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Organic distributed feedback laser for label-free biosensing of ErbB2 protein biomarker

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ABSTRACT

The human epidermal growth factor receptor 2 (ErbB2) protein plays an important role in human malignancies. Its overexpression has been recognized as a feature of a malignant cancerous phenotype in breast cancer cell lines, and has become one of the most widely investigated clinical indicators of breast, ovarian, gastrointestinal and lung cancers. In this work a vertically emitting organic distributed feedback (DFB) laser has been used to detect the ErbB2 protein. This DFB laser consists of a polystyrene (PS) film containing a perylenediimide laser dye, deposited over a second-order one dimensional grating fabricated on fused silica by thermal-nanoimprint lithography and subsequent reactive ion etching processes. Specificity of the system to ErbB2 protein biomarker, achieved by functionalizing the PS with anti-ErbB2 monoclonal antibodies, is demonstrated. A concentration limit of detection for ErbB2 protein of 14 ng/mL has been obtained, and no cross-reactivity has been observed with bovine serum albumin (BSA) and tumor necrosis factor alpha (TNFα) proteins. These findings open the possibility of using this type of biosensors in clinical applications.

1. Introduction

Label-free biosensors must simultaneously provide high sensitivity, large dynamic range and sufficient resolution for detection of mass density changes less than <1 pg/mm^2 in order to have an impact on the most challenging detection applications [1]. Label-free resonant optical sensors generally detect shifts in the resonant wavelength caused by the interaction between the target molecule and the evanescent portion of resonant modes, and the amount of wavelength shift is proportional to the density of captured biomolecule on the sensor surface. The narrow spectral linewidth achieved by using quality factor (Q-factor) (>10^5) passive optical resonators enables sensor systems to resolve smaller wavelength shifts associated with the detection of analytes at low concentration, or biomolecules with low molecular weight, such as drug compounds [2,3]. While detection resolution can be substantially improved through the use of high Q-factor passive resonators, it is generally at a cost of a decrease of the sensitivity and the dynamic range of the system. Only a few examples of passive resonators have achieved high Q-factor and high sensitivity simultaneously [4]. In addition, the implementation of high Q-factor optical resonators typically requires high precision alignment for evanescent light in/out coupling, providing potential limits to their practical application. One way to solve this problem is to use organic distributed feedback (DFB) lasers. These laser biosensors are simultaneously capable of a high sensitivity and a high degree of resolution, since they operate with single mode and narrow linewidth emission [5–7]. A recently reported strategy to work with good figures of merit for both, resolution and sensitivity, consists in using a photonic crystal resonant reflection technique. It works with a simple optical setup and has showed to be useful for studying cell dynamics or the presence of single metallic or dielectric nanoparticles conjugated with antibodies for biosensing [8,9].

DFB laser biosensors show significant advantages for label-free biosensing applications including: (i) simple implementation -these sensors do not require high precision for positioning of optical fibers or waveguides to the resonator perimeters-; (ii) the chip can be fabricated following low-cost replication techniques and active materials can be applied using spin-coating or dip-coating,

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extensible to roll-to-roll manufacturability [10]; (iii) they can be fabricated on flexible plastic substrates which can be incorporated into standard well microplates [10] for multiplex detection. In addition, the recent demonstration of a DFB laser optically pumped by a Light Emitting Diode (LED) [11] can lead to compact biosensing devices and promising setups for their commercialization. A thorough review of the literature only shows a few reports of organic DFB lasers used as biosensing devices [6,10,12,13]. Lu et al. [6] demonstrated the capability of a DFB device to detect 3.4 nM (60 ng/mL) of human immunoglobulin G (IgG) protein. That laser consisted on a Rhodamine 590 doped SU-8 active film spin-coated over a grating engraved over UV-curable polymer and a 30 nm-thick top layer of titanium dioxide (TiO_2). Tan et al. [10] fabricated similar DFB devices, with the organic active film prepared by horizontal dipping and demonstrated their use to detect tumor necrosis factor alpha (TNFα) down to 0.625 μg/mL. In a later work [12], with the same kind of DFB devices they detected 34 nM (600 ng/mL) rabbit IgG. Haughey et al. [13] fabricated DFB lasers based on an oligofluorene truxene active film deposited by spin-coating on top of the grating engraved on an epoxy resist and no TiO_2 layers. They studied the avidin-biotin interaction and demonstrated detection down to 1 μg/mL of avidin using a biotin-functionalized DFB.

Sensitivities (S_b) in the biological range (at $n = 1.33$) of around 20 nm/RIU have been achieved with DFBs used for the detection of proteins [5,14]. Better sensitivities, of the order of 70–150 nm/RIU have been obtained by experiments and simulations [6,15] with DFB laser sensors coated with a thin layer of high refractive index of TiO_2 ($n = 2.1$). However, Lu et al. [6] fitted the wavelength emission versus refractive index curve over a 14 nm tuning range, while Vannahme et al. [15] calculated the sensitivity at refractive indexes close to $n = 1.5$, thus far from the biological range. DFB sensor resolutions of 0.72 pm [15] have been obtained by fitting the spectral peak to a center of mass model over many laser pulses. Lower resolutions, below 0.5 pm, have been reported [12] using spectrometers with higher optical resolution and triggering the spectrometer to detect the laser pulse only, although this may limit the compactness on the optical setup. Considering that the limit of detection (LOD) of the DFB laser sensor is given by the ratio resolution/sensitivity [2], state-of-the art figure-of-merit for DFB laser sensors are around 7×10^{-6} RIU [15]. In a previous work [16], we have fabricated a DFB laser based on a polystyrene (PS) film doped with a highly photostable perylenediimide derivative (perylene orange, PDI-O) and demonstrated its use as a bulk refractive index sensor with a LOD of 2.5×10^{-5} RIU. In the present paper a vertically emitting organic second-order 1D DFB laser composed of PS/PDI-O active film, similar to that recently reported, is demonstrated for label-free biosensing of human epidermal growth factor receptor 2 (ErbB2) protein biomarker. Specific biofunctionalization of the DFB laser has been successfully carried out.

2. Materials and methods

2.1. Materials, reagents and solutions

The thermal-NIL resist mr-I7010R was purchased from Microresist Technology GmbH (Germany). PS (Mw = 35,000 g/mol) and toluene were purchased from Sigma–Aldrich (Spain). PDI-O (purity higher than 99.5%) was purchased from Phiton (Germany). Recombinant human ErbB2 protein, rabbit anti-ErbB2 monoclonal antibody (anti-ErbB2) and rabbit anti-ErbB2 monoclonal antibody conjugated to fluorescein isothiocyanate (FITC) were purchased from Sino Biological Inc. (China). BSA was from Sigma–Aldrich (Spain) and TNFα from Peprotech (United Kingdom). Phosphate buffer saline and carbonate–bicarbonate buffers were prepared by dissolving readily prepared tablets from Sigma–Aldrich. All other chemicals were of analytical grade and ultrapure water (Millipore Milli-Q) was used throughout.

2.2. Device structure

The structure of the device used as a biosensor (see Fig. 1a) is a waveguide-based organic semiconductor laser. It has been built by a relief grating patterned on a transparent fused silica substrate (refractive index $n_s = 1.46$, at $\lambda = 580$ nm) on which the active material, PDI-doped PS ($n_f = 1.59$, at $\lambda = 580$ nm), is coated as a thin film with a thickness of $h_f = 160$ nm. Active films were prepared by spin-coating a toluene containing PS, as inert polymer, and 1.0 wt% (with respect to PS) of PDI-O. Film thickness was determined by the fringe pattern of the absorption spectrum, measured by a Jasco V-650 UV–VIS spectrophotometer. The resonator is a second-order 1D DFB grating with a period of $\Lambda = 376$ nm and a grating depth of $d = 60$ nm, and has been fabricated by thermal-NIL and subsequent reactive ion etching processes [16]. An Atomic Force Microscope micrograph of one of the obtained gratings is shown in Fig. 1b.

2.3. Biosensor surface functionalization

After thorough water rinsing and N_2 drying of the fabricated DFB devices, the specific biofunctionalization of the surface with anti-ErbB2 monoclonal antibodies was carried out by means of an overnight incubation at 4 °C of a 20 μg/mL antibody solution in pH 9.6 carbonate–bicarbonate buffer. After another cleaning step, a 2 h long incubation of BSA (5 mg/mL) in PBS buffer (pH 7.3) ensured perfect surface coverage for minimizing potential non-specific binding. Incubations with ErbB2 protein biomarker were carried out during 2 h at different concentrations ranging from 0 up to 10,000 ng/mL also in PBS buffer. All incubations were performed with a carefully pipetted 10 μL drop covering all the DFB array structure in a humid atmosphere to avoid evaporation.

2.4. Optical characterization

Optical characterization of the DFB sensors, before and after functionalization, was performed by pumping and collecting the emitted light through the substrate (see Fig. 1a), with the sample placed horizontally with respect to the optical table. This geometry, which requires the use of a transparent substrate, avoids disturbing the analyte with the pump beam. The excitation beam shape is elliptical (minor axis of 1.1 mm), and it is incident at an angle of around 20° with respect to the normal to the film plane (green arrow). The emitted light is collected (red arrow) perpendicularly to the sample surface. A pulsed Nd:YAG (YAG-yttrium aluminum garnet, 532 nm, 5.5 ns, 10 Hz) laser was used to excite the sample and the light was collected by an Ocean Optics MAYA 2000 fiber spectrometer, with a nominal resolution in determining the spectral peak wavelength and linewidth of 0.07 nm and 0.13 nm, respectively. The measured spectrum is formed by discrete points separated by steps of 0.035 nm.

3. Results and discussions

The DFB sensor shows one single peak, which before functionalization appears at $\lambda_{DFB} = 555.2$ nm (see Fig. 2). Its lasing threshold and the photostability half-life are 200 kW/cm^2 and around 20 min (1.2×10^4 pump pulses), respectively [16]. The photostability half-life is defined as the time or the number of pump pulses at which the emitted laser intensity decays to half of its maximum value. Its sensitivity in the biological range ($n = 1.33$) is $S_b = 32$ nm/RIU [16], which is comparable to those reported for other single-layer waveguide DFB biosensors [6,10,12,13]. The sensor resolution was calculated by fitting the central wavelength emission by a center of