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Monolithically Integrated Biosensors based on Frequency-Resolved Mach-Zehnder Interferometers for Multi-analyte determinations

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Abstract—**The application of fully monolithically-integrated Mach-Zehnder interferometer arrays fabricated by standard silicon technology to the label-free detection of analytes is introduced. Detection with the presented biosensor is based on a novel concept, the Frequency-Resolved Mach-Zehnder Interferometry (FR-MZI). In addition, a smart encapsulation based on an appropriately designed microfluidic system and performed at the wafer scale scheme for the easy delivery of the samples to be analyzed is demonstrated. Testing of the FR-MZI biosensors with model binding assays demonstrated the detection of streptavidin binding to immobilized biotin at concentrations in the sub nM range. This is the first experimental demonstration of the FR-MZI concept as well as the first demonstration of a monolithically fully-integrated MZI biosensor.**

I. Introduction

THE demand for portable diagnostic tools of increased performance and rapid detection has led to considerable efforts towards the fabrication of photonic biosensors following standard silicon technologies to reach the ultimate goal of integrating all components on the same chip. Despite the advances in the performance of state-of-the-art photonic biosensors and their proven ability for ultra-sensitive detection [1], the inclusion of the excitation light-source to the sensing system has been achieved either through hybrid integration or through the use of bulky external optical components which add to the size, complexity and cost of the system.

Towards this goal, in this work, a novel biosensor based on fully integrated on silicon wafer, Mach Zehnder interferometers (MZIs) and its application for label-free multi-analyte determinations is presented. The Mach-Zehnder concept relies on the phase change occurring due to the binding of biomolecules on the surface of the sensing arm (Fig. 1) [2]. The MZI principle of operation has been explored by several research groups (e.g. [3], [4]) and was proved to be a methodology with very high resolution and sensitivity. The MZI devices are fabricated with standard silicon technologies but the monochromatic light source and the photodetector are not fabricated on the same Si die; on the contrary the light from the external light source should be coupled to the planar waveguide as well as the light coming out should be coupled to the external photodetector. In certain cases e.g. [5], the photodetector has been successfully integrated on the same Si chip, but the monolithic integration of a monochromatic light source on a Si die still remains the Holy Grail for the Photonics community.

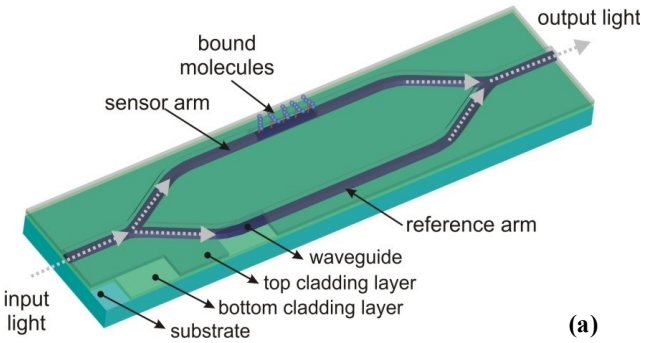

Fig. 1. Schematic representation of a standard planar waveguide MZI configuration.

The MZIs suggested in the present work, are fabricated with standard silicon microfabrication techniques, so as all active (light emitting diodes and photodetectors) and passive (planar waveguides) optical components to be monolithically integrated onto the same silicon chip [6, 7]. The light emitting diode (LED) is a silicon avalanche diode that emits light over a wide spectrum in the VIS-NIR range when biased beyond its breakdown point and the photodector is a common pn-junction photodiode. These two components are optically coupled by a planar waveguide patterned in two arms to create a Mach-Zehnder Interferomer. All MZIs are self-aligned to both the emitter and the detector and present very small losses in the VIS-NIR spectrum range. The basic biosensor scheme allows for the development of arrays that consist of individually operated LED and MZIs converging to a single detector for multiplexing operation. A schematic of the integrated MZI array is presented in Fig. 2.

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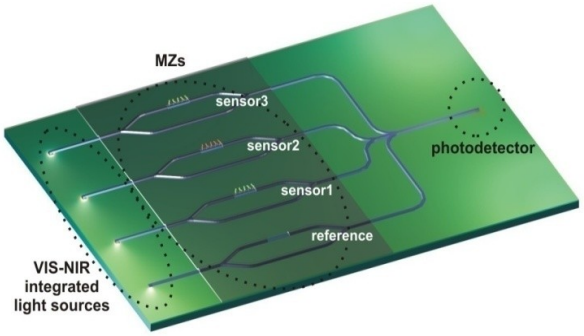

Fig. 2. Schematic of an array of monolithically integrated on silicon MZIs.

Through functionalization of each MZI within an array with a different binding molecule simultaneous determination of multiple analytes into a single sample is possible. As with the standard MZIs, detection is based on the optical path difference between the sensing and reference arms which occur during the binding of specific biomolecules onto the surface of the sensing arm (Fig. 2). However, the proposed sensor takes advantage of a novel concept for label-free detection, the Frequency-Resolved Mach-Zehnder Interferometry (FR-MZI) [8, 9] that has been only very recently introduced. FR-MZI exploits the broad-band input spectrum and through the changes for every input frequency can offer high sensitivity and at the same time overcome the issues of phase ambiguity, signal fading and the need for mm-long sensing arms that plague the conventional single-wavelength MZI.

The developed FR-MZI array is also combined with an appropriately designed microfluidic system which allows for the easy delivery of the samples to be analyzed. The microfluidic system has been designed so as the encapsulation of the biochips to be performed at wafer scale. This approach is expected to greatly facilitate the packaging procedure that is one of the bottlenecks in the development of portable bioanalytical devices based on biosensors. The analytical capabilities of the developed biosensor are exploited through a model binding assay based on biotin/streptavidin interaction.

This is, to our knowledge, the first experimental demonstration of the FR-MZI concept as well as the first demonstration of a monolithically fully-integrated MZI biosensor.

II. MZI ARRAY LAYOUT AND PERFORMANCE

Arrays of FR-MZIs consisting of several monolithically integrated silicon nitride based waveguide MZI biosensors, each one excited by its own source and a common photodector where all MZIs converge are fabricated at the clean room facility of the Institute of Microelectronics of NCSR "Demokritos". The waveguides are covered by a 2-micron thick silicon oxide film which has been removed over one of the MZI arms to create the sensing arm. The sensing arm is only 300 μm-long. The entire array chip is very compact with a size of ~ 50 mm^2.

Each LED on the chip can be addressed independently and in a multiplexing manner allowing continuous collection of data from all sensors onto a single chip through signal multiplexing. As it is shown in Fig. 3, the LED emits a wide spectrum over the VIS-NIR range when biased beyond its breakdown point with an emission maximum at 650 nm.

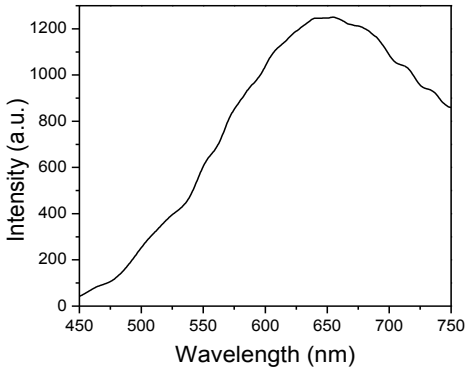

Fig. 3. Emission spectrum of the integrated LED.

For the real-time and label-free monitoring of the bioanalytical reactions taking place on top of the FR-MZI sensing arm, the changes for every input frequency along the broad-band LED spectrum are taken into account. The spectral region of the emission maximum is also the region for which the maximum spectral shifts are expected as it is anticipated by the simulation experiments results presented in Fig. 4. For these calculations, the immobilization of a 0.1 nm-thick adlayer with refractive indices ranging from 1.38 to 1.56 have been used. The used refractive indices values are in the area of expected refractive indices when biomolecules (proteins or single-stranded DNA) will be immobilized onto the sensing arm of the FR-MZI.

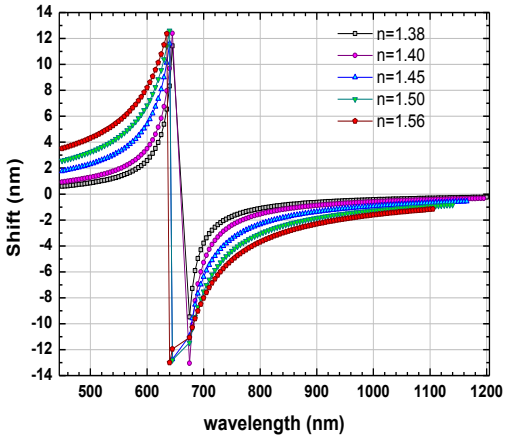

Fig. 4. Anticipated spectral shifts per input wavelength (frequency) of a FR-MZI with 300 μm-long sensing arms induced by the adhesion of 0.1 nm of hypothetical adlayers with refractive indices between 1.38 and 1.56.

The fact that for the signal calculation the changes over the broad-band LED spectrum are used is expected to offer significant advantages over the standard single-wavelength Mach-Zehnder interferometers. In particular, the drawbacks of single-wavelength Mach-Zehnder interferometers that are expected to be solved by FR-MZIs are the following:

a) Optical coupling of active and passive optical components. This major issue is radically dealt with through the monolithic integration of the Si avalanche diode LED which is optically coupled to the self-aligned Si_3N_4 waveguide;
b) Suppression of signal ambiguity since the phase change for every wavelength is different and therefore it is easier to deduce from the full spectrum the exact changes;
(c) For a monochromatic source very careful design of the MZI has to be performed prior to any application in order to avoid operation near the quadrature points that can lead to signal fading. This renders each MZI applicable only to a limited number of sensing schemes. On the contrary, when a broad-band source is employed, there are wavelengths in the spectrum that will be far off from the extreme values of their corresponding transmission curves, enhancing the output signal and allowing thus the BB-MZI to be applied to a large number of detection schemes.

Moreover, the use of a broad-band spectrum is expected to provide higher detection sensitivity since every wavelength is "affected" by the binding effect in the sensing arm to a larger or lesser degree producing a phase change of its own. This in turn is translated into the output spectrum both as changes of the intensity and as peak shifts.

III. MZI CHIP PACKAGING

Encapsulation of the FR-MZI array with an appropriately designed microfluidic system that will allow for the easy delivery of the samples to be analyzed and ensure the facile contact with the external low-noise electronic components is also under way. The encapsulated array is envisioned to be fixed on a cartridge ready to be manually inserted to a small size compact instrument that will comprise the read-out electronics so signal collection and processing through an appropriate software. The first attempt to encapsulate the FR-MZI chips is presented in Fig. 5. The flow cell is made from Polycarbonate with stainless steel tubes glued on it to provide the interface for fluid connection. The flow cell is fixed to the chip through UV cured adhesive that is dispensed around the flow cell. Though the mechanical machining of the flow cells is less cost effective comparing high volume numbers achieved by injection molding, it provides higher flexibility during device development since it allows for rapid adaptations of new designs, materials etc.

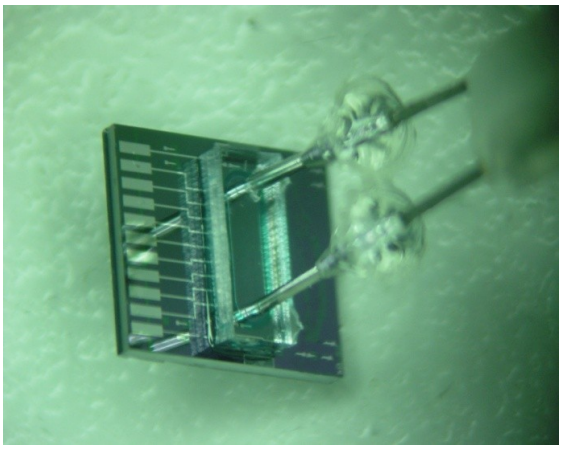

Fig. 5. Optical microscope image of an encapsulated biosensor array of multi monolithically integrated FR-MZIs.

IV. LABEL-FREE DETECTION WITH MONOLITHICALLY INTEGRATED MZI

A biotin-streptavidin model assay (Fig 6), was used to evaluate the analytical capabilities of the developed MZI sensor arrays. For this purpose, packaged chips were coated by flowing a biotinylated bovine serum albumin solution (0.4 μM in 50 mM phosphate buffer, pH 7.4) over the chip surface at a constant rate of approximately 30 μl/min. Then, the surface free protein-binding sites were blocked by running a 10 mg/ml bovine serum albumin solution. After that, the chips were probed by running streptavidin solutions at concentrations down to sub nM concentration range. The binding of streptavidin molecules to immobilized biotin was monitored in real-time and in label-free format. Depending on the streptavidin concentration, maximum plateau values were achieved on different time intervals, however, it is not necessary to extent the reaction time in order to reach plateau values since there is a linear relationship of the signal obtained in the first minute of reaction towards the streptavidin concentration. Thus, the proposed sensor is suitable for extremely rapid detection of biomolecular reactions taking place on the integrated MZI sensing arm.

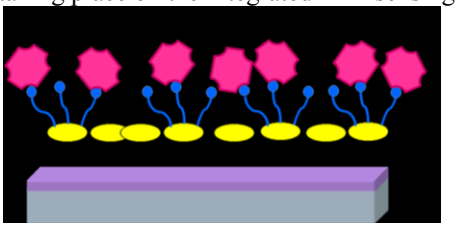

Fig. 6. Schematic representation of the biotin-streptavidin biomolecular interaction.

Further optimization of MZI sensor performance will be pursuit through redesign in order to define the geometrical characteristics that will provide the optimum performance and especially in terms of detection sensitivity. The optimized devices will be then tested both for protein and

DNA detection to determine the application fields where such a device could have the maximum impact.

V. Conclusion

The Frequency-Resolved Mach-Zehnder Interferometric sensor presented here is the only one fully integrated on silicon. These miniaturized devices exhibit a great potential for mass production in order to fabricate the next generation of compact, portable, cost-efficient biosensors which will allow for label-free, real-time detection of biomolecules (like proteins and DNA sequences) at very low concentrations.

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