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Fast label-free detection of C-reactive protein using broad-band Mach-Zehnder interferometers integrated on silicon chips

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ABSTRACT

An immunosensor for fast and accurate determination of C-reactive protein (CRP) in human serum samples based on an array of all-silicon broad-band Mach-Zehnder interferometers (BB-MZIs) is demonstrated. The detection was based on monitoring the spectral shifts during the binding of CRP on the antibody molecules that have been immobilized on the sensing arms of the BB-MZIs. By employing the reaction rate as the analytical signal the assay time was compressed to few minutes. The detection limit was 2.1 ng/mL, the quantification limit was 4.2 ng/mL and the linear dynamic range extended up to 100 ng/mL. The measurements performed in human serum samples with the developed immunosensor were characterized by high repeatability and accuracy as it was demonstrated by dilution linearity and recovery experiments. In addition, the concentration values determined were in excellent agreement with those determined for the same samples by a standard clinical laboratory method. The compact size of the chip makes the proposed immunosensor attractive for incorporation into miniaturized devices for the determination of clinical analytes at the point-of-need.

1. Introduction

The development of miniaturized sensing devices aiming at the point-of-need (PoN) detection of analytes is a long standing objective and its fulfilment is expected to skyrocket the application of biosensors in medical diagnostics [1], environmental monitoring [2], and food analysis [3]. The majority of the sensors that have been developed so far rely on the use of labels (fluorophores, magnetic or metal nanoparticles, etc.). In parallel, several label-free transduction principles have been developed presenting in many cases detection of analytes in complex matrices with sensitivity comparable to that obtained using labels [4]. Most of the currently available transducers for label-free detection are optical, such as surface plasmon resonance (SPR) sensors [5], planar optical waveguides [6,7], optical fibers [8], Mach-Zehnder [9–11], Young [12], and bimodal-waveguide interferometers [13], microring resonators [14,15], and reflectometric interference spectroscopy sensors [16,17]. Amongst them, integrated waveguide interferometers are highly attractive since they present increased potential for miniaturization and are therefore, more suitable for PoN applications [18,19].

To this direction, we have introduced the broad-band Mach-Zehnder interferometry (BB-MZI) principle of operation and we have implemented an array of such sensors on a silicon chip fabricated by mainstream silicon processing technology [20–22]. The array consists of ten planar silicon nitride waveguide Mach-Zehnder interferometers (MZIs), accommodated on an 8.8×4.0 mm^2 silicon chip, each one with its own integrated on-chip broad-band silicon avalanche light emitting diode (LED). This monolithic integration alleviates the need for

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alignment of the light source with the waveguide, as in the standard MZIs, while the use of a broad-band light circumvents two other limitations of standard MZIs; the signal and phase ambiguity. The latter results from the fact that the phase change is different for each wavelength, and thus, it is easier to deduce from the full transmission spectrum, the change in the effective refractive index due to the biomolecular reaction taking place on the sensing arm of the MZI. These innovations were complimented by the transmission spectrum processing method, according to which the spectral shifts recorded during the biomolecular interactions, are tracked as phase changes in the Fourier Domain for both TE and TM polarizations [22]. The whole concept represents a new detection principle, namely the broad-band interferometry, which is ideal for label-free detection of biomolecular interactions [22] offering very high analytical performance in terms of sensitivity and limit of detection.

In the present work, the BB-MZI chip is exploited as a miniaturized immunosensor for the fast label-free quantification of C-reactive protein (CRP) in human serum samples aiming to its potential application at the point-of-need. CRP is the most widely used marker in clinical practice for the diagnosis of inflammation since its levels in blood rise rapidly in numerous pathological situations including infections, tissue damage, renal and cardiovascular diseases [23]. Generally, plasma concentrations lower than 2.0 mg/L are determined in healthy individuals, which can increase up to 1000-fold during the acute phase of inflammation [24]. Apart from been an acute inflammation indicator, CRP is also used as a prognostic marker for cardiovascular events. In particular, the American Heart Association and the United States Centre for Disease Control have suggested three levels of CRP for the evaluation of cardiovascular disease risk: concentrations below 1.0 mg/L represent low risk; concentrations in the range 1.0–3.0 mg/L average risk; and levels above 3.0 mg/L high risk [25]. Therefore, there is a need to develop methods for fast and accurate determination of CRP over a wide range of concentrations. This need has fuelled the development of a great variety of sensors for the quantitative determination of CRP in human serum samples based on immunochemical approaches as well as of point-of-care systems [26–31]. To this end, we present the sensitive quantitative detection of CRP in human serum samples performed by monitoring the direct binding of CRP to the immobilized onto the BB-MZIs antibody. The implementation of kinetic measurements allows suppression of the assay duration to few minutes. The results obtained by the immunosensor developed with respect to CRP determination in human serum samples were compared to that of a commercially available kit run in a standard clinical laboratory analyser (Siemens Dimension® Clinical Chemistry System). Moreover, the analytical characteristics of the BB-MZI based immunosensor are discussed and compared to those of other label-free sensors for the determination of CRP reported in the literature. This comparison revealed that the BB-MZI sensor is ranked amongst the most sensitive label-free sensors and with a clear potential for application at the point-of-need.

2. Materials and methods

2.1. Materials

Bovine serum albumin (BSA), (3-aminopropyl)triethoxysilane (APTES), (3-glycidyloxypropyl)trimethoxysilane (GOPTS) and glutaraldehyde solution (25% w/w in H_2O) were purchased from Sigma Chemical Co. (St. Louis, MO). C-reactive protein (CRP), CRP free-serum, and a goat polyclonal anti-CRP antibody (code GC019) were purchased from Scripps Laboratories (San Diego, CA). All other materials were purchased from Merck (Darmstadt, Germany). Human serum samples from anonymous donors were provided by the Diagnostic Laboratories of “Henry Dunant” Hospital in Athens, Greece (after approval from the hospital's Ethics Committee and informed consent of the patients). All samples have been previously analyzed using the Siemens Dimension® Clinical Chemistry System.

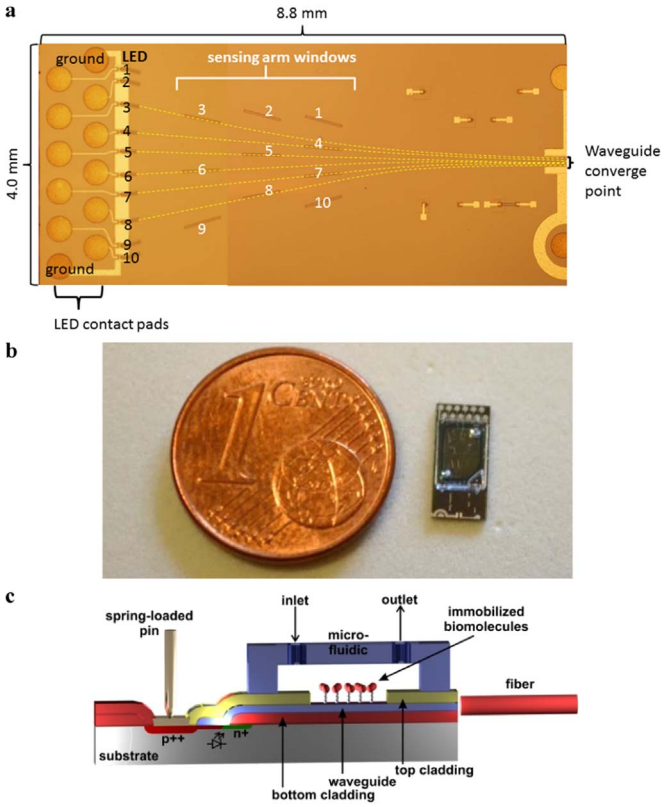

Fig. 1. (a) Optical microscope image of a chip, where the sensing arm windows, the LEDs and their respective contact pads, and the waveguide convergence point are indicated. The waveguides are not visible under the microscope; the yellow dashed lines have been drawn to indicate approximately the position of 6 out of 10 waveguides. The 10 sensing arm windows are numbered 1–10. (b) Photograph of the chip with the fluidic on top compared to 1 cent coin. (c) Cross-section of the BB-MZI sensing window with the fluidic on top showing the light source, the waveguide and the aligned external optical fiber for the coupling to the external spectrometer.

2.2. Sensor chip and fluidic layout

The fabrication of the biosensor chips was realized in the Nanotechnology & MEMS Laboratory (N-MEMS) of the Institute of Nanoscience and Nanotechnology of NCSR “Demokritos” (Athens, Greece), as it has been described in previous publications [21,22]. An optical microscope image of the finalized chip is depicted in Fig. 1a, showing the topological arrangement of the 10 BB-MZIs on the chip, the LED pads and the convergence of the 10 waveguides at the output signal collection area. In this image the 10 (600-micron long, 25-micron wide) sensing arm windows can be observed; the silicon nitride rib waveguides are not visible under a standard optical microscope, therefore yellow dashed lines have been drawn over 6 out of 10 waveguides as a guide to the eye. The chip dimensions, 8.8 mm in length and 4.0 mm in width, are also provided in Fig. 1a. The fluidic module consisted of a poly(methyl methacrylate) foil with drilled inlet and outlet holes onto which a thick film adhesive photoresist was laminated and photolithographically patterned to create the flow channel. The fluidic covers are assembled either on single chips or on wafer scale through the adhesive photoresist defining the fluidic cell. An image of the encapsulated chip with respect to 1-cent coin is provided in Fig. 1b.

2.3. Instrumentation, signal collection and processing

The measuring apparatus consisted of the docking station that