PROCEEDINGS OF SPIE

SPIEDigitalLibrary.org/conference-proceedings-of-spie

Real time and label-free detection of C-reactive protein in serum by long period grating in double cladding fiber

Esposito, Flavio, Sansone, Lucia, Srivastava, Anubhav, Baldini, Francesco, Campopiano, Stefania, et al.

Flavio Esposito, Lucia Sansone, Anubhav Srivastava, Francesco Baldini, Stefania Campopiano, Francesco Chiavaioli, Michele Giordano, Ambra Giannetti, Agostino Iadicicco, "Real time and label-free detection of C-reactive protein in serum by long period grating in double cladding fiber," Proc. SPIE 11772, Optical Sensors 2021, 117720Q (18 April 2021); doi: 10.1117/12.2589969

SPIE.

Event: SPIE Optics + Optoelectronics, 2021, Online Only

Downloaded From: https://www.spiedigitallibrary.org/conference-proceedings-of-spie on 19 Apr 2021 Terms of Use: https://www.spiedigitallibrary.org/terms-of-use

Real time and label-free detection of C-reactive protein in serum by long period grating in double cladding fiber

Flavio Esposito[a], Lucia Sansone[b], Anubhav Srivastava[a], Francesco Baldini[c], Stefania Campopiano[a]*, Francesco Chiavaioli[c], Michele Giordano[b], Ambra Giannetti[c], Agostino Iadicicco[a]
[a]Department of Engineering, University of Naples "Parthenope", 80143 Napoli, Italy;
[b]Institute for Polymers, Composites and Biomaterials, National Research Council of Italy (CNR), 80055 Portici, Italy;
[c]Institute of Applied Physics "Nello Carrara", National Research Council of Italy (CNR), 50019 Sesto Fiorentino, Italy

ABSTRACT

Here, we present a novel label-free biosensor based on fiber optic technology which was tested for the detection of a serum inflammatory marker, the C-reactive protein (CRP). The biosensor is based on a long period grating (LPG) inscribed in a double cladding fiber (DCF) having a W-type refractive index profile. Such DCF fiber permits to tune the sensor working point to the so-called mode transition region through etching of the fiber outer cladding. Therefore, a significant enhancement of the refractive index sensitivity, as well as visibility of the grating spectral features were attained since the mode transition was induced in all-silica fiber structure. Subsequently, the so-prepared LPG was coated with a nano-scale layer of graphene oxide, providing carboxylic functional groups for the covalent immobilization of the biological recognition element for the CRP. As a result, a remarkable limit of detection of 320 pg/mL and a large working range of clinical relevance (0.002-100 µg/mL) were achieved during the real time detection of CRP in human serum.

Keywords: biosensors, C-reactive protein, graphene oxide, fiber optic sensors, long period gratings.

1. INTRODUCTION

Long period gratings (LPG) are excellent fiber optic refractometers which are fabricated through a periodic perturbation of the refractive index (and sometimes geometry) of the fiber using a period of hundreds of micrometers. Such structure produces a resonant coupling between the core mode and co-propagating cladding modes. The transmitted spectrum of the sensor exhibits several attenuation bands associated to the coupling with different cladding modes, which are characterized by an intrinsic sensitivity upon the refractive index of the medium surrounding the fiber (surrounding refractive index, SRI)[1]. Several approaches have been investigated in order to achieve SRI sensitivities up to thousands nm/RIU (RIU = refractive index unit), making of the LPG one of the most promising fiber technological platform to be employed in several biosensing applications[2].

Consequently, different configurations for the detection of various species can be highlighted. For example, immunoglobulins were detected down to few ng/mL by TiO_2 coated LPG working in mode transition[3]. Streptavidin was detected in the ng/mL range by TaO_x-coated LPG working near phase-matching turnaround point in [4]. *Escherichia coli* bacteria detection has been widely investigated leading to the detection of few CFU/mL[5]. The detection of viruses, as Norovirus[6] and AIV H5N1[7], was also demonstrated. Finally, the detection of drugs and human health related compounds, such as fentanyl[8], hemoglobin[9], thyroglobulin[10], and neuropsin[11], was obtained with interesting results.

In this work, we report a novel configuration based on an LPG written in a double cladding fiber (DCF) with W-shaped refractive index profile. The performances of the biosensor were verified using the C-reactive protein (CRP) as exemplary target. CRP is an important serum inflammatory marker[12], whose levels have been also put under attention recently in patients affected by COVID-19 disease[13]. This novel LPG configuration is characterized by high sensitivity and good visibility of the grating spectral features, due to the possibility to induce mode transition by light chemical etching of the DCF outer cladding[14]. Moreover, the device was coated with a thin layer of graphene oxide (GO) providing carboxylic

* corresponding author: stefania.campopiano@uniparthenope.it

Optical Sensors 2021, edited by Francesco Baldini, Jiri Homola, Robert A. Lieberman, Proc. of SPIE
Vol. 11772, 117720Q · © 2021 SPIE · CCC code: 0277-786X/21/$21 · doi: 10.1117/12.2589969

Downloaded From: https://www.spiedigitallibrary.org/conference-proceedings-of-spie on 19 Apr 2021
Terms of Use: https://www.spiedigitallibrary.org/terms-of-use

groups for the immobilization of the biological recognition element (BRE) for the CRP. Finally, the device was tested for the detection of CRP concentrations of clinical relevance in samples of human serum.

2. SENSOR DEVELOPMENT

This section deals with the fabrication of the highly sensitive LPG sensor fabricated in DCF with GO layer, whose schematic picture is reported in Figure 1(a). The sensor was successively functionalized in order to immobilize the BRE for the detection of the target, CRP.

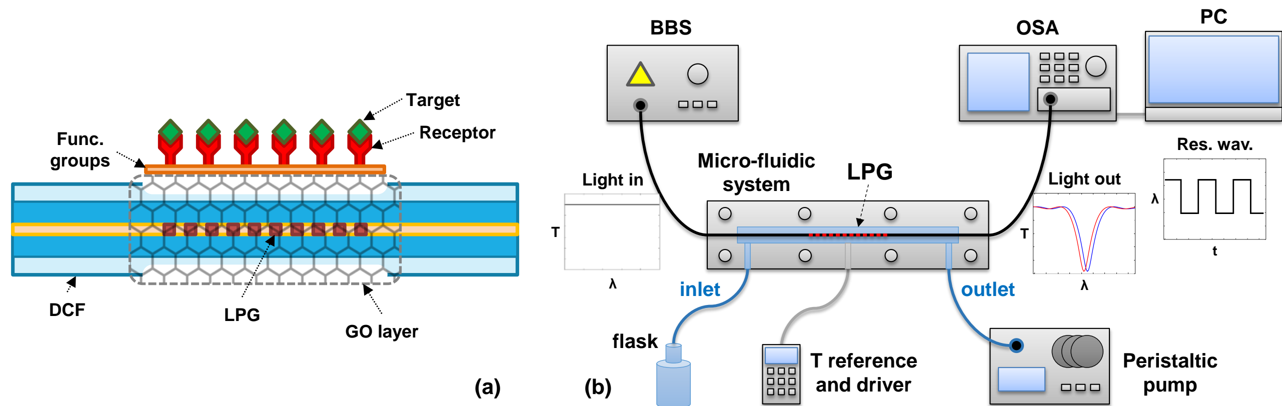

Figure 1. (a) Schematic of the LPG-based biosensor in etched DCF with GO layer. (b) Schematic of the microfluidic and optoelectronic measurement platform for the LPG. BBS, broadband source. OSA, optical spectrum analyzer.

2.1 Testing platform

The platform for testing of the biosensor is reported in Figure 1(b). It consists of a microfluidic system made of an aluminum block with a channel having a length of 40 mm and a section 1 mm^2 (40 µL volume) to host the sensor. The block is sealed with a PMMA cover that is tightened using eight screws. The cover presents inlet and outlet tubes, connected to a peristaltic pump for the management of the different solutions to be injected into the cell. The system was thermally stabilized by using Peltier cells and temperature controllers. Concerning the optoelectronic measurement system, the fiber with the sensor was illuminated by using a broadband light source (range 1300-1700 nm) while the light transmitted from the device was acquired by using an optical spectrum analyzer (OSA). The acquired raw spectral data were further processed in real-time by using a custom LabView software in order to retrieve the resonance wavelength values. More details regarding the whole system can be also found in [3].

2.2 Graphene oxide preparation

Graphene oxide was synthetized by using the Hummers' method, involving the chemical exfoliation of graphite (Asbury Carbons, USA), as reported in [15]. GO was selected due to its attractive properties for biosensing, as the presence of carboxylic functional groups. The resulting 2 mg/mL GO water dispersion was coated in form of few nm thin layer onto the LPG fiber surface, through dip coating procedure at 100 mm/min withdrawal rate.

2.3 LPG sensor fabrication and characterization

A double cladding fiber with W-shaped refractive index profile was selected for the purpose of the work, as we recently demonstrated the possibility to induce mode transition phenomenon by controlled etching of the outer cladding of such fiber[14]. The model S1310 manufactured by Nufern was a good candidate for the purpose, since it presents core and outer cladding diameters matching those of standard fiber and an inner cladding with diameter of around 95 µm. Moreover, the core and outer cladding regions are pure-silica while the inner cladding is doped with Fluorine, which lowers the silica refractive index and makes the RI profile as W-shaped. Due to the pure-silica core nature of the fiber, we fabricated the LPG in this fiber by using the electric arc discharge method, implemented in a well-settled platform at our laboratory. Additional details and relevant results regarding this technique can be found in [16–18].

Downloaded From: https://www.spiedigitallibrary.org/conference-proceedings-of-spie on 19 Apr 2021
Terms of Use: https://www.spiedigitallibrary.org/terms-of-use

The design of the device was supported by numerical simulations based on the model reported in [15,19]. Specifically, the period of the LPG was selected equal to Λ = 400 µm to have the coupling with the 6th order cladding mode around 1375 nm after fabrication, as reported in Figure 2(a) with black line. To induce the mode transition and enhance the sensitivity of this LPG, we considered the approach reported in [14], consisting of etching the fiber outer cladding. Specifically, it was done by chemical process with 24% (v/v) HF acid solution. Figure 2(b) and 2(c) illustrate the resonance wavelengths and resonance amplitudes of the LPG related to the 4th, 5th, and 6th order cladding modes during the etching process (i.e., diameter reduction), reported with markers. The main effects were red shifting of the resonance wavelengths and increasing in resonance peak depths. Moreover, it is worth highlighting the shift rate was maximum when mode transitions occurred, e.g., for the 5th mode they are visible for diameter values around 116 µm and 107 µm. In the same figures, numerical data were also reported with solid lines showing a satisfactory agreement with experimental values. The etching process was terminated when the diameter reached 107 µm, i.e., at 2nd transition. The related spectra are reported in Figure 2(a): when the grating was in air the attenuation band was close to the limit of our interrogation window (blue dashed line), anyway when the grating was immersed in water a clear resonance peak was visible at 1561 nm with depth of 15 dB (orange dashed line). Subsequently, the LPG was coated with a thin layer of GO (details in section 2.2), which shifted the same resonance to 1533 nm with 10 dB depth (orange line). Finally, the grating was characterized towards SRI changes, exhibiting a sensitivity of -1035 nm/RIU in the range 1.33-1.37, which was a 13x increase due to etching and mode transition.

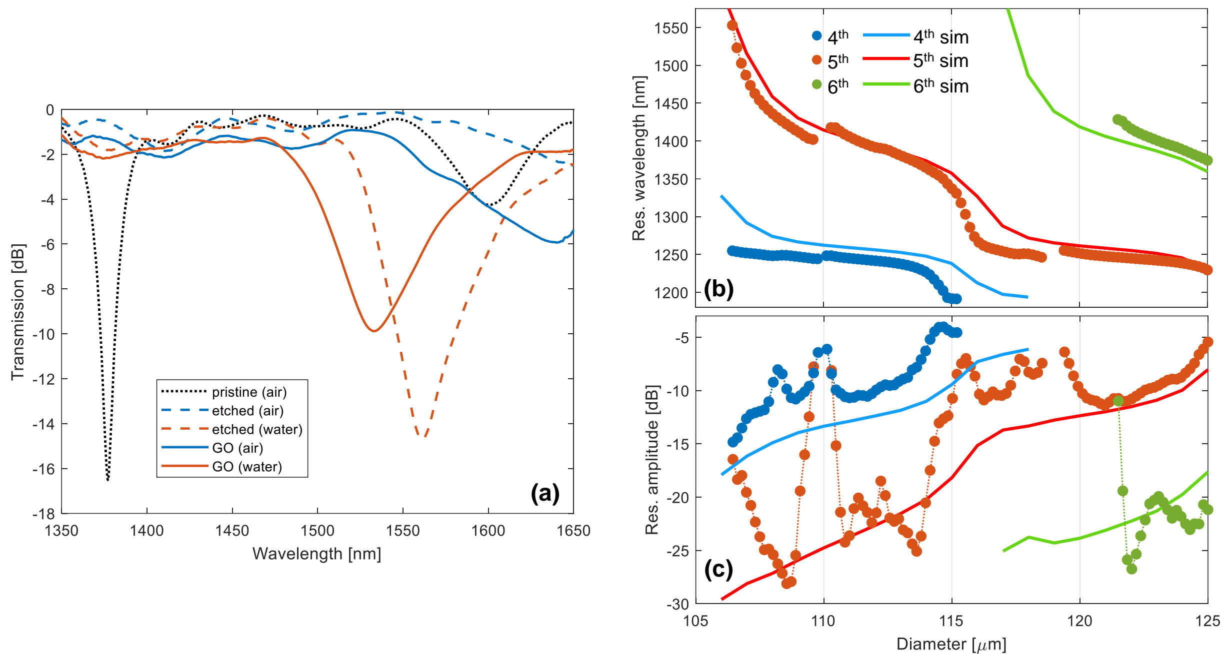

Figure 2. Fabrication of the LPG sensor in DCF: (a) spectral results of bare grating, after etching and after GO deposition (air and water); Resonance wavelength (b) and resonance depth (c) changes during etching process (markers for experimental data and solid lines for simulations).

3. RESULTS

Here, the functionalization and testing procedures of the biosensor are reported, as well as the results achieved during the detection of CRP in human serum. The procedures were performed using the setup in Figure 1(b).

3.1 Functionalization

The resonance wavelength of the sensor versus time during the functionalization procedure, following a well-assessed protocol by the authors[20], is reported in Figure 3. Specifically, the GO provides the carboxylic functional groups used for the covalent immobilization of the capture antibody, without the need for any additional functional layer (i.e., silane, polymer, etc.). These groups were activated using an EDC/NHS (200/50 mM) solution for 20 minutes. Subsequently, the BRE for the CRP, i.e., Anti-CRP (monoclonal antibody clone C5, 500 µg/mL), was covalently bound to the sensor surface

Downloaded From: https://www.spiedigitallibrary.org/conference-proceedings-of-spie on 19 Apr 2021
Terms of Use: https://www.spiedigitallibrary.org/terms-of-use

for about 1 h using a flow rate of 15 μL/min. The unbounded antibodies were removed by washing in phosphate buffered saline (PBS, 40 mM pH 7.4) for 10 minutes at a faster flow rate of 150 μL/min. Finally, the sensor surface was passivated with 1% (w/v) bovine serum albumin (BSA) in PBS for 20 minutes. Such step is useful to block the remaining activated carboxylic groups and prevent non-specific adsorption. Finally, the sensor was washed in PBS.

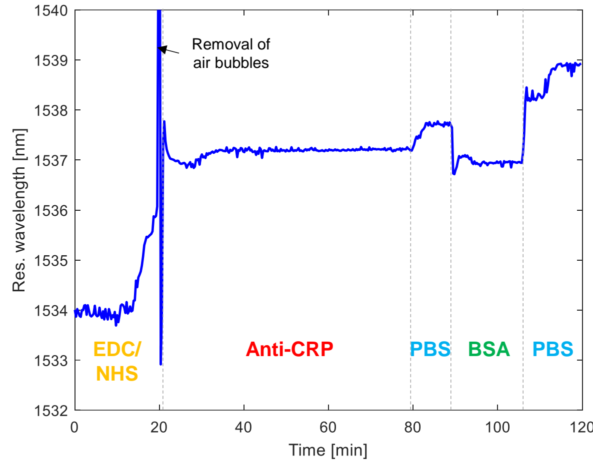

Figure 3. Resonance wavelength of the LPG during the functionalization procedure for the immobilization of the biological recognition element, Anti-CRP.

3.2 Detection

The performances of the biosensor were evaluated by detecting increasing concentrations of CRP (in range 0.01-100 μg/mL) in human serum (diluted 1:10 (v/v) in PBS). Each incubation step lasted around 30 minutes (at 25 μL/min flow rate) and was followed by a washing step in PBS for 15 minutes. The resonance wavelength of the LPG versus time during the test is reported in Figure 4(a), where it can be clearly observed that the resonance blue shifted when washing in PBS after the incubation of each CRP concentration.

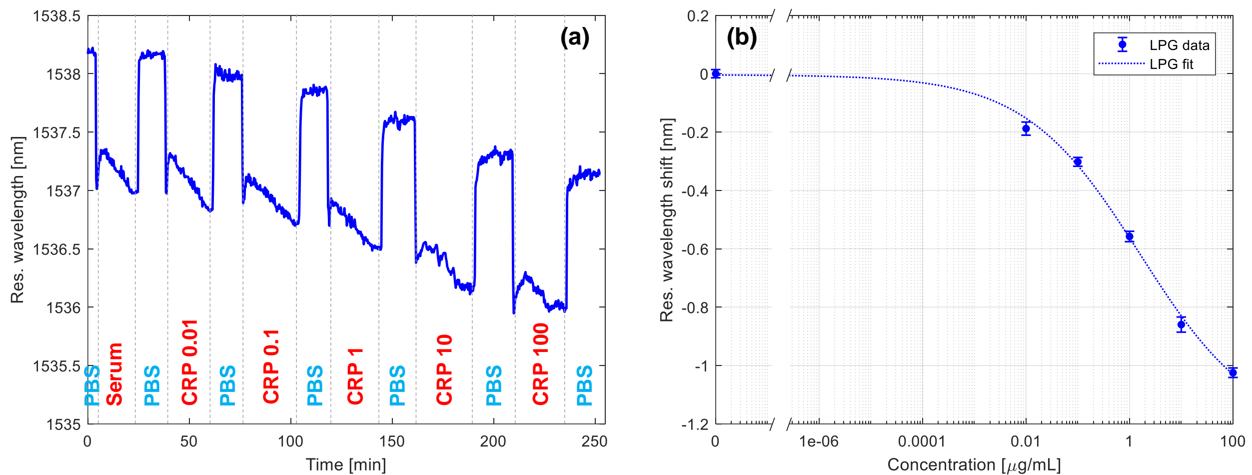

Figure 4. (a) Resonance wavelength of the LPG during testing with CRP (concentrations reported as μg/mL). (b) Calibration curve of the LPG with fitting of the experimental data (markers) using the Logistic function (line).

The calibration curve of the biosensor was obtained and reported in Figure 4(b). Each point accounts for the shift in PBS after the incubation of each CRP concentration, with respect to the value at zero concentration of the analyte. Moreover,

Downloaded From: https://www.spiedigitallibrary.org/conference-proceedings-of-spie on 19 Apr 2021
Terms of Use: https://www.spiedigitallibrary.org/terms-of-use

each point is reported in terms of mean value and standard deviation (errorbars) considering 20 subsequent measurements. In the same figure, fitting of the experimental data by using Logistic function was also reported[21]. The overall wavelength shift in the considered range of CRP concentrations was equal to -1.02 nm. The limit of detection (LOD), defined as the sensor signal related to zero concentration of the analyte plus three times its standard deviation[22], was equal to 320 pg/mL and is one of the lowest reported so far in fiber biosensors[23]. Finally, the biosensor was able to cover a large working range (0.002-100 µg/mL) of clinical relevance for CRP[12].

4. CONCLUSION

Here, we demonstrated a new fiber biosensor configuration based on LPG fabricated in DCF with W-type refractive index profile. Significant enhancement of the SRI sensitivity, as well as high visibility of the attenuation bands, were achieved through selection of the sensor working point in mode transition region, acting on the DCF outer cladding by chemical etching. The device does not require additional coatings on the fiber to enhance the sensitivity (e.g., polymers or oxides) and presents an all-silica structure with further advantages of stability and long-term operation. For functionalization, a nanometric GO coating was applied to provide carboxylic groups for the grafting of the capture antibody. The biosensor was tested for the detection of CRP concentrations in human serum, obtaining a very low LOD of 320 pg/mL together with the possibility to operate in a large working range of clinical interest (0.002-100 µg/mL).

REFERENCES

[1] Chiavaioli, F., Baldini, F., Tombelli, S., Trono, C. and Giannetti, A., "Biosensing with optical fiber gratings," Nanophotonics 6(4), 663–679 (2017).

[2] Esposito, F., Srivastava, A., Sansone, L., Giordano, M., Campopiano, S. and Iadicicco, A., "Label-free Biosensors based on Long Period Fiber Gratings: A Review," IEEE Sens. J. (2020).

[3] Chiavaioli, F., Biswas, P., Trono, C., Jana, S., Bandyopadhyay, S., Basumallick, N., Giannetti, A., Tombelli, S., Bera, S., Mallick, A. and Baldini, F., "Sol–Gel-Based Titania–Silica Thin Film Overlay for Long Period Fiber Grating-Based Biosensors," Anal. Chem. 87(24), 12024–12031 (2015).

[4] Piestrzyńska, M., Dominik, M., Kosiel, K., Janczuk-Richter, M., Szot-Karpińska, K., Brzozowska, E., Shao, L., Niedziółka-Jonsson, J., Bock, W. J. and Śmietana, M., "Ultrasensitive tantalum oxide nano-coated long-period gratings for detection of various biological targets," Biosens. Bioelectron. 133, 8–15 (2019).

[5] Kaushik, S., Tiwari, U., Nilima, Prashar, S., Das, B. and Sinha, R. K., "Label-free detection of Escherichia coli bacteria by cascaded chirped long period gratings immunosensor," Rev. Sci. Instrum. 90(2), 025003 (2019).

[6] Janczuk-Richter, M., Gromadzka, B., Richter, Ł., Panasiuk, M., Zimmer, K., Mikulic, P., Bock, W. J., Maćkowski, S., Śmietana, M. and Niedziółka Jönsson, J., "Immunosensor Based on Long-Period Fiber Gratings for Detection of Viruses Causing Gastroenteritis," Sensors 20(3), 813 (2020).

[7] Luo, B., Liu, Z., Wang, X., Shi, S., Zhong, N., Ma, P., Wu, S., Wu, D., Zhao, M. and Liang, W., "Dual-peak long period fiber grating coated with graphene oxide for label-free and specific assays of H5N1 virus," J. Biophotonics 14(1) (2021).

[8] Liu, L., Grillo, F., Canfarotta, F., Whitcombe, M., Morgan, S. P., Piletsky, S., Correia, R., He, C., Norris, A. and Korposh, S., "Carboxyl-fentanyl detection using optical fibre grating-based sensors functionalised with molecularly imprinted nanoparticles," Biosens. Bioelectron. 177, 113002 (2021).

[9] Liu, C., Xu, B. J., Zhou, L., Sun, Z., Mao, H. J., Zhao, J. L., Zhang, L. and Chen, X., "Graphene oxide functionalized long period fiber grating for highly sensitive hemoglobin detection," Sensors Actuators B Chem. 261, 91–96 (2018).

[10] Quero, G., Consales, M., Severino, R., Vaiano, P., Boniello, A., Sandomenico, A., Ruvo, M., Borriello, A., Diodato, L., Zuppolini, S., Giordano, M., Nettore, I. C., Mazzarella, C., Colao, A., Macchia, P. E., Santorelli, F., Cutolo, A. and Cusano, A., "Long period fiber grating nano-optrode for cancer biomarker detection," Biosens. Bioelectron. 80, 590–600 (2016).

[11] Delgado-Macuil, R., González-León, K. and Beltrán-Pérez, G., "Neuropsin (Opn5) detection in the brain tissue of a murine model using long period fiber grating (LPFG)," Opt. Laser Technol. 139, 106972 (2021).

[12] Sproston, N. R. and Ashworth, J. J., "Role of C-Reactive Protein at Sites of Inflammation and Infection," Front. Immunol. 9 (2018).

[13] Liu, F., Li, L., Xu, M., Wu, J., Luo, D., Zhu, Y., Li, B., Song, X. and Zhou, X., "Prognostic value of interleukin-6, C-reactive protein, and procalcitonin in patients with COVID-19," J. Clin. Virol. 127, 104370 (2020).

Downloaded From: https://www.spiedigitallibrary.org/conference-proceedings-of-spie on 19 Apr 2021
Terms of Use: https://www.spiedigitallibrary.org/terms-of-use

[14] Esposito, F., Srivastava, A., Sansone, L., Giordano, M., Campopiano, S. and Iadicicco, A., "Sensitivity Enhancement in Long Period Gratings by Mode Transition in Uncoated Double Cladding Fibers," IEEE Sens. J. 20(1), 234–241 (2020).
[15] Esposito, F., Sansone, L., Taddei, C., Campopiano, S., Giordano, M. and Iadicicco, A., "Ultrasensitive biosensor based on long period grating coated with polycarbonate-graphene oxide multilayer," Sensors Actuators B Chem. 274, 517–526 (2018).
[16] Esposito, F., Ranjan, R., Campopiano, S. and Iadicicco, A., "Arc-Induced Long Period Gratings from Standard to Polarization-Maintaining and Photonic Crystal Fibers," Sensors 18(3), 918 (2018).
[17] Esposito, F., Srivastava, A., Iadicicco, A. and Campopiano, S., "Multi-parameter sensor based on single Long Period Grating in Panda fiber for the simultaneous measurement of SRI, temperature and strain," Opt. Laser Technol. 113, 198–203 (2019).
[18] Esposito, F., Campopiano, S. and Iadicicco, A., "Arc-Induced Long Period Gratings in Erbium-Doped Fiber," IEEE Photonics J. 11(1), 1–8 (2019).
[19] Esposito, F., Zotti, A., Ranjan, R., Zuppolini, S., Borriello, A., Campopiano, S., Zarrelli, M. and Iadicicco, A., "Single-Ended Long Period Fiber Grating Coated With Polystyrene Thin Film for Butane Gas Sensing," J. Light. Technol. 36(3), 825–832 (2018).
[20] Aray, A., Chiavaioli, F., Arjmand, M., Trono, C., Tombelli, S., Giannetti, A., Cennamo, N., Soltanolkotabi, M., Zeni, L. and Baldini, F., "SPR-based plastic optical fibre biosensor for the detection of C-reactive protein in serum," J. Biophotonics 9(10), 1077–1084 (2016).
[21] Esposito, F., Sansone, L., Srivastava, A., Baldini, F., Campopiano, S., Chiavaioli, F., Giordano, M., Giannetti, A. and Iadicicco, A., "Long period grating in double cladding fiber coated with graphene oxide as high-performance optical platform for biosensing," Biosens. Bioelectron. 172, 112747 (2021).
[22] Chiavaioli, F., Gouveia, C., Jorge, P. and Baldini, F., "Towards a Uniform Metrological Assessment of Grating-Based Optical Fiber Sensors: From Refractometers to Biosensors," Biosensors 7(2), 23 (2017).
[23] Salvo, P., Dini, V., Kirchhain, A., Janowska, A., Oranges, T., Chiricozzi, A., Lomonaco, T., Di Francesco, F. and Romanelli, M., "Sensors and Biosensors for C-Reactive Protein, Temperature and pH, and Their Applications for Monitoring Wound Healing: A Review," Sensors 17(12), 2952 (2017).

Downloaded From: https://www.spiedigitallibrary.org/conference-proceedings-of-spie on 19 Apr 2021
Terms of Use: https://www.spiedigitallibrary.org/terms-of-use