Integrated Bimodal Waveguide Interferometric Biosensor for Label-free Analysis

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Abstract—The performance of an interferometric sensing device based on integrated Bimodal Waveguides (BiMW) is demonstrated. Fabricated using standard silicon technology, the sensors can achieve a detection limit of $2.5 \cdot 10^{-7}$ RIU for homogeneous sensing, rendering in a high sensitive device. The applicability of the bimodal waveguide interferometers as label-free biosensors has been demonstrated by the real-time detection of the biomolecular interaction of BSA and anti-BSA. Due to their simplicity, the devices could be further integrated in complete lab-on-a-chip platforms for point-of-care diagnostics showing as a powerful instrument for biochemical analysis.

Index Terms—Bimodal waveguide, optical biosensors, integrated optics, evanescent wave detection, waveguides

I. INTRODUCTION

Most diagnostics 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 tags. There is an unmet need of having reliable diagnostic tools that ensure a sensitive, rapid, affordable and simple analysis, particularly in the clinical practice. Such reliable diagnostic tools could afford the decentralization of diagnostics (clinical or environmental) to point-of-care (POC) settings, allowing tests in workplaces, homes or primary care facilities, among others.

Last advances in micro- and nanosensing technology are offering the implementation of diagnostic tools with increased sensitivity, specificity, and reliability for in vivo and in vitro applications. The application of a portable, easy-to-use and highly sensitive biosensor lab-on-a-chip platform for real-time diagnosis could offer significant advantages over current methods [1]. Photonic biosensors based on evanescent wave detection are one of the best candidates for such platform due to their high sensitivity.

Among evanescent wave optical biosensors, interferometric sensors are recognized to be one of the most sensitive devices which can be used for label-free analysis. The sensitivity to homogeneous changes in the refractive index can reach $10^{-8}$ of refractive index unit (RIU) which is at least two orders of magnitude better than, for example, the sensitivity of Surface Plasmon Resonance (SPR) sensors [see for example Ref.2, pp. 466-470]. But problems of stability, complex manipulation and read-out have prevented the general use of integrated interferometers biosensors for real field applications.

Integrated interferometric sensor devices have been extensively studied over the last two decades. The configurations based on Mach Zehnder (MZI) [1] and Young (YI) [3] interferometers are the most known. The principal difference between them is that the interference pattern readout is on chip in the case of the MZI and off chip in the case of the YI. Interferometers with MZI configuration have the advantage of a signal readout using a single photodetector, but they require high stability in the light coupling, which is the main bottleneck for their practical implementation. In the Young configuration the relative changes in the interference pattern are measured in far field. They are almost independent on the light intensity, making them more reliable and easier to use. Both MZI and YI use two waveguide channels, one for sensing and one for reference. In differential interferometers (DI) [4] the propagation of light of two orthogonal polarizations in a unique single-mode waveguide channel is analyzed. Two orthogonal polarizations are also used in a technique based on mixture of YI and DI methods, called Dual Polarization Interferometry (DPI) [5]. The DPI sensor allows simultaneous measuring of thickness and refractive index of a film adsorbed on the sensor surface.

We present the results of a single channel waveguide interferometer operated on interference of two waveguide modes of the same polarization. The characterization of sensitivity was done for both homogeneous and surface sensing types. The simplicity of the design of the bimodal waveguide (BiMW) interferometers is attractive for mass-fabrication of integrated sensors as there is no need for the design and fabrication of Y-shape splitters, which are the most complex component of MZI and YI devices, responsible for
the modulation depth of the output signal and, subsequently, for the sensitivity.

II. PRINCIPLES OF OPERATION OF BIMODAL WAVEGUIDE INTERFEROMETER

The devices dealing with modal interference employ a single waveguide supporting the interfering modes [6-10]. The sketch of the device is presented in Fig. 1. Light from a coherent source is coupled into a ridge waveguide supporting a single transversal mode. After some distance this mode is coupled into another waveguide which supports two transversal modes. Due to the vertical asymmetry of the junction, the fundamental mode of the first waveguide splits in two, the fundamental and the first order modes, which are propagating till the output of the chip. The modes propagating with different velocities create at the exit an interference pattern, where the intensity distribution depends on the physical parameters of the structure, and, in particular, on the refractive index of the cladding layer through the interaction with the evanescent field. This intensity distribution is monitored off chip using a photodetector array [9].

For implementation of the BiMW device, a light source with a narrow spectral line, stable in time, and with a coherence length of several millimeters is required. This helps to create high contrast interference at the output of the BiMW. Temperature sensitivity of BiMW device is an intrinsic property, which is due to a fact that propagation constants of the modes are differently affected by temperature changes. To take advantage of this, the temperature is used for initial adjustment of the interference pattern and for preliminary characterization of the chip (see Fig. 2). By changing the temperature, the modulation depth of the output signal and the speed of modulation in temperature domain can be observed, and can help in the estimation of the quality of the modal splitter and the bimodal part of the waveguide. The interference pattern is independent on the light intensity and the output signal is not sensitive to fluctuations in the coupling efficiency, which is an advantage as compared to classical integrated MZI devices containing a single 3 dB splitter and a combiner as a one described in [1]. Fluctuations of intensity of light source or coupling efficiency are transferred in the output signal and can be interpreted as a result of a monitored reaction. This problem can be easily solved by monitoring of light coupled into the interferometer, but an additional measuring channel and an on-chip splitter would be required to realize that. The optical quality of the end facet, either polished or cleaved, must be good enough to allow the read-out of the interference pattern of the modes with low distortions.

The waveguide should be maintained defects-free as much as possible in order to reduce scattering. The defects result in reduction of the modulation depth of the output signal.

III. EXPERIMENTAL

A. The chip

The sensing chip was fabricated onto a silicon substrate using standard microelectronics technologies. A silicon nitride layer of 2.00 refractive index and thickness of 350 nm was deposited using LPCVD technique on a thermally grown silicon dioxide buffer layer (1.46 refractive index, 2 µm thickness). A ridge type channel waveguides with width of 3 µm and ridge height of 2 nm was formed by BHF etching through a photoresist mask patterned by conventional photolithography.

The single mode part of the waveguide was fabricated using conventional photolithography and dry etching. The single mode part in our device was 150 nm thick. According to our calculations in order to obtain more than 70% modulation, single mode part must have a thickness below 160 nm. For modeling we used transfer matrix approach in order to construct profiles of electric fields for each mode in both single and bimodal parts. Then we used overlap integrals to estimate coupling efficiency of light from the zero mode of the single mode part to the zero and first order modes of the bimodal part. Finally, integrating the intensities of the fields at the exit over the upper and the lower half-planes we obtained the modulation of the output signal as a function of phase shift between the modes. Using the information on distribution of
electro-magnetic fields we calculated the confinement factors for both the fundamental and first order modes which correspond to 0.94 and 0.67. These numbers help to understand that the fundamental mode is less sensitive to the cladding layer parameters, because the major part of energy is located inside the waveguide.

The structure was covered by a silicon dioxide (refractive index 1.48 at 633 nm) cladding layer deposited by PECVD technique. A sensing window was etched in the cladding on an area of 150x0.5 mm² by wet etching.

The total length of the waveguide (chip) including both single and bimodal parts was 30 mm. The total length of the bimodal part was 25 mm which included the sensing window with a length of 15 mm, placed 5 mm off the chip exit. The chip was 30x10 mm² in size and contained 16 independent interferometers.

Polishing of the chip edges was done by the consecutively use of sandpapers with different grain size (9, 3 and 0.3µm grain size) with a Polishing Machine (Logitech CL50) until an optical quality for end grain size) with a Polishing Machine (Logitech CL50) until an optical quality for end grain size) with a Polishing Machine (Logitech CL50) until an optical quality for end grain size) with a Polishing Machine (Logitech CL50) until an optical quality for end grain size) with a Polishing Machine (Logitech CL50) until an optical quality for end grain size) with a Polishing Machine (Logitech CL50) until an optical quality for end grain size) with a Polishing Machine (Logitech CL50) until an optical quality for end grain size) with a Polishing Machine (Logitech CL50) until an optical quality for end grain size) with a Polishing Machine (Logitech CL50) until an optical quality for end grain size) with a Polishing Machine (Logitech CL50) until an optical quality for end grain size) with a Polishing Machine (Logitech CL50) until an optical quality for end grain size.

Cleaning of the chip was performed by flushing acetone, ethanol and water and drying with N2.

B. Measuring setup

Linearly polarized light with electric vector oriented in chip plane was coupled from a He-Ne laser (λ=632.8 nm) into the waveguide by means of a microscope objective (40x). The interference pattern created at the output of the bimodal waveguide part was monitored using a two sectional photodetector (S5870, Hamamatsu) with each section connected to a current amplifier (PDA 200C, Thorlabs).

Light exiting the waveguide generates the currents $I_{up}$ and $I_{down}$ in the upper and the lower sections of the photodetector, respectively. The currents were used for calculation of a parameter $S$, according with the expression:

$$S = \frac{I_{up} - I_{down}}{I_{up} + I_{down}}$$  \hspace{1cm} (1)

A photo of the chip mounted in the setup is shown in Fig. 3. The chip is mounted on a copper base and covered by a PMMA microfluidic header.

The chip was mounted on a copper base mounted over a thermoelectric element (TEC1.4-6, Thorlabs, located under the copper base in Fig. 3) connected to a Temperature Controller (TED 200C, Thorlabs). The system provided temperature stabilization of the chip with 0.01 degrees accuracy. Temperature control allows controlling intermodal phase difference. In Fig. 4 the change in intermodal phase difference induced by temperature variation is plotted. In this experiment, the sensing area was sealed by a polymer with refractive index of 1.42. Temperature variation induced chip displacement due to the expansion of the chip holder. Linear reduction of the total output signal by 45% was obtained (dash line on the graph). This affected the coupling efficiency, but did not affect the modulation depth of the output signal: the phase difference linearly increases with the temperature (solid line on the graph). The output signal is the sum of the signals obtained after the current-to-voltage conversion of the currents generated by both photodiode sections. The temperature $T$ and parameter $S$ have been registered simultaneously in this experiment. The function $S(T)$ was approximated by harmonic function and then it was recalculated into phase shift using the expression

$$\varphi(T) = \alpha \sin \left[ S(T) \right] - \alpha \sin \left[ \frac{S(T + \Delta T)}{S_m} \right]$$  \hspace{1cm} (2)

where $\Delta T$ corresponds to the temperature interval used for sampling of $S(T)$. $T_0$ is the lower limit of the temperature range used in this experiment.

The same chip with the polymer sealing the sensing area was used for testing the drift in the setup. The temperature was maintained with stability of 0.01 degree while the fluctuation of the $S$ parameter did not exceed 2% during 15 minutes.
IV. HOMOGENEOUS SENSING

In order to check the sensitivity of our device, a calibration curve has been carried out by injections of different concentrations of HCl (0.2M, 0.1M, 0.05M and 0.025M) while keeping a continuous water flow. Refractive indexes of the HCl solutions employed are summarized in Table 1. Refractive index measurements of the solutions were carried out with an ABBE Refractometer (Optic Ivymen System, Spain). Changes of the refractive index over the sensor area between water and HCl solutions induce a phase change through the evanescent field interaction. Figure 5 show the monitoring of the phase change in real time when a concentration of 0.05 M of HCl (n=1.3327) is introduced.

TABLE I
VALUES OF THE ABSOLUTE REFRACTIVE INDEX (N) OF HCl CONCENTRATIONS AND CHANGE IN REFRACTIVE INDEX (Δn)

<table>
<thead>
<tr>
<th>HCl concentration</th>
<th>n</th>
<th>Δn</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.2</td>
<td>1.3342</td>
<td>1.9E-3</td>
</tr>
<tr>
<td>0.1</td>
<td>1.3333</td>
<td>1E-3</td>
</tr>
<tr>
<td>0.05</td>
<td>1.3331</td>
<td>6E-4</td>
</tr>
<tr>
<td>0.025</td>
<td>1.3329</td>
<td>4E-4</td>
</tr>
</tbody>
</table>

Figure 5: Real time monitoring of the ∆ϕ caused by the injection of 250 µL of HCl 0.05M in a continuous water flow over the sensor surface.

The change in the bulk refractive index (Δn=5.9×10⁻⁴ RIU) produces a change in the phase until the injected 0.05 M HCl is totally out of the sensor area and then the signal recovers its initial value (marked with dashed line in the figure).

Phase variation is plotted versus index variation (Δn) and the experimental sensitivity is d(ϕ·2π)/dn=2026 which means a detection limit of Δn_min=2.5×10⁻⁷ RIU. The lowest phase shift measurable is considered to be three times the S/N ratio which is 5×10⁻⁴ 2π rad, corresponding to a surface sensitivity of 3×10⁻⁵ nm⁻¹.

V. SURFACE SENSING

Bovine Serum Albumin (BSA) protein and its specific antibody anti-BSA have been commonly used as a test biomolecular system to demonstrate the applicability of novel waveguide devices as label-free biosensors [11-13].

Figure 6: Calibration curve of the BiMW sensor plotted by measuring the phase change as a function of variation in the refractive index due to the injection of HCl concentrations. Inset: Figure A) Output signal for the detection of HCl 0.025 M corresponding to a phase change of 0.45×2π rad, Figure B) Output signal for the detection of HCl 0.05 M corresponding to a phase change of 0.83×2π rad, Figure C) Output signal for the detection of HCl 0.1 M corresponding to a phase change of 1.65×2π rad, Figure D) Output signal for the detection of HCl 0.2M corresponding to a phase change of 3.47×2π rad (In all the Figures only HCl entrance is showed).

Acetone, ethanol, Phosphate Buffer Saline (PBS; 10 mM phosphate, 2.9 mM KCl, 137 mM NaCl, pH 7.4), albumin bovine 95-99% (BSA) and monoclonal anti-bovine serum albumin antibody (mAb-BSA) produced in mouse were purchased from Sigma. The employed water was Milli-Q grade (Millipore).

Physical adsorption of BSA protein onto the sensor area of the BiMW was done by flowing 250 µL of a PBS solution with 50 µg/mL BSA, at a flow rate of 10µL/min in water flow. Phase variation (7.86×2π rad) due to the refractive index bulk change by physical adsorption of the protein is observed in Fig. 7. The phase change becomes slower until reaches a stable signal, which means that the surface is totally saturated with proteins. Next, the water flow induces the detachment of non-adsorbed protein (0,15×2π rad).

Total phase change due to the receptor attachment was 4.41×2π rad corresponding to a final covering of 0.42 ng/mm² [14] of BSA receptors.
biosensor; the sensitivity is independent of the light coupling efficiency and moreover the device is easy to be operated by non-experienced users. The problem of the strong temperature dependence is solved by thermal stabilization of the chip. At the same time the temperature control can be used as a test technique for the interferometer real time characterization. We have demonstrated that the device has a high sensitivity (2.5×10⁷ RIU) for label-free sensing, which makes it a suitable candidate for further integration in point-of-care instruments.

Further work is being carried out for developing a complete lab-on-a-chip microsystem by integrating the bimodal sensors, a microfluidics network, a phase modulation system to convert the periodic interferometric signals into direct phase measurements, and grating couplers for the in and out coupling of the light.

REFERENCES


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