IEEE Photonics Journal
An IEEE Photonics Society Publication

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Invited Paper

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Volume 5, Number 2, April 2013

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DOI: 10.1109/JPHOT.2013.2250943
1943-0655/$31.00 ©2013 IEEE

IEEE photonics SOCIETY

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Breakthroughs in Photonics 2012: 2012 Breakthroughs in Lab-on-a-Chip and Optical Biosensors

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(Invited Paper)

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DOI: 10.1109/JPHOT.2013.2250943

Manuscript received February 11, 2013; accepted February 20, 2013. Date of publication March 7, 2013; date of current version May 2, 2013. Corresponding author: L. M. Lechuga (e-mail: laura.lechuga@cin2.es).

Abstract: We review the most important achievements published in 2012 in the field of lab-on-a-chip (LOC) and optical biosensors. We will specially focus on optical label-free biosensors and their implementation into lab-on-a-chip platforms, with an emphasis on manuscripts demonstrating bioanalytical applications.

Index Terms: Biosensors, integrated nanophotonic systems, waveguide devices.

1. Introduction

Lab-on-a-chip (LOC) platforms are miniaturized devices, which integrate all functionalities on a single chip: fluid handling, sample preparation (filtration, homogenization, and dilution), target detection, transducer readout, and signal processing. Taking advantage of the miniaturization, LOC platforms can perform high throughput screening while consuming only tiny amounts of samples, reagents, space, and energy. They should also contain enough hard-wired intelligence and robustness to be used by nonskilled personnel (for instance the patient itself) and should deliver in real time a multitude of data directly to a central database.

Optical biosensors appear as one of the most promising candidates for LOC platforms as they offer outstanding sensitivity and the possibility of miniaturization. They can be divided in two categories, depending on the use or not of labels. Most of the labeled biosensors are based on fluorescence detection and generally imply laborious sample or reagents preparation and, due to the off-chip detection, the need for an external readout system. On the contrary, label-free biosensors, most of them using evanescent field detection, afford a real-time and on-chip detection [1]. In addition, the merging of photonic sensors with microfluidic, the so-called optofluidic, provides enhanced functions and performances [2], [3].

To develop a LOC platform, three main steps must be followed. First, a type of transducer must be selected, optimized, and fabricated for its intended use. Then, it must be validated with a proof of concept of a bioanalytical application and, finally, the transducer has to be integrated with all the required components to become a standalone LOC platform and validated again for the same application. For LOC based on photonic biosensors, the first step is clearly the one where most progresses have been done in the last years, while the second and the third steps are still in their early stages. In this context, we report on the progress made along 2012 in the field of photonic

biosensors and LOC platforms, focusing mainly on publications demonstrating bioanalytical detections. The lure of these research areas is demonstrated by the wide number of publications on the subject: According to the ISI Web of Science, more than 600 papers have been published in 2012 on the topic "lab-on-a-chip" and nearly 200 on "optical biosensor." In this review, particular attention is dedicated to IO and nanoplasmonic biosensors and their integration into LOC platforms as we do believe they represent the major breakthrough of 2012 in this field.

2. Optical Biosensors

The term biosensor includes 1) a bioreceptor (DNA, proteins, antibodies, etc.) which will capture the analyte in a specific way and 2) a transducer, which will convert the biomolecular detection into a physical signal. In the last decades, a wide range of optical transducers have been successfully developed for biosensing applications: Surface Plasmon Resonance (SPR) and nanoplasmonics devices, integrated interferometers, microring resonators, grating-coupled waveguide sensors, photonic crystal- or silicon-wire-based sensors, among others. Nowadays, the emergence of new transducer schemes has clearly slowed down, and the tendency is more to: i) apply existing transducers to specific bioanalytical problems, ii) translate existing transducers to another range of wavelengths, and iii) combine two detection schemes into hybrid devices to enhance the performance.

When dealing with bioanalytical studies, surface functionalization is of great importance to enhance the transducer capabilities. The biofunctionalization process should guarantee efficient bioreceptor coverage of the transducer surface and should ensure the sensitivity, selectivity, and stability of the biosensor even in complex media such as blood or urine. The immobilization strategy is selected according to the application, i.e., the nature of the bioreceptor to be immobilized, and the transducer surface. For instance, Bailey's group has reported the detection of Bean Pod Mottle Virus (BPMV) in buffer and in complex leaf extracts [4] as well as the detection and identification of transfer-messenger RNA (tmRNA) [5] using a platform based on silicon microring resonators. The difference between both applications arises from the immobilization strategy: In the first case, antibodies specific to BPMV were conjugated to amino-reactive crosslinking reagents to be attached to the microring surface, while in the second case, specific ssDNA capture probes were immobilized using hydrazone-bond linker chemistry. In both cases, label-free and multiplexed detections have been demonstrated, highlighting a high sensitivity and specificity. Silicon microring resonators have also been used in [6] as a label-free methylation specific sensor for detection and quantification of DNA methylation biomarkers in bladder cancer with the ability to distinguish a change of few nucleotides between the methylated and the unmethylated targets. As shown by these previous examples and those reported in the 2012 review [7], microresonators are appealing biosensors for the multiplexed detection of a wide range of analytes (bacteria, virus, protein biomarkers, cancer biomarkers, DNA, RNA, etc.) with limits of detection comparable to those of commercially available multiplexed assays such as the well-known label-based enzyme-linked immunosorbent assays (ELISA).

Optical biosensors, and in particular SPR, have been first implemented for wavelengths in the visible range. Then, taking advantage of the extended know-how developed for telecommunications, optical transducers working in the near infrared have emerged. The best example of this technological transfer is the microring resonator described above, a component of the telecommunication industry, which is now widely employed as a biosensor working at 1.5 μm. However, to take advantage of the low-cost optical components available in the visible range, novel waveguide technologies have been investigated in 2012 to develop ring resonators at lower wavelength [8]. Conversely, a shift to larger wavelengths is also observed. For instance, Mach–Zehnder interferometers (MZIs) based on GaAs/AlGaAs waveguides for the midinfrared range (3–12 μm) was introduced by Mizaikoff's group [9]. Even if the biosensing capabilities of this device have not been confirmed yet, it seems particularly interesting as it takes advantage of a much larger penetration depth of the evanescent field while providing the inherent molecular selectivity of this spectral window. This concept can be extended until microwave frequencies: Lee *et al.* have reported the use as biosensors of radio-frequency planar ring resonators excited by a local high-impedance

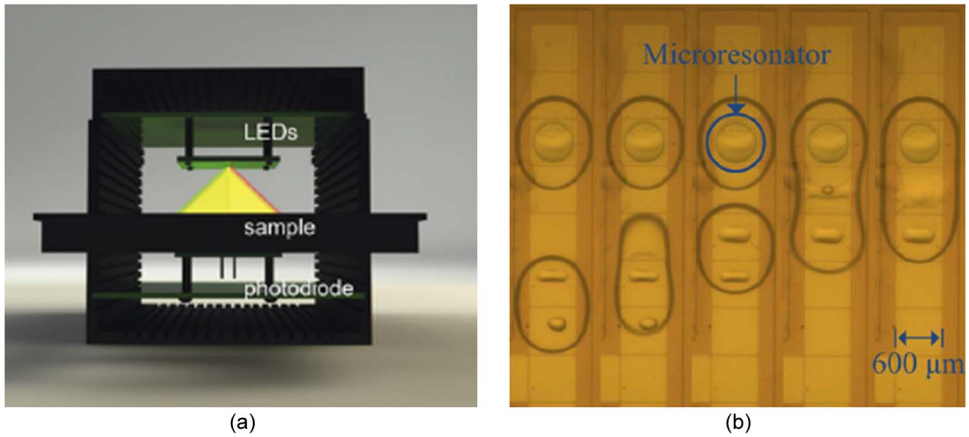

Fig. 1. (a) Scheme of the T-LSPR platform (reprinted from [13] with permission) and (b) sequence of photomicrographs showing the droplet merging operation (left to right) in the electrowetting system integrated with polymeric microresonators (reprinted from [19] with permission).

microstrip. Biosensing capabilities of the device have been verified with the label-free detection of the prostate specific antigen (PSA) and the cortisol stress hormone at low concentrations $(1 \times 10^{-10}$ g/mL) [10].

Another interesting research line relies on the development of hybrid transducers able to solve some of the limitations of the standard methods of detection. For example, current strategies for ultrasensitive detection require sophisticated instruments preventing their use in developing countries. The plasmonic ELISA-based biosensor proposed by de la Rica and Stevens can circumvent this problem as they have demonstrated the ultrasensitive detection $(1 \times 10^{-18}$ g/mL) with the naked eye of disease biomarkers such as PSA or HIV-1 capsid antigen p24 in whole serum; the color of the solution depends of the presence of the analyte through the aggregation of gold nanoparticles controlled by an enzyme label of an ELISA [11]. Even if the method is not quantitative, it has a strong potential for achieving low-cost LOC platforms for point-of-care (POC) diagnostics. Another limitation of current transducers is their difficulty to detect analytes of low molecular weight. This has been achieved by Dantham *et al.*; they have reported the detection of the smallest individual RNA virus (only 6 ag) using a whispering-gallery-mode nanoplasmonic hybrid device composed of a spherical dielectric microcavity with a nanoplasmonic receptor at the equator [12]. This configuration provides an enhancement of the field intensity and the wavelength shift, which results in a significantly improved signal to noise ratio, allowing the detection of a single particle with such low mass.

3. Integration of Optical Biosensors Into Complete LOC Platforms

To achieve a standalone LOC platform, the optical transducer in a multiplexed configuration must be integrated with all the required units (as light sources, photodetectors, microfluidics, processing electronics, etc.) as well as with robust biofunctionalization protocols for the biological receptors. Even though the individual components are well known, there are still major barriers to overcome for their assembly, among others, because the subsystem interfaces between them are difficult to optimize.

In the case of LOC based on plasmonic biosensors, one the main limitation when using the standard SPR transducer is the need for moving elements to observe the changes in the reflectance angle. Localized SPR (LSPR) transducers are particularly interesting as they can operate in transmission configuration (T-LSPR), which only requires the alignment between the light source, the sample, and the detector, without moving parts. Cappi *et al.* have presented a portable T-LSPR device based on low-cost and low-consumption components [see Fig. 1(a)]. The key element of this device is a novel data analysis approach that extracts the key signature of the LSPR

spectrum employing only three LEDs as light sources [13]. A proof of concept of the surface sensitivity has been shown through the detection of the peak shift due to the immobilization of a layer of ssDNA on the sensor surface, but its ability for biosensing detection has still to be demonstrated.

LOC using IO sensors can circumvent the disadvantages inherent to fluorescence- or SPR-based detection as they combine highly sensitive, label-free, and on-chip detection with the possibility of miniaturization and photonic integration of components on one single chip. This is particularly true for IO biosensors based on silicon and silicon-related materials as they can benefit of the know-how of the semiconductor microfabrication industry and its potential for mass production with a consequent reduction of costs. However, IO-based LOC is still very much a work in progress when compared to other techniques. Advances in this field have been reported by Lechuga's group through the implementation of a LOC based on novel silicon nitride bimodal waveguide interferometers. The interferometers have been integrated with submicronic grating couplers for efficient light in-coupling in the visible range and with a 3-D network of SU-8 polymer microfluidics assembled at the wafer level to ensure perfect sealing and compact packaging [14]. In addition, to solve some of the drawbacks inherent to the interferometric readout, they have implemented a novel all-optical wavelength modulation system, which provides a linear response and a direct readout of the phase variation without additional fabrication processes or instrumentation [15].

Multiplexed label-free detection is mandatory for diagnostics applications where we need to perform high throughput analysis of multiple biomarkers in real time and using small volume of the patient sample. To reach this goal, Chen's group has developed a method to create large-scale chip-integrated multiple photonic crystal (PC) microcavities arrayed along the same PC waveguide. Multiplexed capability has been verified through the simultaneous interrogation of five PC microcavities that recognized different specific binding interactions between target and probe antibody conjugates [16]. Moreover, they applied this multiplexed platform to the specific detection of a relevant cancer-associated protein in lung cancer cell lysates with sensitivity down to two cells per microliter [17]. This result is particularly interesting as the detection has been done in complex mixtures containing more than 20 000 to 50 000 proteins derived from a whole cell lysate of a lung cancer cell line.

To decrease even more the costs of the fabrication processes, an alternative approach consists in the use of a low-cost polymer material combined with fabrication processes such as UV-based soft imprint lithography. In 2012, ring resonators in polymer showing high performance for biosensing have been implemented for the first time [18], [19]. Jokerst's group has integrated these polymeric microresonators with a digital electrowetting-on-dielectric microfluidic system [see Fig. 1(b)] [19] and with a thin-film InGaAs-based metal–semiconductor–metal photodetector [20], demonstrating that the use of a polymer waveguide does not impede the integration with complex elements. However, the biosensing capabilities of this intricate system have not been reported yet.

As mentioned previously, LOC based on IO biosensors have not yet reached the level of development of other detection methods. As a logic consequence, while LOC platforms based on lateral flow tests and electrochemical or fluorescence detection have been commercialized since the 1990s [21], the first LOC based on IO transducers has been launched on the market only in July of 2012 by the company Genalyte [22]. The Maverick platform is composed of silicon microresonators in a multiplexed configuration implemented in a simplified one-step workflow, which provides accurate results within 15 min. Even though this platform is not truly portable, it is an important achievement in the field of IO-based LOC, and it is expected that other platforms will soon follow the pathway for commercialization.

4. Nonconventional LOC Platforms

An alternative strategy for LOC platforms consists in using standard consumer electronic devices such as CDs or cell phones, taking advantages of their versatility and widespread dissemination. Lab-on-a-disc (LOAD) platforms, which use discs as centrifugal microfluidics, have a clear potential,

even though they usually require an external reader. To overcome this limitation, Czugala *et al.* have integrated a wireless paired emitter detector diode device (PEDD) on a portable multichannel LOAD platform, getting rid of the external reader [24]. This platform has been applied to the *in situ* monitoring of the pH of river water, but its working principle is of interest for biomedical POC applications in remote location as data can be directly delivered to a central monitoring station.

Another approach employs the cell phone as source or/and detector. Indeed, with more than 5 billion devices worldwide, the cell phone is a ubiquitous device that, in combination with the current status of wireless technologies, exhibits a promising potential for healthcare application at the POC, even in the less favored countries. Preechaburana *et al.* have implemented an angle-resolved SPR device, which temporarily adheres to the phone screen surface during the analysis: Light from the cell screen is coupled into the SPR sensor and then coupled out to the cell camera [23]. The performance of this device has been studied with a commercial gold chip from Biacore functionalized with a monoclonal mouse-anti-human β_2 microglobulin. Another interesting use of the cell phone has been reported in [25] where a compact and cost-effective digital rapid-diagnostic-test reader of only 65 g has been designed to be mechanically attached to the camera unit of a cell phone. The proposed reader platform works with lateral flow immunochromatographic assays for the detection of malaria, tuberculosis, and HIV. This detection scheme is not completely into the scope of this article, but it seems worth mentioning it for the proposed cellphone application that could also clearly be implemented for IO-based LOC. This application allows the automated reading of the diagnostic result and the transmission of the data to a central server, which presents the result on a world map through geotagging. Such dynamic spatiotemporal map is highly promising as it might assist healthcare professionals and policymakers to track emerging epidemics worldwide.

5. Conclusion

Research in LOC platforms based on optical biosensors experienced significant activities in 2012. In particular, IO transducers emerge as an appealing solution for achieving a portable and automated LOC platform for POC diagnostics as they provide highly sensitive, label-free, and real-time on-chip detection. However, their commercialization is still limited, mainly because of problems in the integration of all the components into a single platform as well as in the demonstration of multiplexed detection of analytes of clinical interest. Proof of this challenge is the announcement in January of 2012 of the Qualcomm Tricorder X Prize, a $10 million prize for the first research team, which will achieve a standalone LOC device (without restriction on the transducer scheme but limited to a total weight of 5 lb) able of accurately diagnosing 15 diseases in patients while providing a strong consumer experience in the areas of usability and understandability [26].

References

[1] M. C. Estevez, M. Alvarez, and L. M. Lechuga, "Integrated optical devices for lab-on-a-chip biosensing applications," *Laser Photon. Rev.*, vol. 6, no. 4, pp. 463–487, Jul. 2012.
[2] F. B. Myers and L. P. Lee, "Innovations in optical microfluidic technologies for point-of-care diagnostics," *Lab Chip*, vol. 8, no. 12, pp. 2015–2031, Dec. 2008.
[3] X. Fan and I. M. White, "Optofluidic microsystems for chemical and biological analysis," *Nat. Photon.*, vol. 5, no. 10, pp. 591–597, Oct. 2011.
[4] M. S. McClellan, L. L. Domier, and R. C. Bailey, "Label-free virus detection using silicon photonic microring resonators," *Biosens. Bioelectron.*, vol. 31, no. 1, pp. 388–392, Jan. 2012.
[5] O. Scheler, J. T. Kindt, A. J. Qavi, L. Kaplinski, B. Glynn, T. Barry, A. Kurg, and R. C. Bailey, "Label-free, multiplexed detection of bacterial tmRNA using silicon photonic microring resonators," *Biosens. Bioelectron.*, vol. 36, no. 1, pp. 56–61, Jun./Jul. 2012.
[6] Y. Shin, A. P. Perera, J. S. Kee, J. Song, Q. Fang, G.-Q. Lo, and M. K. Park, "Label-free methylation specific sensor based on silicon microring resonators for detection and quantification of DNA methylation biomarkers in bladder cancer," *Sens. Actuators B, Chem.*, vol. 177, pp. 404–411, Feb. 2013.
[7] C. Barrios, "Integrated microring resonator sensor arrays for labs-on-chips," *Anal. Bioanal. Chem.*, vol. 403, no. 6, pp. 1467–1475, Jun. 2012.
[8] R. Heideman, M. Hoekman, and E. Schreuder, "TriPleX-based integrated optical ring resonators for lab-on-a-chip and environmental detection," *IEEE J. Sel. Topics Quantum Electron.*, vol. 18, no. 5, pp. 1583–1596, Sep./Oct. 2012.

[9] M. Sieger, F. Balluff, X. Wang, S.-S. Kim, L. Leidner, G. Gauglitz, and B. Mizaikoff, "On-chip integrated mid-infrared GaAs/AlGaAs Mach–Zehnder interferometer," *Anal. Chem.,* Nov. 2012, DOI: 10.1021/ac302551s.
[10] H.-J. Lee, J.-H. Lee, H.-S. Moon, I.-S. Jang, J.-S. Choi, J.-G. Yook, and H.-I. Jung, "A planar split-ring resonator-based microwave biosensor for label-free detection of biomolecules," *Sens. Actuators B, Chem.*, vol. 169, pp. 26–31, Jul. 2012.
[11] R. de la Rica and M. M. Stevens, "Plasmonic ELISA for the ultrasensitive detection of disease biomarkers with the naked eye," *Nat. Nanotechnol.*, vol. 7, no. 12, pp. 821–824, Dec. 2012.
[12] V. R. Dantham, S. Holler, V. Kolchenko, Z. Wan, and S. Arnold, "Taking whispering gallery-mode single virus detection and sizing to the limit," *Appl. Phys. Lett.*, vol. 101, no. 4, pp. 043704-1–043704-4, Jul. 2012.
[13] G. Cappi, E. Accastelli, V. Cantale, M. A. Rampi, L. Benini, and C. Guiducci, "Peak shift measurement of localized surface plasmon resonance by a portable electronic system," *Sens. Actuators B, Chem.*, vol. 176, pp. 225–231, Jan. 2013.
[14] D. Duval, A. B. González-Guerrero, S. Dante, J. Osmond, R. Monge, L. J. Fernández, K. E. Zinoviev, C. Domínguez, and L. M. Lechuga, "Nanophotonic lab-on-a-chip platforms including novel bimodal interferometers, microfluidics and grating couplers," *Lab Chip*, vol. 12, no. 11, pp. 1987–1994, May 2012.
[15] S. Dante, D. Duval, B. Sepúlveda, A. B. González-Guerrero, J. R. Sendra, and L. M. Lechuga, "All-optical phase modulation for integrated interferometric biosensors," *Opt. Exp.*, vol. 20, no. 7, pp. 7195–7205, Mar. 2012.
[16] Y. Zou, S. Chakravarty, W.-C. Lai, C.-Y. Lin, and R. T. Chen, "Methods to array photonic crystal microcavities for high throughput high sensitivity biosensing on a silicon-chip based platform," *Lab Chip*, vol. 12, no. 13, pp. 2309–2312, Jul. 2012.
[17] S. Chakravarty, W.-C. Lai, Y. Zou, H. A. Drabkin, R. M. Gemmill, G. R. Simon, S. H. Chin, and R. T. Chen, "Multiplexed specific label-free detection of NCI-H358 lung cancer cell line lysates with silicon based photonic crystal microcavity biosensors," *Biosens. Bioelectron.*, vol. 43, pp. 50–55, May 2013.
[18] L. Wang, J. Ren, X. Han, T. Claes, X. Jian, P. Bienstman, R. Baets, M. Zhao, and G. Morthier, "A label-free optical biosensor built on a low-cost polymer platform," *IEEE Photon. J.*, vol. 4, no. 3, pp. 920–930, Jun. 2012.
[19] M. W. Royal, N. M. Jokerst, and R. B. Fair, "Integrated sample preparation and sensing: Polymer microresonator sensors embedded in digital electrowetting microfluidic systems," *IEEE Photon. J.*, vol. 4, no. 6, pp. 2126–2135, Dec. 2012.
[20] L. Lin, M. W. Royal, R. Evans, R. B. Fair, and N. M. Jokerst, "Chip scale optical microresonator sensors integrated with embedded thin film photodetectors on electrowetting digital microfluidics platforms," *IEEE Sens. J.*, vol. 12, no. 6, pp. 1794–1800, Jun. 2012.
[21] C. D. Chin, V. Linder, and S. K. Sia, "Commercialization of microfluidic point-of-care diagnostic devices," *Lab Chip*, vol. 12, no. 12, pp. 2118–2134, Jun. 2012.
[22] Genalyte, Inc., last visited Feb. 2013. [Online]. Available: www.genalyte.com
[23] P. Preechaburana, M. C. Gonzalez, A. Suska, and D. Filippini, "Surface plasmon resonance chemical sensing on cell phones," *Angew. Chem.*, vol. 51, no. 46, pp. 11 585–11 588, Nov. 2012.
[24] M. Czugala, R. Gorkin Iii, T. Phelan, J. Gaughran, V. F. Curto, J. Ducree, D. Diamond, and F. Benito-Lopez, "Optical sensing system based on wireless paired emitter detector diode device and ionogels for lab-on-a-disc water quality analysis," *Lab Chip*, vol. 12, no. 23, pp. 5069–5078, Dec. 2012.
[25] O. Mudanyali, S. Dimitrov, U. Sikora, S. Padmanabhan, I. Navruz, and A. Ozcan, "Integrated rapid-diagnostic-test reader platform on a cellphone," *Lab Chip*, vol. 12, no. 15, pp. 2678–2686, Aug. 2012.
[26] Qualcomm Tricorder X Prize, last visited Feb. 2013. [Online]. Available: www.qualcommtricorderxprize.org