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Porous silicon-based optical biosensors and biochips

Ivo Rendina*, Ilaria Rea, Lucia Rotiroti, Luca De Stefano

Institute for Microelectronics and Microsystems, National Council of Research, Via P. Castellino 111, 80131 Napoli, Italy

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Abstract

Porous silicon multilayered microstructures have unique optical and morphological properties that can be exploited in chemical and biological sensing. The large specific surface of nanostructured porous silicon can be chemically modified to link different molecular probes (DNA strands, enzymes, proteins and so on), which recognize the target analytes, in order to enhance the selectivity and specificity of the sensor device. We designed fabricated and characterized several photonic porous silicon-based structures, which were used in sensing some specific molecular interactions. The next step is the integration of the porous silicon-based optical transducer in biochip devices: at this aim, we have tested an innovative anodic bonding process between porous silicon and glass, and its compatibility with the biological probes.

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1. Introduction

Recently, lot of experimental work, concerning the worth noting properties of nanostructured porous silicon (PSi) in chemical and biological sensing has been reported, showing that, due to its morphological and physical properties, PSi is a very versatile sensing platform [1–5]. A key feature for a recognition transducer is a large surface area: PSi has a porous structure with a specific area of the order of 200–500 m^2/cm^3, so that it is very sensitive to the presence of biochemical species which penetrate inside the pores. Moreover, PSi is an available, low-cost material, completely compatible with VLSI and micromachining technologies, so that it could usefully be employed in the fabrication of MEMS, MOEMS and smart sensors.

The PSi optical sensing features are based on the changes of its photonic properties, such as photoluminescence or reflectance, on exposure to the gaseous or liquid substances. Unfortunately, these interactions are not specific, so that the PSi cannot be used as a selective optical transducer material. One way to overcome this limit is to chemically or physically modify the PSi hydrogenated surface in order to enhance the sensor selectivity through specific biochemical interactions. The reliability of the biosensor strongly depends on the functionalization process: how simple, homogenous and repeatable it can be. This fabrication step is also crucial for the stability of the sensor: it is well known that as-etched porous silicon has a very reactive Si–H terminated surface due to the Si dissolution process [6]. The substitution of the Si–H bonds with Si–C ones guarantees a much more stable surface from the thermodynamic point of view.

Testing and demonstrating the PSi capabilities as a useful functional material in the optical transduction of biochemical interactions is only the first action in the realization of an optical biochip based on this nanostructured material. In this case, all the fabrication processes should be compatible with the utilization of biological probes and the feasibility of such devices must be proven. This means that the standard integrated circuit microtechnologies should be modified and adapted to this new field of application. A very strong interdisciplinary is required to match and resolve all these problems.

*Corresponding author.
E-mail address: ivo.rendina@na.imm.cnr.it (I. Rendina).

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In this work, we review our recent experimental results in optical biosensing using PSi-based photonic devices and report our newest results about the design and fabrication of optical biochip by exploiting the PSi nanotechnology [7–12].

2. Materials and methods

Following the pioneering work of Mike Sailor's group [13], we started using the PSi monolayers, which optically act as Fabry–Perot interferometers, as basic transducer in biosensing experiments. This is a very convenient choice since PSi monolayers are very easy to fabricate and to characterize. These devices have also a high sensitivity to changes in refractive index, up to 70 ppm [14]. By exploiting the optical features of PSi Fabry–Perot interferometers, we studied several bio-molecular interactions: DNA single strands with their complementary strands, Glutamine binding protein (GlnBP) from *Escherichia coli* with Glutamine; GlnBP with Gliadine [8,11,12]. The high-quality optical response of PSi multilayers allows the fabrication of resonant optical devices and photonic crystals that are very attractive for sensing applications [15].

In biochemical sensors based on 1-D photonic bandgap (PBG) structures, usually there is both a reduced group velocity of light within the sample, which increases the interaction with the analyte and thus the sensitivity, and the presence of a resonant characteristic wavelength. In this case, sensors whose operation is based on the measurement of the shift of a very narrow resonance (for instance, a transmission or reflection peak having a full-width at half-maximum of few tens of microns) are characterized by increased sensitivity and resolution. We have used a PSi optical microcavity to study the molecular binding of GlnBP and glutamine with a higher sensitivity with respect to the interferometric characterization [10].

Another typical PBG structure used in optical sensing is the distributed Bragg mirror which is obtained by alternating high (A) refractive index layers (low porosity) and low (B) refractive index layers (high porosity). The thicknesses of each layer satisfy the following relationship: $n_A d_A + n_B d_B = m\lambda/2$, where m is an integer and λ is the Bragg resonant wavelength. The main optical feature of a Bragg mirror is the presence of a single, degenerate photonic band gap in a period of the reciprocal space. As a consequence, the reflectivity spectrum is characterized by a principal ($m = 1$) stop band, centred around λ, whose width depends on the refractive indexes contrast (n_A/n_B), while its height, depends on the number of the layers. The higher orders of the resonance ($m = 2,\ 3,\ 4,\ldots$) are characterized by a narrow width and a lower reflectivity [16].

We have designed, simulated and realized a low contrast, 20-layer-Bragg mirror, with the first-order ($m = 1$) resonant wavelength at 4800 nm. A highly doped p^+-silicon, $\langle 1\,0\,0 \rangle$ oriented, 0.01 Ω cm resistivity, 400 μm thick was used as substrate to realize the structures. The Bragg low-porosity layers (effective refractive index $n_L \cong 1.56$, thickness $d_L \cong 641$ nm) were produced by an etching current density of 125 mA/cm^2 for 2.58 s, while an etching current density of 150 mA/cm^2 for 3.62 s has been used for the high-porosity layers ($n_H \cong 1.50$, $d_H \cong 933$ nm).

For the biosensing experiments, a strong base post-etch treatment was used to increase the pore size and remove the superficial nano-residue due to the electrochemical etch process, so to improve the infiltration of biomolecular probes into the pores [17]. Unfortunately, this process removes most of the native Si–H bonds, required by the subsequent functionalization treatment, from the porous silicon surface: to restore these bonds, we rinsed the porous silicon device in a very diluted HF-based solution for 30 s.

The reflectivity spectra were measured over the range 800–1600 nm with a resolution of 0.2 nm by using an Y optical fibre connected to a tungsten lamp, as a light source, and to an optical spectrum analyzer.

We have estimated the refractive index sensitivity, i.e., the response of the sensor to the changes of the average refractive index, by measuring the peak shift of a higher order Bragg resonance on exposure to several organic volatile substances with different and increasing refractive indexes. Each measurement starts after that a small amount of volatile substance is added to reaction chamber and saturates with its vapours the atmosphere surrounding the sensor. The vapours penetrate into the nanometric pores, substituting the air. The average refractive index of the layers changes, so that the reflectivity spectrum of the device shifts towards higher wavelengths.

Procedures for the immobilization of biomolecules on porous silicon are usually based on a chemistry that involves the silanization of the oxidized PSi surface [18]. A promising recently proposed alternative is that exploiting the reaction of acids molecules with the hydrogen-terminated porous silicon surface in order to obtain a more stable organic layer covalently attached to the PSi

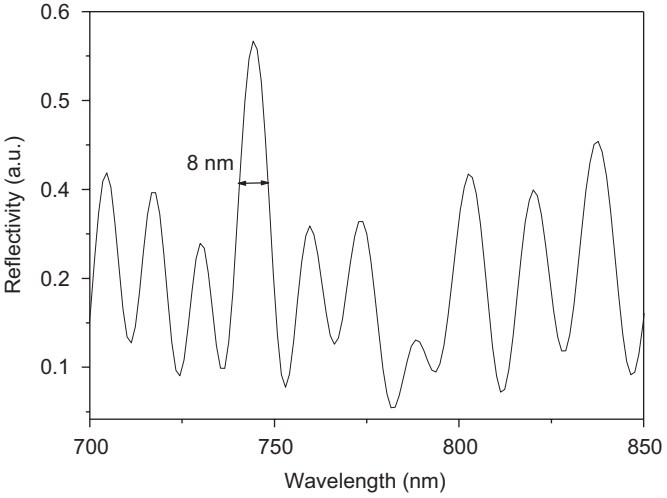

Fig. 1. Reflectivity spectra of a porous silicon Bragg mirror.

surface through Si–C bonds. We have exploited a photo-activated modification of PSi surface based on the UV exposure of an *N*-hydroxysuccinimide ester (UANHS) solution [19]. This kind of treatment is very fast since the time required for the complete surface passivation is of the order of few minutes. Moreover, after UV exposition the chip can be analysed by FT–IR microscopy to verify the state of the process. In view of a possible integration of the PSi-based biosensor into a lab-on-chip, we have verified the thermal stability of the Si–C bonds up to the temperature adopted in the packaging process of the chip. Once functionalized, the PSi optical transducer was bonded to a glass slide by an innovative anodic bonding process that takes into account the very peculiar characteristic of this material. Details of the process and of the microfluidic solutions adopted are elsewhere described [7].

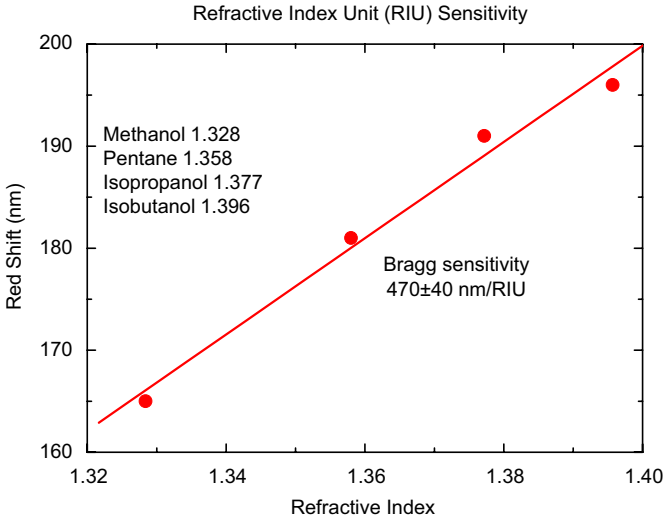

Fig. 2. Red shift of the Bragg peak at 750 nm versus the refractive index of vapor substances.

Even if our aim is the realization of a label-free optical biosensor based on the PSi nanotechnology, we have used a fluorescent protein to control the distribution of the biological matter on the chip surface and to preliminary test the chemical stability of the covalent link between the protein and the PSi surface.

3. Experimental results and discussion

Fig. 1 shows the reflectivity spectrum of the PSi Bragg mirror in the range 700–850 nm: we have found that despite the high order of the resonance ($m = 6$), the resonant peak at $\lambda = 750$ nm has a reflectivity which is the 60% of the first order and the width is less than 10 nm. Note that due to refractive index and thickness fabrication uncertainties the $m = 6$ Bragg wavelength is about 750 nm instead of 800 nm.

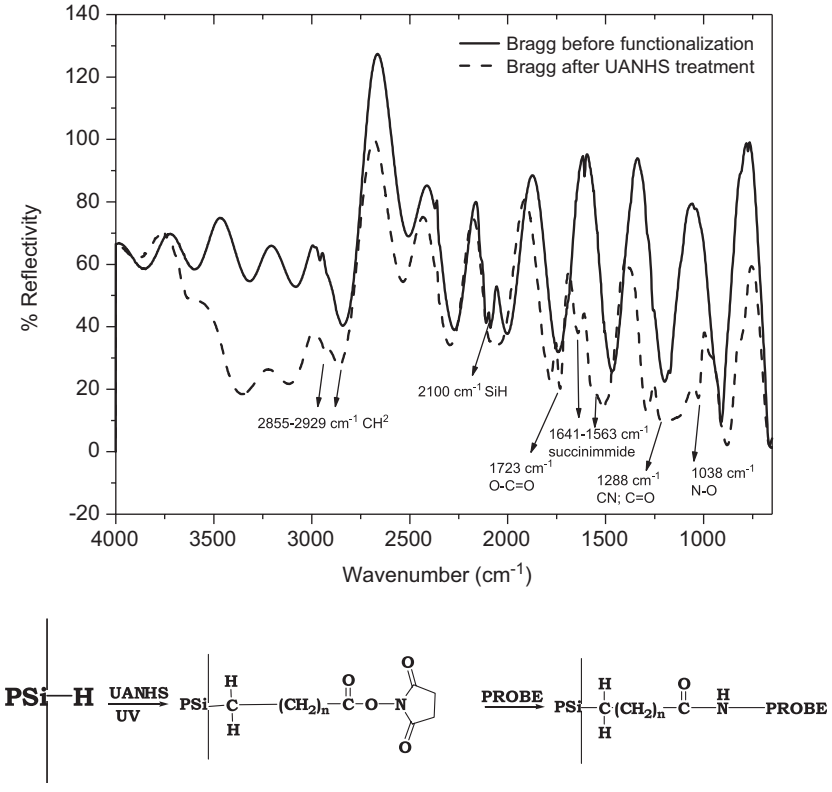

Fig. 3. FT–IR spectra of the realized porous silicon Bragg structure before and after the photoinduced functionalization process based on UV exposure, together with the reaction scheme.

In Fig. 2, the red shifts of the $m = 6$ Bragg resonance peak at 750 nm are reported as function of the refractive index of each volatile substance employed. A well linear response is observed and a sensitivity of 470 (40) nm/RIU can be estimated, where RIU is the acronym for refractive index unit. Since the resolution of our spectrometer is 0.05 nm, a limit of detection for the refractive index change of 1.06×10^{-4} can be estimated for this kind of optical device.

The UV-induced surface passivation process results in covalent attachment of UANHS to the porous silicon surface, as clearly shown in the FT–IR spectrum reported in Fig. 3 together with the reaction scheme: all the characteristic absorption peaks due to the organic compounds are present in the sample after the functionalization process. The chip was also optically characterized: a red shift of about 54 nm in the reflectivity spectrum is observed.

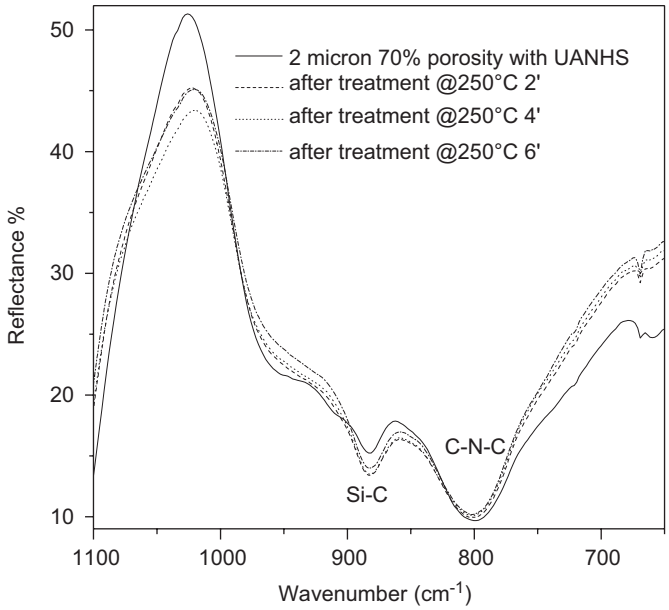

Fig. 4. FT–IR spectra of a PSi chip after a thermal treatment at different times.

Then the device was incubated over night with the biological probe. The selective recognition of the biomolecular target is optically verified by a further red shift (data will be published elsewhere).

The FT–IR spectrum of a PSi chip after functionalization at different temperatures is reported in Fig. 4: it is well evident that the link between the inorganic and organic phases is stable up to 250 °C. The Si–C and C–N–C bonds have high thermodynamic strength so that the passivated chip is still working after the anodic bonding process.

In Fig. 5, a real view of the hybrid porous silicon-glass chip (A) is reported, while in the insets (B) and (C) the fluorescent images observed by a Leica Z16 APO fluorescence macroscopy system after incubation of the labelled bioprobes bonded to the chip, are shown. By illuminating the chip spotted with labelled proteins (fluorescent glutamine binding protein, purified by *E. coli* [8]) by a 100 W high-pressure mercury source, we found that the fluorescence is very high and homogeneous on the whole surface (see Fig. 5(B)). In Fig. 5(C) a detail of a scratch on the chip surface can be seen, showing that the fluorescent probes also penetrate into the inner pores of the chip. After several flushing run with demineralized water, injected by a micro-syringe, we found that the fluorescence is still bright.

4. Conclusions

In this work, our recent experimental results in optical biosensing using PSi-based photonic devices are reviewed. Newest results about the fabrication and characterization of PSi sensors based on the exploitation of distributed Bragg reflectors operating at higher order harmonics are also presented. The PSi surface functionalization of such structures by UV stimulated processes, allowing the attachment of linkers through stable covalent Si–C bonds, is also demonstrated to be compatible to the high-temperature anodic bonding processes used for the realization of silicon-glass biochips.

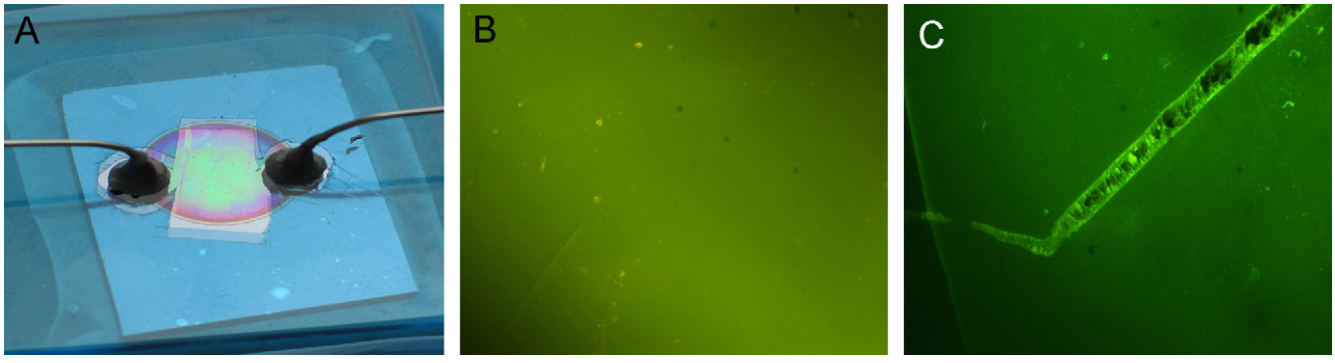

Fig. 5. Images of the basic element of a lab-on-chip based on a PSi optical transducer: (A) a real view of the porous hybrid silicon-glass chip; (B) fluorescent images of labelled proteins; (C) particular of a scratch on the chip surface.

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