Ultrasensitive interferometric detection of beta-lactam specific IgE antibodies

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Driven by the technological advances in lithography and material science, integrated photonic biosensors have been presented in the most recent decades as small, label-free, high-sensitivity alternatives to more traditional label-based biodetection methods such as the different immunoassay techniques. Moreover, photonic solutions offer unmet detection limits with the added advantage of real-time monitorization.
This work presents an interferometric sensor based on the well-stablished silicon-on-insulator platform with state-of-the-art figures of merit, developed at Bioherent (Malaga, Spain). The chosen platform ensures high production reliability while maintaining fabrication costs low. The sensor was tested detecting amoxicillin specific IgE antibodies (AXO-sIgE), illustrating its potential use for drug allergy diagnostics, an area where in-vivo tests, such as the drug provocation, are still the gold standard, not without risk for allergic patients.

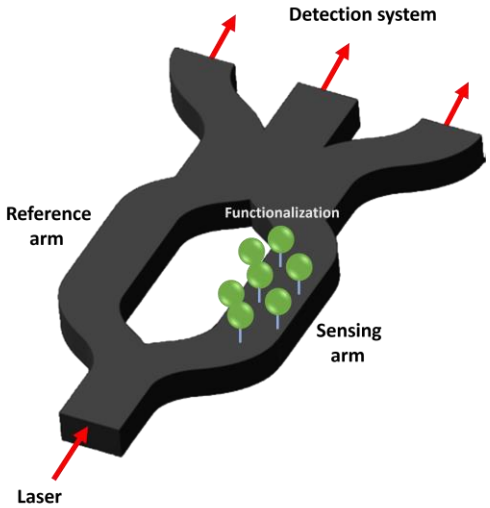

Figure 1. Basic structure of a Mach-Zehnder biosensor with 2x3 coherent readout system.

The sensor is based on a silicon nitride Mach-Zehnder architecture with a 2x3 coherent readout, operating at a wavelength of 1550 nm. In this structure, the incoming light gets split into two waveguides. One acts as reference arm, completely covered by SiO_2, encapsulated from the environment, while the other waveguide, the sensing arm, is exposed to the sample solution that flows over the surface of the chip. Refractive index changes close to the sensing arm surface affect the light modes propagation, as a small part of the modes power penetrates the surrounding medium (evanescent field sensing). Mode velocity changes, due to the refractive index variation in the medium, being this change linearly translated into a phase shift at the end of the sensing arm, when compared with the modes of the reference arm.
The 2x3 multimode interference coupler splits the incoming light of reference and sensing arm into three interferometric light signals with a relative phase offset of 120° between one another. The linear phase response is derived from the periodic interferometric signals of these three channels.
Prior to its use in biosensing, the performance of the device was characterized by homogeneous refractive index changes. Sensitivity was studied first. For this, the sensor was exposed to sodium chloride aqueous solutions of increasing concentrations ranging from 1.5% to 6% (w/w). A washout injection with water was introduced between NaCl injections. The phase obtained from the experiment is presented in figure 2.

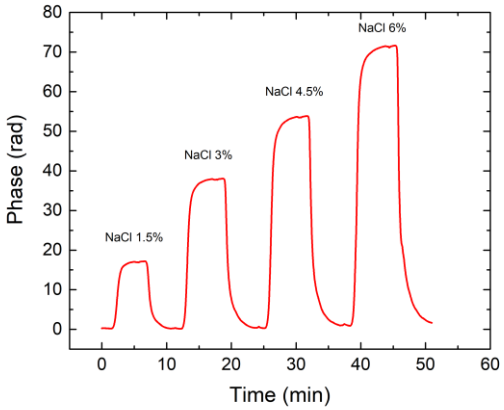

Figure 2. Phase response of the sensor to increasing concentrations of NaCl in water.

The homogeneous sensitivity of the sensor is the slope of the phase shift obtained as a result of the refractive index change introduced. To achieve this, we mapped the NaCl concentrations to their respective refractive indices based on the relationship studied by Saunders [1]. A sensitivity value of S = 7061 rad/RIU was reported, being RIU the refractive index units.
A most appropriate figure of merit to compare our sensor with competing technologies is the limit of detection (*LOD*), defined as the smallest detectable physical parameter change, generally derived by the ratio between three times the sensors uncertainty (noise) and its sensitivity:

$$LOD = 3\sigma/S$$

The measured noise level during the experiment was $6.5 \cdot 10^{-4}$ rad, which results in a *LOD* of $2.7 \cdot 10^{-7}$ RIU. The noise level can be further reduced by

applying digital filters to the signal. This way, a *LOD* of ~1·10^{-8} RIU can be obtained. This is a state-of-the-art value that outperforms commercial competitors such as surface plasmon resonance (~1·10^{-7} RIU, Biacore) and ring resonators (~1·10^{-5} RIU, Genalyte), and the most recent, and usually more complex, advances in the academic space, that are not available in the diagnostics market [2].

Our sensing structure has also been tested in a practical biosensing scenario. The goal was to detect specific IgE antibodies against beta-lactam drugs, which are clinically related to immune-mediated, and potentially life-threatening, allergic reactions.

The concentration of drug specific IgE antibodies is extremely low in individuals (0.2% of total IgE) compared to other allergens [3]. This explains why there are commercially available in vitro diagnostic tests for a wide variety of inhalant and food allergens with high specificity and sensitivity, while for drugs, there are few tests available. Notably, average beta-lactam specific IgE values in patients' serum samples often fall below the detection limit of the few available in vitro techniques like ImmunoCAP® (ThermoFisher, USA). This underscores the need for more sensitive technologies, such as the ultra-sensitive photonic biosensor presented in this work.

For IgE biorecognition, a specific functionalization of the sensing waveguide was performed using amoxicillin, but amoxicillin alone does not elicit an immune response. The allergic response in sensitive individuals is triggered when the amoxicillin bioconjugates to a serum transport protein like albumin, forming the hapten-carrier. Our surface chemistry introduces a linker molecule that mimics the function of these serum proteins, allowing the binding of the AXO-sIgE present in patients' serum samples.

The experiment performed registered the sensor response to the interaction between the hapten-carrier immobilized on the surface and mouse AXO-sIgE in a PBST buffer solution at increasing antibody concentrations. These concentrations spanned from clinically relevant values (0.24 to 2400 ng/ml, equivalent to 0.1 to 100 kUI/L). In between antibody samples injections, the transiently bonded molecules were washed out of the system by injecting buffer solution. The phase shift that corresponds to the binding process was calculated by subtracting the phase baseline prior to the IgE injection to that well after the IgE pass, during the washout phase. The phase response of the sensor can be completely characterized by its IgE concentration vs phase curve (figure 3).

At low IgE concentrations, the steep slope of the curve corresponds with a sensitivity to IgE concentrations of 88 mrad/(ng/ml). Even non-clinical levels of IgE result then in lectures well above the noise level of the sensor. Beyond antibody concentrations of 100kUI/L, the rate of phase change per unit concentration decreases, suggesting the onset of saturation on the sensor's active surface.

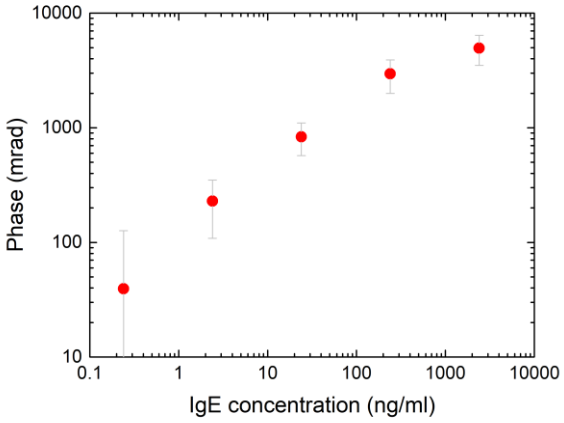

Figure 3. Phase shift vs mouse AXO-sIgE at increasing concentrations.

The specificity of the reported response was assessed through the repetition of the experiment where the mouse AXO-sIgE was replaced with a non-specific mouse IgE variant.

To summarize, the biosensing architecture presented here shows state-of-the art *LOD*s compared not only with commercial competitors but also with equivalent academic research. Preliminary experiments for specific IgE quantification demonstrate that the photonic biosensing technology developed at Bioherent, with minimal optimization, reached detection limits comparable with other in vitro commercial techniques based on label-based immunoassays.

While this work illustrates the potential use of the sensor for drug allergy diagnostics, the photonic technology is label free. The use of the sensor can be extended to any potential biomarker, given a reliable surface functionalization that guarantees the efficient recognition of the target.

In addition to that, this technology inherits all the well-known benefits derived from the silicon-on-insulator platform on which it is based. Those are the maturity of the processes that ensure high reliability and low cost, being an optimal option for commercial scalation.

References

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