8,9]. Clear advantage of grating couplers is their tiny dimensions, which makes them easily to integrate in lightwave structures. Typical length of diffraction grating coupler does not exceed 100 μm, what provides ability to accommodate many of them within a small area of several square millimeters[10]. However, the compatibility and complexity of the technologies for batch fabrication of the DG couplers and the waveguides on same wafer is challenging.

In order to solve the known problems of DGC, we proposed a novel approach where grating couplers were fabricated separately and then were attached to the waveguides. We used hard dielectric thin film couplers supported by polydimethylsiloxane (PDMS) film. We developed a technique to mount thin film dielectric grating couplers on planar waveguides. The couplers were batch fabricated, fixed on the elastomer film, characterized and then installed on the chip containing the waveguides (Figure 5). The property of the elastomer film to adapt or, in the other words, to stick to even nonplanar surfaces is employed to press the couplers against the waveguides. Similar to the prism couplers concept, the gratings can be reliably pressed against the waveguides by the elastomer and can be safely released from it. This robust and reliable method has been demonstrated before with pure elastomer gratings[11]. The disadvantage of using only pure elastomer couplers is that their parameters might change because of inherited tendency to deformation of the elastomer. Our method assured a good and reproducible contact of the waveguides with the thin film grating couplers fabricated separately. We called this approach "mountable gratings".

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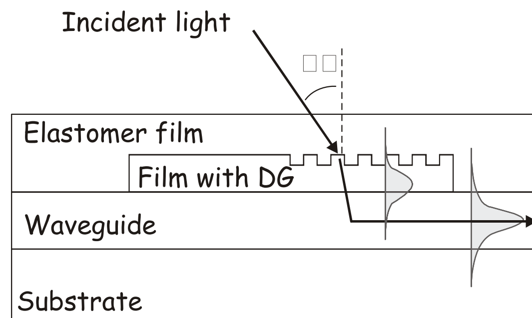

Figure 5. Schematic view of thin film dielectric coupler attached to a sample with waveguides using PDMS.

Thin film dielectric stripes with the embedded gratings were fabricated by combination of holography and silicon technologies. The silicon nitride stripe probes, 180 nm thick, 40 μm wide, and up to 200 μm long, were stuck to a piece of elastomer film. The corrugation period, duty cycle, and depth of the grating were 500 nm, 0.35, and 40 nm, respectively. The characterisation of the probes was done using excitation of waveguide modes and AFM scanning of the grating profile. The experiments confirmed that the technique represented a flexible and robust method for pin-point injection of light into integrated optics circuits. For testing the probes we attached them to a chip with rib loaded silicon nitride waveguides.

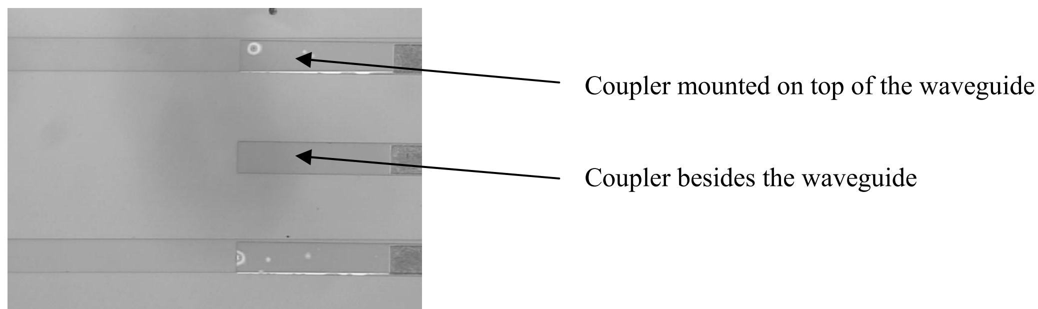

Figure 6. Photograhp of the couplers attached to rib waveguides. It can be observed two couplers which are coincident with two different waveguides and one coupler inbetween the waveguides.

A photograph of the couplers attached to the waveguides can be seen in Figure 6. No air gaps were observed at the interface between the gratings and the waveguides, confirming that the DGC was in contact with the waveguide surface, except on defects (white spots on the photo). The out-of-plane light coupling into the rib waveguide using the proposed hybrid DGC-PDMS system was obtained focusing a He-Ne laser (632.8 nm) on the grating using a plano–convex lens. Light beam from the laser was focused and directed to one of the couplers at a certain angle (see Figure 7).

A maximum coupling efficiency of 5% was obtained when the fundamental mode of the TE polarisation was excited. Although this efficiency could be considered low, these were preliminary results which could be enhanced by an appropriate design of the coupler and by optimisation of the parameters of the focusing optics. According our simulations the excitation length of the structure used in the experiment was 50 μm, that is, the spot size along the waveguide should be adjusted to this value. Then the coupling efficiency is expected to increase by a factor of three. The experiments for the enhancement of the coupling efficiency are in progress but the showed results have confirmed the validity of the proposed configuration of the mountable grating coupler.

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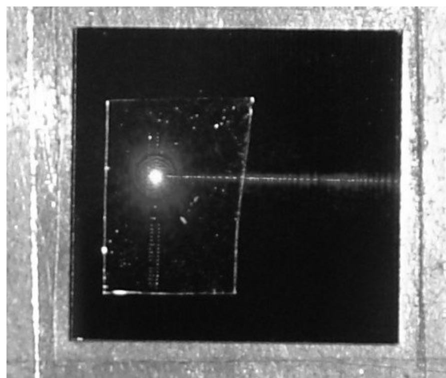

Figure 7. Out of plain coupling of light into a waveguide. A piece of PDMS with the DG couplers was attached to a chip with silicon nitride waveguides.

4. CONCLUSION

We are working in the implementation of a lab-on-a-chip device with on-chip detection for the fast, simple, direct and cost-effective testing of clinical samples. The LOC is pursued by the integration of: (i) ultrasensitive and miniaturised photonic Mach-Zehnder interferometer sensor based on integrated optical waveguides ($Si/SiO_2/Si_3N_4$) of micro/nanodimensions. These sensors have already shown that they are able to detect, in a real-time and a label-free way, DNA single point mutations at nanomolar level. (ii) 3D-polymeric microfluidic network which has been successfully integrated with the biosensors by using a fabrication method of embedded microchannels. 50 µm height, 100 µm wide and 2 cm length SU-8 microchannels have been successfully integrated on top the MZI nanophotonic devices. The device is able to support steady-state flow rates from 1-100 µL/min and up to 10 bar without liquid leakage. (iii) A novel mountable diffraction grating coupler device which is a combination of dielectric coupling diffraction element on PDMS film. The elastomer allowed for a precise attachment of the coupler elements to the photonic circuitry. Excitation of the waveguides modes using this novel grating coupler was demonstrated.

The combination of mountable diffraction grating couplers and microfluidic systems made on polymer with integrated optical circuits is promising for LOC development with on-chip detection. Further development including the integration of optical light sources, photodetectors and CMOS electronics at the wafer level will offer the possibility to achieve a truly portable clinical diagnostic tool with increased sensitivity, specificity, and reliability for early disease detection.

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