Novel optoelectronic platform for label-free biosensing of influenza detection based on interferometric transducers

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ABSTRACT: The main goal of this project is to present an Optical Label-free Point-of-Care Device based on a novel read-out methodology that enhances significantly the biosensing response in terms of sensitivity and LoD, using a simple, fast and reliable interrogation process. The performance of this PoC device is verified by carrying out the calibration curve for the indirect immunoassay of Influenza Virus and comparing it with high-resolution spectrometry using the same Fabry-Perot interferometers as biosensors.

Key words: Interferometric detection system, Label-free biosensing, infectious diseases, and Influenza detection

1. – Introduction

Devices for in-vitro diagnosis provide valuable information on a patient's health status by performing a simple assay and are used in large-scale population screening. For example, diabetes patients use regularly an in-vitro device to monitor their blood glucose. Commonly used for pregnancy and for the detection of infectious disease such as hepatitis or HIV, the importance of in-vitro devices relies on the prevention and early intervention in healthcare [1].

In-vitro diagnostic tests classify in clinical laboratory testing, near patient testing or self-testing. Point-of-care (PoC) devices belong to near patient testing and offer several advantages such as minimal sample volume, reduced cost and rapid analysis time. Moreover, tests are performed at home, in a doctor's office or in paramedical support vehicles using a simple protocol for the analysis of urine or blood [2] .

Label-free optical in-vitro diagnosis offers a direct detection of specific biological species in absence of the chemical amplification commonly used in the clinical laboratory test (Enzyme-Linked Immunosorbent Assay - ELISA). This particularity allows reducing the cost and complexity of the diagnostic by simplifying the process. Therefore, the challenge will rely on achieving a competitive LoD.

This work will focus on the development of an optical Point-of-care device that enhances the LoD in comparison with high precision spectrometry. The device performance will be tested and compare by the recognition of influenza virus.

2. – Theoretical simulation

The theoretical behavior of the read-out system proposed is based on the interference of two Fabry-Perot interferometers (FPI), one as signal and one as reference, and it can be understood in Figure 1, where the interference profiles for both signal (Isig) and reference (Iref) are shown [3]. These signals are calculated as the optical power of each interferometer by the following equation (1).

$$IROP(\%) = \left(\frac{I_{Sig}}{I_{Ref}} - 1\right) \cdot 100 \qquad (1)$$

The reference interferometer remains constant during the immunoassay meanwhile the immobilization or recognition of biomolecules takes place in the signal or sensing interferometer. It can be observed that the optical power corresponding to Iref is a minimum and the optical power corresponding to Isig is a maximum before the deposition of any biofilm thickness but it suffers a displacement and therefore get closer to the Iref signal as the biofilm thickness increases [4].

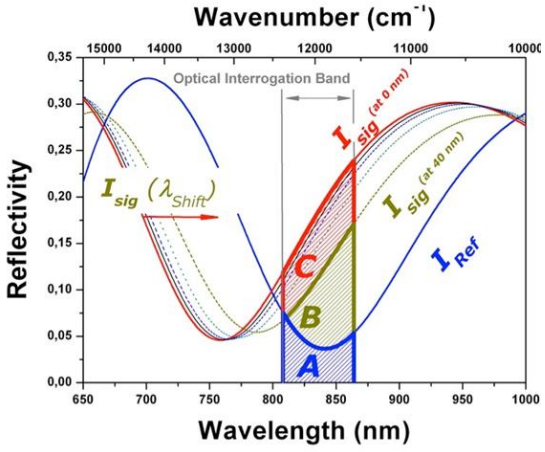

Figure 1. IROP-based read-out system theoretical behavior

With this configuration, ΔIROP will be defined as the difference between the IROP signals for a null biofilm thickness (IROP0) and when there is a certain biofilm thickness (IROP1) and it is the variable used for the calibration curves of biomolecules recognition.

3. – Read-out device and transducers implementation

For the practical implementation of the read-out system, we employed an infrared Light Emitting Diode (Hamamatsu L3989-01) and a Photodiode (Hamamatsu S2386-18L) to collect the reflectance, in a configuration with an angle of incident over the sensing area of 11.9º. Both led and photodiode are coupled in two mounts featuring three high accuracy screws to control the platform tip, tilt and translation in the optical axis and precisely and easily carry out the alignment (Figure 2).

The electronic hardware is based on the Cookies platform [5] [6], a modular design with three capabilities: sensing, power supply and data processing. The processing layer deals with the signal coming from the other stages and processes the information. The microcontroller used (C8051F930 – Silicon Laboratories) is a 10-bit, 20 MHz processor with low power consumption capabilities. It contains several serial ports (SPI, UART), a real time clock and a 6-bit programmable current reference, among other capabilities. For this application, the SPI is used to communicate with the external ADC in the sensing layer, the programmable current reference is connected to the analog circuit in the sensing layer to set the current though the light source, the UART is used to send information to a computer, and the real time clock defines the periodicity of measurements.

The power supply layer is in charge of providing the voltage levels requested (3.3V for the sensing layer and 2.5V for the processing layer). It also contains a UART to USB chip in order to send the information from the processor to a computer via USB connection, which is also used to power the device. The sensing layer consists of the power supply circuit for the light source and the conditioning signal circuit for the photodetector. Light source demands a constant and stable optical power and the circuit is

based on unidirectional and MOSFET configurations. The conditioning of the photodetector signal uses a transimpedance amplifier configuration providing an output voltage proportional to the current generated by the photoreceptor. All components are high precision and low noise in order to increase the device SNR, especially the operational amplifiers used (LMV791/LMV792 - Texas Instruments).

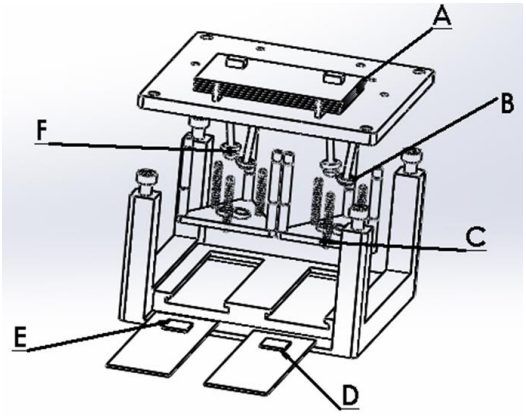

Figure 2. A-Electronic unit; B- Optical Signal channel; C- Optical Alignment mount; D- Signal FPI; E- Reference FPI; F- Optical Reference channel.

Regarding the transducers used in this application, both signal and reference FPI are based on thin layers of SU8 and Nitrocellulose over a Si substrate [7]. For the fabrication of the FPI transducers, the substrate is cleaned with DMSO by sonication and is washed with Milli-Q water and dried with particle-free air. Next step is to deposit the SU8 layer by spin coating and apply a soft-bake treatment at 120 degrees for 30 seconds. After that, the nitrocellulose is also deposited by spin coating and is applied a soft-bake treatment in two steps: first at 120 degrees during 8 seconds and then at 60 degrees for 1 hour. For the measurement of the thin layers in each step it is used Filmetrix (FV20-UV) and FT-VIS-NIR spectrometer (BRUKER Vertex 70 adapted to visible range).

In order to be able to measure the FPIs in the device and manipulate them easily, they are packaged over a glass substrate. Finally, to define the well where the sample drop would be deposited, a vinyl sticker with the design required by the sample volume is fabricated. Figure 3 shows this KIT design.

Figure 3. Bio-KITs

4. – Results

4.1. - Results for ALD FPIs

The first step to verify the device performance is to carry out the measurement of several transducers which simulate the film thickness of different biomolecules and compare these measurements with a high-resolution spectrometer (FTIR-BRUKER). These FPIs are based on silicon oxide interferometers and different thickness of Al2O3 fabricated using Atomic Layer Deposition (ALD).

Results are shown in Figure 4, including the system response for different thickness and the correlation with FTIR. The theoretical sensitivity for the read-out system based on IROP, calculated with these interferometers is, on average, 1.75 %/nm and the uncertainty is 0.69 %; reaching a limit of detection of 0.5 nm.

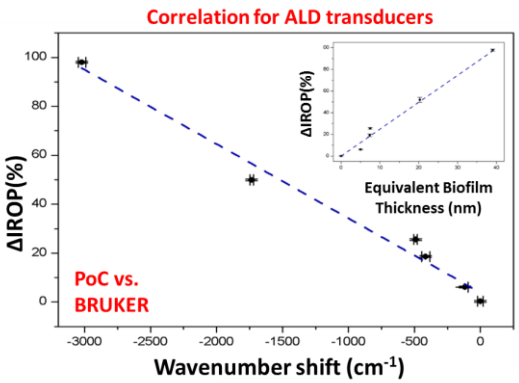

Figure 4. Correlation results for ALD transducers

4.2. - Result for direct immunoassay

For the recognition of the influenza virus antigen over the sensing surface of the FPI, several steps were performed [8]. Firstly, the antibody was incubated onto the chip surface, placing a drop of sample with a concen-

tration of 50 µg·mL^{-1} overnight, at 37 degrees and avoiding evaporation. After incubation, the surface is washed with Milli-Q water and dried with particle-free air. Then, different concentrations of the influenza virus were incubated, diluting 8 times the pure virus sample.

Results for this recognition curve are shown in Figure 5, as well as the correlation of these results with the high-resolution spectrometry equipment (BRUKER). The figure shows how the recognition curve follows a sigmoidal curve and how the correlation fits a linear adjustment, as it was expected for this immunoassay.

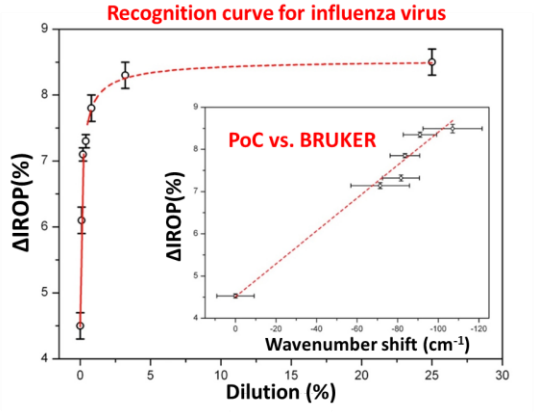

Figure 5. Recognition curve and correlation for Influenza virus

5. – Conclusion

The IROP read-out methodology presented within this work allows developing a compact, easy-to-use and cost effective Point-of-care with a competitive performance in terms of LoD and time in comparison with high-resolution spectrometry. This devices provides quantitative results for the recognition of influenza virus in only 2 minutes with 50 µL of sample. All these features fulfil the requirements for an ideal PoC device.

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References

[1] F.S. Ligler, C.R. Taitt, *"Optical Biosensors: Today and Tomorrow"*. Elsevier; 2008.

[2] Kumar Vashist S, Luppa PB, Yeo LY, et al. "*Emerging technologies for next generation point-of-care testing"*. Trends Biotechnology, 2015.

[3] M. Holgado, F.J. Sanza, A. López, A. Lavín, R. Casquel, M.F. Laguna; *"Description of an Advantageous Optical Label-Free Biosensing Interferometric Read-Out Method to Measure Biological Species"*; Sensors 2014

[4] M. Holgado, M. V. Maigler, B. Santamaría, A. López, A. Lavín, M. F. Laguna, F. J. Sanza, D. Granados, R. Casquel, J. Portilla, T. Riesgo; *"Towards reliable optical label-free Point-of-Care (PoC) biosensing devices";* Sensors and Actuators B: Chemical; Volume 236, 29 November 2016

[5] J. Portilla, T. Riesgo, A. Abril, A. de Castro, *"Rapid prototyping for multi-application sensor networking"*, 12 November 2007, SPIE Newsroom. DOI: 10.1117/2.1200711.0851

[6] J. Portilla, A. de Castro, E. de la Torre, T. Riesgo, *"A Modular Architecture for Nodes in Wireless Sensor Networks",* Journal of Universal Computer Science (JUCS), vol. 12, nº 3, pp. 328 – 339, March 2006.

[7] M. Holgado, C.A.Barrios, F.J. Ortega, F.J. Sanza, R. Casquel, M.F. Laguna, M.J. Bañuls, D. López-Romero, R. Puchades, A. Maquieira, *"Label-free biosensing by means of periodic lattices of high aspect ratio SU-8 nano-pillars"*, Biosens. Bioelectron. 25 (2010) 2553-2558.

[8] M.F. Laguna, M. Holgado, F.J. Sanza, A. Lavín, A.L. Hernandez, R. Casquel, *"Optimization of dengue immunoassay by label-free interferometric optical detection method"*, Sensors (2014) 6695-6700.

Other:

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