Coherent silicon photonic interferometric biosensor with an inexpensive laser source for sensitive label-free immunoassays

**Jonas Leuermann,**1,2,* Vladimir Stamenkovic,**1,3 Patricia Ramirez-Priego,**4 Alejandro Sánchez-Postigo,**2 Adrián Fernández-Gavela,**5 Cole A. Chapman,**6 Ryan C. Bailey,**6 Laura M. Lechuga,**4 Ezequiel Perez-Inestrosa,**1,3 Daniel Collado,**1,3 Robert Halir,**1,2 and Íñigo Molina-Fernández1,2*  

1 Bionanod Center for Nanomedicine and Biotechnology, Parque Tecnológico de Andalucía, 29590 Málaga, Spain  
2 Departamento de Ingeniería de Comunicaciones, University of Málaga, ETSI Telecomunicación, Campus de Teatinos, 29071 Málaga, Spain  
3 Departamento de Química Orgánica, Facultad de Ciencias, University of Málaga, 29071 Málaga, Spain  
4 Nanobiosensors and Bioanalytical Applications Group, Catalan Institute of Nanoscience and Nanotechnology (ICN2), CSIC, BIST and CIBER-BBN Campus UAB, 08193 Barcelona, Spain  
5 Departamento de Física, Universidad de Oviedo, C/Federico García Lorca, 33007 Oviedo, Spain  
6 Department of Chemistry, University of Michigan, 930 North University Avenue, Ann Arbor, Michigan 48109, USA  
*Corresponding author: jonas.leuermann@uma.es

Received 2 October 2020; revised 7 November 2020; accepted 9 November 2020; posted 10 November 2020 (Doc. ID 411635); published 8 December 2020

Over the past two decades, integrated photonic sensors have been of major interest to the optical biosensor community due to their capability to detect low concentrations of molecules with label-free operation. Among these, interferometric sensors can be read-out with simple, fixed-wavelength laser sources and offer excellent detection limits but can suffer from sensitivity fading when not tuned to their quadrature point. Recently, coherently detected sensors were demonstrated as an attractive alternative to overcome this limitation. Here we show, for the first time, to the best of our knowledge, that this coherent scheme provides sub-nanogram per milliliter limits of detection in C-reactive protein immunoassays and that quasi-balanced optical arm lengths enable operation with inexpensive Fabry–Perot-type laser sources at telecom wavelengths. © 2020 Optical Society of America under the terms of the OSA Open Access Publishing Agreement

https://doi.org/10.1364/OE.411635

Integrated photonic biosensors have been the subject of intense research in the last two decades, due not only to their miniturizability but also because they can quantitatively detect extremely low concentrations of analytes in real time and without the need of labeling tags [1–3]. Silicon-based sensors provide particularly compact footprints, enabling multiplexed detection of different biochemical substances, and CMOS compatible fabrication, which paves the way towards low-cost mass production [4]. Therefore, they are candidates for point-of-care (POC) solutions in which pre-functionalized, disposable chips are used in conjunction with a read-out system to provide diagnostics that would otherwise require specialized laboratories and trained personnel [5]. Most photonic integrated sensors rely on the principle of evanescent field sensing: the analyte attaches to a biorecognition layer on the waveguide surface, where it interacts with the evanescent field of the guided wave, changing its phase [6–8]. Since changes in the optical phase cannot be measured directly, they are converted into changes in optical power, using on-chip resonant or interferometric structures.

Resonant structures convert the phase change into a resonance wavelength shift, thus often requiring a read-out system with a tunable external cavity laser (ECL) [9–14], or a broadband light source combined with additional wavelength filtering [15]. For homogeneous refractive index sensing, these systems offer moderate limits of detection (LODs), on the order of $10^{-6}$ refractive index units (RIUs), but can achieve very compact footprints [9,16,17]. Regarding the detection of biomolecules, in benchmark primary C-reactive protein (CRP) binding assays, resonant sensors achieve LODs on the order of 10 ng/mL (0.4 nM) [18]. Interferometric sensors compare the optical wave in the sensing waveguide to a wave in a reference waveguide to reconstruct the phase shift. These sensors generally operate with a fixed-spectrum laser source, which can be a superluminescent diode, with asymmetric sensing and reference arm and optical wavelength filtering in the read-out [2,19,20], or a narrow-linewidth laser source, e.g., a He–Ne laser at visible wavelengths [21–23] or ECLs at telecom wavelengths [10,24,25]. Detection limits down to 2ng/mL (84 pM) with a label-free primary binding immunoassay for detecting CRP have been reported for Mach–Zehnder interferometers (MZIs) [16,26]. It is noteworthy that shortcomings commonly associated with these interferometric sensors, such as sensitivity fading when not operated at the quadrature point and phase ambiguity, have been overcome, either by adding a...
anced interferometer, the wavelength response is flat [Fig. 1(a)].

Intuitively, this can be understood because for a perfectly balanced interferometer, the wavelength response is flat when the optical arm lengths are balanced. (b) Approximated laser spectra of a narrow-linewidth external cavity laser and a Fabry–Perot laser, with center wavelength $\lambda_0$, linewidth $\delta \lambda$, and spectral spacing $\Delta \lambda$.

In our experiments, the reference and sensing arms have the same length $L = 8.8$ mm and a group index difference of $\Delta n_{g,15} = |n_{g,s} - n_{g,r}| \approx 0.01$ at $\lambda_0 = 1.55 \mu$m.

In our read-out system, the light source is connected to an optional erbium-doped fiber amplifier and a variable optical attenuator to obtain the desired input power. A polarization rotator is used to achieve TE polarization that is coupled into the silicon nitride sensor chip via grating couplers, with coupling losses of $\sim 10$ dB [Fig. 1(a)]. The sensor chip is fabricated with a multi-project wafer approach in a 300 nm thick silicon nitride film [36]. The sensing arm is covered with a polydimethylsiloxane (PDMS) microfluidic channel, with a width of 1 mm, length of 9.8 mm, and height of 0.5 mm, which is connected to a syringe pump. In between, a six-port valve system enables the injection of an analyte volume of $V_{\text{inj}} \approx 120$ $\mu$L into the channel. The three optical output signals are coupled to a fiber array using on-chip identical grating couplers and are detected by separate photodiodes (PDs) with integrated transimpedance amplifiers. The resulting voltages are quantized by a data acquisition board, and signal processing is used to estimate the phase change $\Delta \varphi$ [31]. A detailed description and bulk LOD optimization of the experimental setup can be found in [24]. To assess the impact of the laser linewidth on sensor performance, two different light sources were employed in the experiments. As a high-quality reference, we used the WSL-100 from SANTEC (Japan), a single-mode ECL bought for approximately 10,000€, with a linewidth of $\delta \lambda < 80$ $\text{fm}$ (100 kHz) [Fig. 1(b)]. As a low-cost alternative, we used a hand-held Fabry–Perot laser (FPL) with a center wavelength of $\lambda_0 = 1543$ $\mu$m, $\Delta \lambda = 1.36$ $\text{nm}$ wavelength spacing, and a bandwidth of $\delta \lambda \sim 3.6$ $\text{nm}$ (450 GHz) [Fig. 1(b)], purchased on Amazon for 34€.

Two types of experiments were chosen to determine the quality of the sensor, bulk refractive index sensing, and protein CRP immunodetection. We used two different power levels: low, with a total optical power of approximately $-30$ $\text{dBm}$ received by the PDs, and high, with a power of approximately $-10$ $\text{dBm}$. Homogeneous refractive index sensing experiments were performed by injecting four different mass percentage solutions of sodium chloride (3%, 6%, 9%, 12%) resulting in changes of $(0.5, 1.0, 1.5, 2.0) \times 10^{-2}$ $\text{RIU}$.

In Fig. 2, bulk sensing using the reference ECL source (continuous blue curve) and the low-cost FPL (dashed orange curve) for 3%, 6%, 9%, and 12% mass percentage sodium chloride injections and low input power.
A flow rate of 20 µL/min was blocked with bovine serum albumin (BSA, 10 mg/mL), (BS3). Before each detection experiment, the sensor surface anti-CRP antibodies were covalently attached to the surface with (3-aminopropyl)trimethoxysilane (APTMS). Finally, in piranha solution, and subsequently amine-functionalized [39]: the sensor chip surface was first oxidized by immersion fully established offline chemical procedure was carried out [38]. Prior to each experiment, the following already success-

to validate the performance of the device as a biosensor, benchmark CRP protein detection experiments were performed with the high power setting. CRP was chosen because it is a widely used biomarker for inflammatory conditions [38]. Prior to each experiment, the following already success-

to the four different sodium chloride concentrations evaluated with low power by both light sources is shown in Fig. 2. The blue solid and the orange dashed curves correspond to the ECL and the FPL source, respectively. Both are highly corre-

Table 1. Detection Limits in RIU for All Homogeneous Refractive Index Sensing Experiments, Categorized by Optical Source and Power Level

| Optical Source | Low Power | High Power |
|----------------|-----------|------------|
| ECL            | $5 \times 10^{-7}$ | $9 \times 10^{-8}$ |
| FPL            | $1 \times 10^{-6}$ | $5 \times 10^{-7}$ |

*Each LOD represents the average value of three experiments.

200 ng/mL follow a linear trend. However, as CRP accumu-
lates on the surface, the biorecognition layer starts to saturate, and increasing concentrations yield smaller phase shifts. The corresponding mean calibration curves for CRP detection, for both the low-cost FPL and the reference ECL source, are represented in Fig. 4. The data of the cumulative introduced phase changes $\Delta \phi_{cum}$ for each experiment were fitted to the function $BC_{cum}/(D + C_{cum})$ achieving an excellent fitting ($R^2 > 0.99$ for each calibration), with $C_{cum}$ the cumulative sum of the injected CRP concentrations, and $B, D$ the fitting parameters. The dots represent the mean cumulative phase shift and the error bars the standard deviation for the calibration curve for the corresponding cumulative concentration. The high standard deviation of the sensitivity can be explained by two factors. First, for each experiment, a different chip was used, each of which had been functionalized several times before,
possibly degrading the antibody immobilization efficiency. Second, but more importantly, our functionalization strategy requires further optimization to achieve reliable homogeneous surface coverage. A worst-case sensitivity of 2 mrad/(ng/mL) and detection limit just below 300 pg/mL were extracted from the data with the reference ECL source. The mean sensitivity was $S = 3.1$ mrad/(ng/mL) with a LOD of 184 pg/mL. This is significant improvement compared to previous CRP detection assays reported in the literature [16,18,26] and highlights the potential of the coherent sensing approach. Using the low-cost FPL source, a sensitivity $S = 5.2$ mrad/(ng/mL) and an LOD of 1.9 ng/mL were observed. This moderate degradation is attributed mainly to the thermal instability of the source.

In this work, we have demonstrated the potential of coherently detected balanced MZIs for biosensing. We have demonstrated competitive detection limits, below 300 pg/mL for CRP, with a reference high performance optical source. Using a low-cost FPL, we were still able to achieve LODs below 2 ng/mL for CRP. We believe that these developments are an important step towards portable, low-cost, POC biosensor devices.

**Funding.** Horizon 2020 Framework Programme (EuroNanoMed 3-H2020 DrNanoDAII); Ministerio de Economía y Competitividad (2019/PCI 2019-2, CTQ2017-86994-R, PID2019-104293GB-I00, RD16/0006/0012, TEC2016-80718-R, UMA18-FEDERJA-007, UMA18-FEDERJA-219); H2020 Marie Skłodowska-Curie Actions (713721).

**Disclosures.** The authors declare no conflicts of interest.

**REFERENCES**

1. M. C. Cardenosa-Rubio, H. M. Robison, and R. C. Bailey, Curr. Opin. Environ. Sci. Heal. 10, 38 (2019).
2. P. Ramírez-Priego, D. Martens, A. A. Elamin, P. Soetaert, W. V. Roy, R. Vos, B. Anton, R. Bockstaele, H. Becker, M. Singh, P. Biestman, and L. M. Lechuga, ACS Sens. 3, 2079 (2018).
3. A. F. Gavela, D. G. García, J. Ramírez, and L. Lechuga, Sensors 16, 285 (2016).
4. R. Baets, A. Z. Subramanian, S. Clemmen, B. Kuyken, P. Biestman, N. L. Thomas, G. Roelkens, D.-X. Xu, P. Cheben, B. Lamontagne, and J. H. Schmid, in Optical Fiber Communication Conference (Optical Society of America, 2016), pp. 1–3.
5. B. Chocarro-Ruiz, A. Fernández-Gavela, S. Herranz, and L. M. Lechuga, Curr. Opin. Biotechnol. 45, 175 (2017).
6. J. G. Wangüemert-Pérez, A. Hadji-EHouati, A. Sánchez-Postigo, J. Leuermann, D.-X. Xu, P. Cheben, A. Ortega-Moñux, R. Halir, and I. Molina-Fernández, Opt. Laser Technol. 109, 437 (2019).
7. P. Cheben, R. Halir, J. H. Schmid, H. A. Atwater, and D. R. Smith, Nature 560, 565 (2018).
8. D. M. Kita, J. Michon, S. G. Johnson, and J. Hu, Optica 5, 1046 (2018).
9. M. Isqbal, M. A. Gleeson, B. Spaugh, F. Tybor, W. G. Gunn, M. Hochberg, T. Baehr-Jones, R. C. Bailey, and L. C. Gunn, IEEE J. Sel. Top. Quantum Electron. 16, 654 (2010).
10. R. J. J. van Gulik, B. M. de Boer, and P. J. Harmsma, IEEE J. Sel. Top. Quantum Electron. 23, 433 (2017).
11. J. Fueckiger, S. Schmidt, V. Donzella, A. Sherwali, D. M. Ratner, L. Chrostowski, and K. C. Cheung, Opt. Express 24, 15672 (2016).
12. S. Hu, Y. Zhao, K. Qin, S. T. Retterer, I. I. Kravchenko, and S. M. Weiss, ACS Photon. 1, 590 (2014).
13. Q. Liu, X. Tu, K. W. Kim, J. S. Kee, Y. Shin, K. Han, Y.-J. Yoon, G.-Q. Lo, and M. K. Park, Sens. Actuators B Chem. 188, 681 (2013).
14. A. D. Falco, L. O’Faolain, and T. F. Krauss, Appl. Phys. Lett. 94, 063503 (2009).
15. T. Claes, W. Bogaerts, and P. Biestman, Proc. SPIE 8099, 80990R (2011).
16. E. Luan, H. Shoman, D. Ratner, K. Cheung, and L. Chrostowski, Sensors 18, 3519 (2018).
17. D. Martens and P. Biestman, Nanophotonics 6, 703 (2017).
18. M. S. Luchansky, A. L. Washburn, M. S. McClellan, and R. C. Bailey, Lab Chip 11, 2042 (2011).
19. D. Martens, P. Ramírez-Priego, M. S. Murib, A. A. Elamin, A. B. González-Guerrero, M. Stehr, F. Jonas, B. Anton, N. Hlawatsch, P. Soetaert, R. Vos, A. Stassen, S. Severi, W. V. Roy, R. Bockstaele, H. Becker, M. Singh, L. M. Lechuga, and P. Biestman, Anal. Methods 10, 3066 (2018).
20. K. Schmitt, B. Schirmer, C. Hoffmann, A. Brandenburg, and P. Meyrueis, Biosens. Bioelectron. 22, 2591 (2007).
21. S. Danto, Opt. Pura y Appl. 45, 87 (2012).
22. K. Zinoviev, L. G. Carrascosa, J. S. del Rio, B. Sepúlveda, C. Dominguez, and L. M. Lechuga, Adv. Opt. Technol. 2008, 1 (2008).
23. N. Skivesen, A. Tétu, M. Kristensen, J. Kjems, L. H. Frandsen, and P. I. Borel, Opt. Express 15, 3169 (2007).
24. J. Leuermann, A. Fernández-Gavela, A. Torres-Cubillo, S. Postigo, A. Sánchez-Postigo, L. M. Lechuga, R. Halir, and I. Molina-Fernández, Sensors 19, 3671 (2019).
25. A. Denmore, D.-X. Xu, S. Janz, P. Waldron, T. Mischki, G. Lopinski, A. Delage, J. Lapointe, P. Cheben, B. Lamontagne, and J. H. Schmid, Opt. Lett. 33, 596 (2008).
26. A. Psaroul, A. Botisalas, A. Salapatas, G. Stefanitsis, D. Nikita, G. Jobst, N. Chaniotakis, D. Goustouridis, E. Makarona, P. S. Petrou, I. Raptis, K. Misiakos, and S. E. Kakabakos, Talanta 165, 458 (2017).
27. S. Dante, D. Duval, B. Sepúlveda, A. B. González-Guerrero, J. R. Sendra, and L. M. Lechuga, Opt. Express 20, 7195 (2012).
28. A. B. González-Guerrero, J. Maldonado, S. Herranz, and L. M. Lechuga, Anal. Methods 8, 8380 (2016).
29. Y. E. Marin, V. Toccafondo, P. Velha, Y. Jeong, S. Scarano, A. Nottola, S. Tirelli, H. P. Jeon, M. E. Minunni, F. D. Pasquale, and C. J. Oton, Proc. SPIE 10510, 1051005 (2018).
30. B. Luff, J. Wilkinson, J. Piehler, U. Hollenbach, J. Ingenhoff, and N. Fabricius, J. Lightwave Technol. 16, 583 (1998).
31. R. Halir, L. Vivien, X. L. Roux, D.-X. Xu, and P. Cheben, IEEE Photon. J. 5, 6800906 (2013).
32. J. Milvich, D. Kohler, W. Freude, and C. Koos, in IEEE Photonics Conference (IPC) (IEEE, 2017).
33. M. Knoerzer, C. Szyszek, G. Ren, C. S. Huertas, S. Palmer, P. Tang, T. G. Nguyen, L. Bui, A. Boes, and A. Mitchell, Opt. Express 27, 21532 (2019).
34. I. Molina-Fernández, J. Leuermann, A. Ortega-Moñux, J. G. Wangüemert-Pérez, and R. Halir, Opt. Express 27, 12616 (2019).
35. P. J. Reyes-Iglesias, I. Molina-Fernández, A. Moscoso-Mártil, and A. Ortega-Moñux, Opt. Express 20, 5725 (2012).
36. VLC Photonics. Available online (https://www.vlcphotonics.com/).
37. J. E. Saunders, C. Sanders, H. Chen, and H.-P. Loock, Appl. Opt. 55, 947 (2016).
38. N. R. Sproston and J. J. Ashworth, Front. Immunol. 9, 754 (2018).
39. H. M. Robison and R. C. Bailey, Curr. Protoc. Chem. Biol. 9, 158 (2017).