Manufacturing and Optimization of Sol-gel-based TiO_2-SiO_2 thin Films as High Refractive Index Overlays for Long Period Grating-based Biosensing

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Abstract: The manufacturing procedure and the optimization of high refractive index overlays for long period grating-based sensors are reported. The overlay consists of a sol-gel-based TiO_2-SiO_2 thin film. By carefully tuning the overlay thickness and refractive index, it is possible to bring the LPG in the so-called transition mode working region, and to optimize and maximize the LPG sensing performances. LPGs are here characterized as optical refractometers, and, after a suitable functionalization of the sol-gel coated fiber surface, as biosensors performing an IgG/anti-IgG bioassay.

1 INTRODUCTION

The optical label-free detection of chemical compounds or biological species is based on the modulation of the refractive index (RI) occurring at the liquid/solid sensor interface, where the biochemical interaction between the sensing layer and the analyte of interest takes place (Fan, 2008). Generally, the RI modification modulates the evanescent wave component of the total optical power. The literature accounts for several optical configurations, mainly based on surface plasmon resonance (SPR) (Homola, 2008), on localized SPR (Willets and Van Duyne, 2007), on interferometry (Queirós et al., 2011) or on optical resonance-based structures (Kindt and Bailey, 2013). The substrate on which the sensing layer is deposited is generally an optical waveguide that allows both the interaction light-analyte and the transport of the optical signal.

Recently, optical fiber long period gratings (LPGs) have been proposed as a promising tool for label-free biosensors (Chiavaioli et al., 2015; Baldini et al., 2012). They exploit the typical peculiarities and advantages of optical fiber sensors, such as compactness, lightweight, intrinsic miniaturization, high compatibility with optoelectronic devices, remote measurement capabilities and multiplexing thanks to the spectral modulation of the signal.

An LPG is produced by inducing periodic RI perturbations in the core of a single-mode optical fiber. When the light normally guided into the fiber core interacts with the grating, the fundamental core mode couples to co-propagating cladding modes at well-defined resonance wavelengths (LPG λ_{res}) which satisfy the phase-matching condition expressed by the characteristic equation of LPGs (Erdogan, 1997):

$$\lambda_{res(m)} = (n_{eff,core} - n_{eff,clad(m)}) \Lambda \quad (1)$$

where Λ is the grating period (usually in the range from 100 μm to 600 μm), $n_{eff,core}$ and $n_{eff,clad(m)}$ represent the effective RIs of the fundamental core mode (i.e. LP_{01}) and the m-th cladding mode (i.e. LP_{0m}), respectively. Therefore, the transmission spectrum of an LPG will be characterized by one or more attenuation bands (Figure 1), in which the minimum of each band corresponds to the coupling with a selective m-th cladding mode. The cladding refractive index $n_{eff,clad}$ will therefore depend on the RI of the surrounding medium (n_{sur}) and this feature allows to use LPGs as RI sensors.

The optical mechanism of LPG-based biosensing can be explained considering that the binding interactions on the fiber surface produce a change of

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the $n_{eff,clad}$ and, consequently, a shift of the LPG λ_{res}.

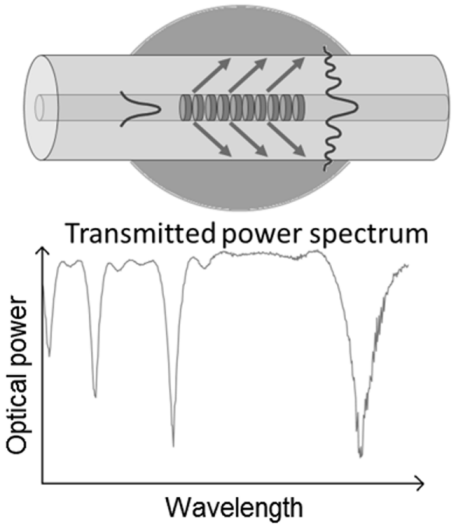

Figure 1: LPG working principle (top) and a typical LPG transmission spectrum (bottom).

The best RI sensitivity of a standard LPG is reached when n_{sur} is close to the RI of fiber cladding (i.e. 1.44–1.46 RIU, RI units), thus quite far from the RI of water or aqueous solutions (i.e. 1.33–1.34 RIU) (Patrick et al., 1998), in which practically all the biochemical reactions occur. To overcome this problem, the literature accounts for a general approach that consists in the deposition over the fiber of a nm-thick film overlay of RI higher than the cladding RI (Del Villar et al., 2005). In this way the RI range in correspondence of the maximum sensitivity can be adjusted around 1.33 RIU. In this case, values of RI sensitivity of the order of thousands of nm RIU^{-1} can be experimentally achieved (Pilla et al., 2012).

Sol-gel-derived coatings have been used for years to enhance the performance of evanescent wave sensors. The sensor manufacturing is quite simple thanks to the dip-coating (DC) technique (MacCraith, 1993), and the possibility of doping sol-gel coatings with high refractive index materials provides the chance for implementing high RI (HRI) film overlays (Smietana et al., 2015; Davies et al., 2009).

In the present paper, the manufacturing procedure and the optimization of a sol-gel-based TiO_2-SiO_2 thin film as HRI overlay for LPG-based biosensing applications were investigated. The HRI overlay, deposited by means of the DC technique along the sensing portion containing the LPG, improved the sensor performance in terms of volume RI sensitivity (as optical refractometer), and of bio-layer formation sensitivity (as biosensor). An IgG/anti-IgG assay implemented on the fiber sol-gel coated sensing region was used to characterize the proposed LPG-based device as a feasible and effective biosensor.

2 MATERIALS AND METHODS

2.1 LPG Fabrication

LPGs were manufactured in a standard single-mode fiber (SMF28e of Corning Inc.) using point-to-point technique (Hill and Meltz, 1997) by means of a KrF pulsed Excimer laser (Braggstar-500, TUI laser, Germany, λ = 248 nm, repetition rate = 200 Hz, pulse energy = 10 mJ) with Λ = 342 μm. The fiber was previously hydrogen loaded at a pressure of 10.3 MPa and a temperature of 100 °C for 48 hours to enhance the photosensitivity. After the inscription, the fiber was annealed at 150–170 °C for about six hours for the stabilization of the optical characteristics of LPGs. In order to deposit a glassy coating (e.g. a sol-gel based thin film) over an optical fiber containing an LPG, the grating region must be heated at around 450 °C during sintering the gel film. In general, at that temperature, the LPG attenuation bands almost reduce to zero. To overcome this problem, during the grating manufacturing, the cladding mode of interest (LP_{07}) was intentionally overcoupled with a greater coupling strength to achieve stable LPGs when heated (Biswas, 2014). The LPGs were stabilized at 550 °C and then gratings with a λ_{res} of roughly 1590 nm, with a visibility of the attenuation band of roughly -12 dB, and with a full width at half maximum (FWHM) bandwidth of roughly 20 nm were attained.

2.2 Sol-gel Preparation and Deposition

The preparation of the sol-gel was performed according to previous literature (Chiavaioli et al., 2015). Briefly, for the silica sol preparation, tetraethyl orthosilicate (TEOS, reagent grade, 98%) was used at a molar ratio water:TEOS:HCl of 2:1:0.001; for the titania sol, tetraisopropylorthotitanate (TIOT, 97%) was used at a molar ratio acetyl acetone:TIOT of 1:2. The two sols were mixed by stirring for about 4 hours and then kept to age for additional 24 hours. The Ti:Si ratio and the total equivalent oxide weight percentage (wt.%) were 1:1 and 7.3, respectively. To obtain a thicker film, the viscosity of the sol was increased from 3.2 mPa·s up to 27 mPa·s by controlling the evaporation of the solvents through the warming of the sol. Afterwards, the LPG was heated in a furnace in order to allow the oxide formation in the deposited

film. At the end of the process, the attenuation band related to the LP_{07} cladding mode was recorded both in air and in phosphate buffered saline (PBS) solution.

Considering the Ti:Si volumetric ratio (1:1), a layer RI of ~1.7 RIU can be estimated as the mean value between the titania RI (i.e. 1.91–1.96 RIU) and silica RI (i.e. 1.42–1.44 RIU) (Davies et al., 2015). To optimize the sensor performance and to reach the optimum overlay thickness (OOT), the film thickness was varied by changing both the sol viscosity during the sol preparation and the withdrawal speed during the film deposition. A single deposition step was found to be good enough to move the selected cladding mode to its transition region (Cusano et al., 2006).

During the heat treatment, the temperature of the furnace was increased from 26 °C to 450 °C at a rate of about 1.2 °C min^{-1} and then the LPG was kept at 450 °C for about 2.5 hours. Later, the furnace temperature was slowly cooled down with the same rate of 1.2 °C min^{-1} in order to avoid any crack due to thermal shock. At the end of the process, the LPG wavelength of LP_{07} mode was measured in air and in phosphate buffered saline (PBS) solution.

2.3 Experimental Setup

The experimental setup is detailed in Figure 2. The flow-cell consists of two parts (Trono et al., 2011): the upper one is a 4 mm thick PMMA transparent layer, and the bottom one is a 6 mm thick aluminium layer placed in thermal contact with a thermo electric cooler (TEC) element. The flow cell is 80 mm long, 15 mm wide, and 10 mm high, and the flow-channel total volume is roughly 50 μL. The temperature stabilization section of the flow cell makes use of a thermistor, inserted into the aluminium bottom part as close as possible to the flow channel, which acts as a feedback element on the Peltier elements that are driven by a suitable controller (ILX Lightwave LDC-3722B TEC controller). The optical fiber containing the LPG is glued at both the edges of the flow cell and a thermocouple (Lutron TM-917) records the temperature of the sensing environment during the measurements. Each sample contained into the flask is pumped inside the flow cell by means of a peristaltic pump (Gilson Minipuls 3). The light is launched by a broadband superluminescent diode (SLD) INPHENIX IPSDD1503. The transmitted spectrum is acquired by an optical spectrum analyzer (OSA) Anritsu MS9030A – MS9701B (0.1 nm spectral resolution).

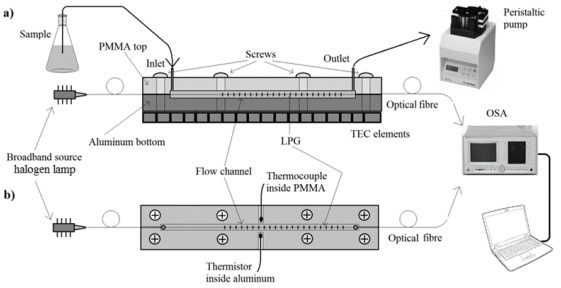

Figure 2: schematic view of the experimental setup. Longitudinal cross section (a) and top view (b) of the flow-cell.

2.4 Data Processing

The optical bandwidth of the OSA is set at 20 nm with the central wavelength roughly corresponding to the expected LPG λ_{res}. After recording the spectrum, the procedure firstly evaluates the starting LPG λ_{res} that is the wavelength corresponding to the minimum of the data set; then the fitting using the Lorentzian function is carried out (normally a correlation greater than 0.996 can be obtained) and provides the final LPG λ_{res} with an error of 7 pm – 8 pm. The whole procedure is performed every 20 s, which is the sensing system acquisition time. For each experimental point, the minimum wavelength is acquired at least 15 times. Therefore, each experimental point is characterized by its own mean value and the respective standard deviation. In this way, the experimental standard deviation takes into account the noise sources coming from not only the extraction procedure of the LPG λ_{res} but also all the other noise sources related to the experimental setup (e.g. temperature fluctuations). In addition, each experimental point is recorded when the flow is stopped, thus the temperature fluctuations can be maintained lower than 0.05 °C during the measuring time.

2.5 Bioassay Protocol

The step-by-step protocol followed to implement the bioassay (Chiavaioli et al., 2014) is depicted in Figure 3. The functionalization of the optical fiber in correspondence of the LPG was achieved by the deposition of a layer of a methacrylic acid/methacrylate copolymer (Eudragit L100) for antibody immobilization.

Once the LPG was functionalized, the optical fiber was placed inside the temperature-stabilized flow cell. All the steps for the implementation of the bioassay were performed using the flow cell

connected to a peristaltic pump and keeping the temperature of the flow cell at 23 °C. The preparation of the biolayer consisted of the following steps (Figure 3): activation of –COOH (carboxylic) groups by cross-linking chemistry (1-Ethyl-3-[3-dimethylaminopropyl] carbodiimide hydrochloride (EDC) and N-hydroxysuccinimide (NHS)), covalent immobilization of mouse IgG (1000 mg L^{-1} in PBS), washing with PBS for removing the un-reacted antibodies, and surface passivation with bovine serum albumin (BSA) (3% in PBS) in order to block the remaining activated carboxylic groups and to prevent non-specific adsorption onto the surface.

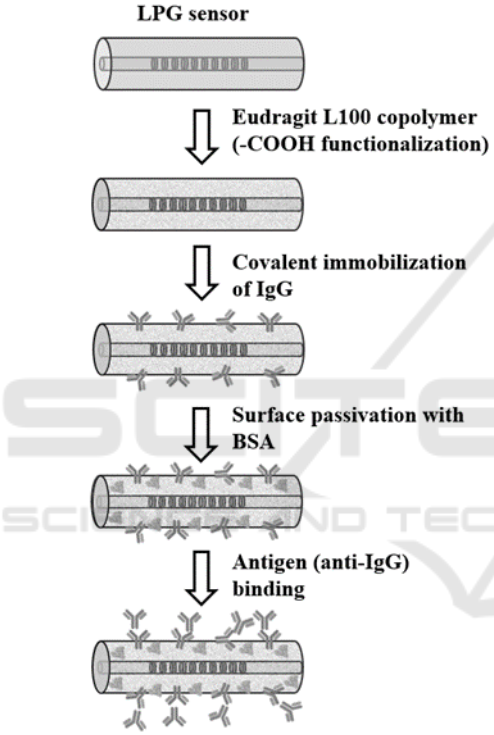

Figure 3: schematic representation of the biolayer preparation on the fiber surface and of the antibody-antigen binding phase.

The assay was performed in human serum spiked with increasing concentrations of goat anti-mouse IgG ranging from 1 µg L^{-1} up to 100 mg L^{-1}. Serum was used at a final dilution of 1:10 (v/v) in PBS.

3 RESULTS

3.1 Thickness Optimization

The LPG RI sensitivity can be significantly enhanced when covered with an overlay of a material having RI higher than the one of the fibre. In these conditions, the selected cladding mode starts to be guided by the overlay and the LPG is working in the so-called transition mode region (Del Villar et al., 2005). The overlay thickness and RI must be precisely controlled in order to reach the optimum overlay thickness (OOT), which occurs when the cladding mode resonance wavelength is positioned at the centre between the original value and that one of the next lower cladding mode (LP_{06} in this case). The behaviour of the LP_{07} cladding mode as a function of the thickness of the sol–gel overlay was calculated considering sol-gel RI of 1.698 RIU and PBS buffer (1.334 RIU) as surrounding medium. The simulated curve, reported in Figure 4, demonstrates that the sensors can work around the most sensitive linear region when the overlay thickness is comprised between 140 and 180 nm. In this way, the LP_{07} mode will be in the transition region when the surrounding medium is PBS buffer, and the sensor will show a higher sensitivity as a function of the biochemical interaction of the target biomolecule with the sensing layer.

Based on these simulations, the thickness of the sol–gel-based TiO_2-SiO_2 film overlay was optimized by varying the viscosity of the sol and the withdrawal speed during the film deposition. Three different batches were realized, changing the sol viscosity (batch A: 3.2 mPa·s; batch B: 27 mPa·s; batch C: 24.5 mPa·s) and the withdrawal speed (batch A: 2.5 mm sec^{-1}; batch B: 2.2 mm sec^{-1}; batch C: 2.95 mm sec^{-1}). The corresponding wavelength shift for the LP_{07} mode due to the film deposition was 8, 13.5 and 20 nm for batch A, batch B and batch C, respectively.

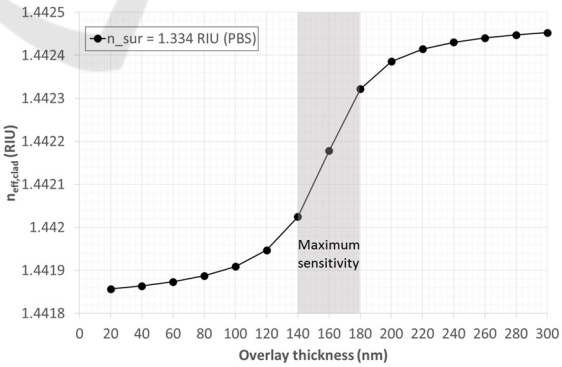

Figure 4: simulated curve, $n_{eff,clad}$ vs. overlay thickness (1.698 RIU) considering the LP_{07} mode PBS buffer as surrounding medium.

The overlay RI can also slightly vary from the desired value depending on:

-the sol composition;

-the ageing time of the sol that can directly influence the viscosity of the sol:

-the withdrawal speed during dip coating;

-the thermal curing temperature.

In order to evaluate the sensor response dependence on this parameter, the effective RI of the LP_{07} mode was simulated as a function of the overlay thickness, considering a ±1% change in the overlay RI (1.681, 1.698 and 1.714 RIU) and considering PBS as surrounding medium. The results are shown in Figure 5. The ±1% variation of overlay RI slightly changes the sensitivity without shifting the sensor from the most sensitive region. Considering a ±1% variation of the overlay RI, a tolerance on the overlay thickness of about 25–35 nm is expected.

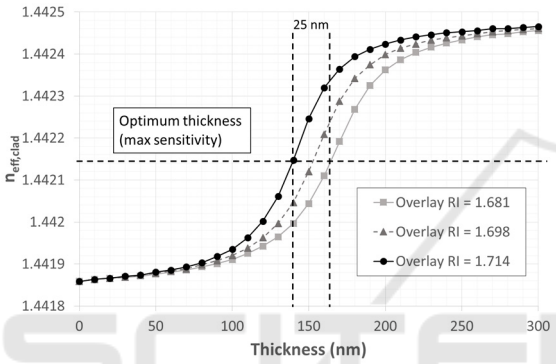

Figure 5: Simulated curves (overlay thickness vs $n_{eff,clad}$) used for studying the influence of a change of 1% in the overlay RI (1.698 RIU, triangles grey dotted line; 1.681 RIU, squares, grey line; 1.714 RIU, circles, black line) considering the LP_{07} mode when the surrounding medium is the PBS buffer.

The film overlay was analysed by means of a field emission scanning electron microscope (FESEM; Supra 35VP, Carl Zeiss). Figure 6 shows an image of the cross-section of an optical fiber coated with the sol-gel based TiO_2-SiO_2 film overlay (sensor of batch C). The thickness of the deposited sol-gel film overlay was estimated to be (159 ± 10) nm (Chiavaioli et al., 2015).

3.2 Bulk Refractive Index Sensitivity

For the RI characterization, the LPGs were immersed in NaCl-in-water solutions of known RI: from 0.1% wt., 1.333 RIU up to 0.6% wt., 1.334 RIU with step of 0.1% wt. Each measurement was taken at stable temperature of 23 °C with fluctuations lower than 0.03 ºC. This allows to discard the contribution coming from the thermo-optic effect acting on solutions (roughly -8×10^{-5} RIU °C^{-1}). After each

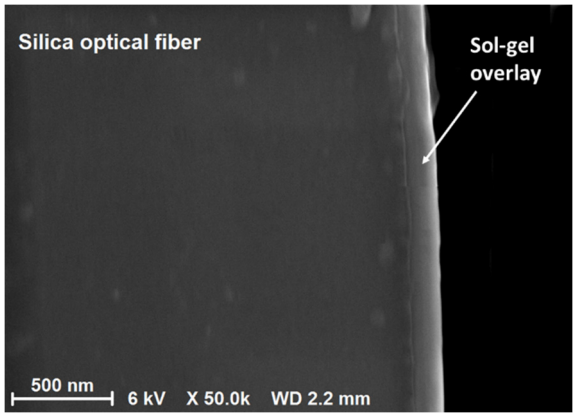

Figure 6: FESEM image of the cross-section of the optical fiber coated with sol-gel based TiO_2-SiO_2 film overlay (batch C).

measurement, the fiber was washed with deionized water in order to remove any remaining NaCl on the overlay surface.

A comparison considering the same cladding mode was carried out for three different LPGs (not coated, batch A and B) in the RI range between 1.333 RIU and 1.334 RIU.

The results are summarized in table 1, while the sensor response curve for a coated LPG of batch B is reported in Figure 7. It is worth pointing out that the sensitivity was evaluated considering the slope of the linear regression approach of the sensor response curve (Figure 7 as an example), whereas the resolution was attained considering three times the standard deviation divided by the sensitivity (Trono et al., 2011).

Table 1: Comparison of the results achieved using different sol-gel based titania-silica coated LPGs and conventional not-coated LPG (same LP_{07} mode order) in terms of volume RI characterization.

Batch	Sensitivity (nm RIU^{-1})	σ (pm)	Resolution (RIU)
not coated LPG	-29.8	8	8.1×10^{-4}
A	-2044.5	10	1.5×10^{-5}
B	-7075.3	11	4.6×10^{-6}

As shown in Table 1, there is a great improvement (both sensitivity and resolution), going from a simple not coated LPG to a coated LPG. Moreover, by optimizing the thickness of the film overlay with values around 130–175 nm considering PBS as surrounding medium (RI of 1.334 RIU), it is possible to achieve very good performances.

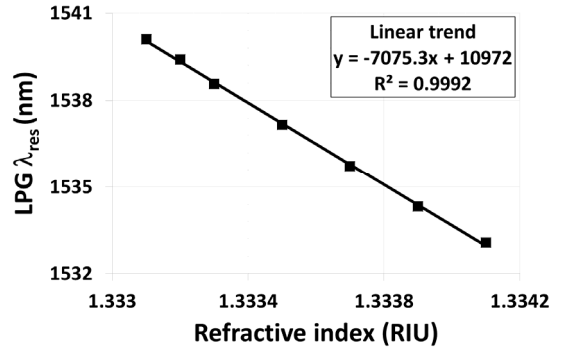

Figure 7: LPG wavelength shift (batch B) as a function of the external volume refractive index.

3.3 Bioassay

The performances of sol-gel coated LPGs were evaluated also by carrying out an IgG/anti-IgG immunoassay on the functionalized surface of the fiber. The sol-gel based TiO_2-SiO_2 coated LPG of batch C was used in this measurement. The sensorgram achieved by spiking the antigen (from 0.1 mg L^{-1} up to 10 mg L^{-1}) in human serum is reported in Figure 8. The duration of the whole immunoassay, including the antibody immobilization step, was of several hours, but the long-term stability of the sensing system (Trono et al., 2011), guarantees the absence of any disturbance coming from long-term drifts.

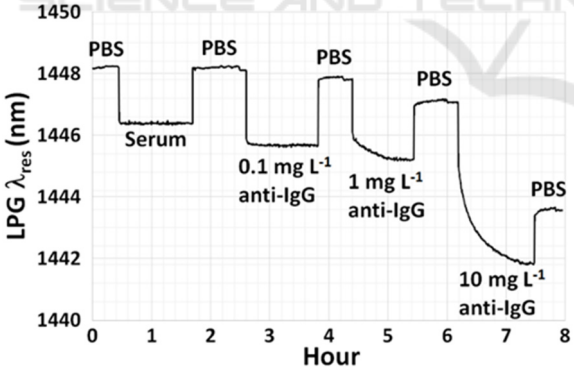

Figure 8: Response of a sol-gel based TiO_2-SiO_2 coated LPG of batch C in human serum. Sample spiked with the antigen (anti-IgG) at 0.1 mg L^{-1}, 1 mg L^{-1} and 10 mg L^{-1}.

A limit of detection (LOD), defined as three times the standard deviation of the blank measurement, of 8 μg L^{-1} was attained (Chiavaioli et al., 2015). The LOD is nine-fold lower than that achieved in the same experimental conditions (i.e. human serum) but using a not coated LPG (Chiavaioli et al., 2014).

4 CONCLUSIONS

The manufacturing procedure and the optimization of high refractive index sol-gel-based TiO_2-SiO_2 thin film overlay for LPG-based sensors have been discussed. The sol-gel characteristics (composition and viscosity) and the withdrawal speed during the dip-coating technique have been chosen in order to have the best combination of RI and thickness. Sensors with overlay thickness of 130–160 nm and RI of 1.7 RIU were manufactured and characterized. The LPG sensors performances were evaluated, as optical refractometer, with the volume refractive index characterization, and as biosensor, with the IgG/anti-IgG bioassay. The best performance was achieved with an overlay thickness of roughly 159 nm, with a bulk refractive index sensitivity of roughly 7000 nm RIU^{-1}, a resolution of the order of 10^{-6} RIU in water environment (refractometer), and a LOD of 8 μg L^{-1} (5.3 x 10^{-11} M) in serum matrix (biosensor).

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