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Doubly resonant porous silicon microcavities for enhanced detection of fluorescent organic molecules

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ABSTRACT

The synthesis of porous silicon-based photonic structures for bio-sensing applications has been widely investigated in the last years. Thanks to its spongiform structure, porous silicon can efficiently host many organic molecules dispersed in solutions having proper chemical affinity. Fluorescent emission of organic-dyes embedded in porous silicon can be enhanced if a photonic structure like a Fabry–Pérot resonator is employed as a host solid matrix. In this work we present experimental evidence of a fluorescence enhancement effect obtained by means of doubly resonant microcavities tuned on both the excitation and the emission wavelengths. The use of doubly resonant cavities allows both a resonant excitation of dyes and a resonant amplification of the emission. We demonstrate that small concentrations of fluorescent dyes down to few picomoles can be detected. The bio-sensing capabilities of such a structure are tested on larger molecules of fluorescein-labelled protein A, yielding to an evident lowering of the detection limit by 2 orders of magnitude.

1. Introduction

In the last decade, porous silicon (PSi) multilayers have been applied for sensing issues in chemical and biological domains. Several research groups worldwide have demonstrated the capabilities of porous silicon for detecting biomolecules adsorbed on the porous silicon surface. In particular, all-PSi optical microcavities, Bragg mirrors and rugate filters have been proposed for detection schemes based on effects such as the changes of refractive index or photoluminescence [1–6]. As far as fluorescence analysis is concerned, several research groups demonstrated that PSi multilayers are able to host efficiently organic fluorescent dyes [7,8]. An efficient emission of dyes impregnated within photonic microcavities have been reported, thus providing a high-sensitivity detection tool for chromophore-marked biological assays [8,9].

The confinement effect of light in the PSi Fabry–Pérot structure constitutes the basic enhancement mechanism of spontaneous emission of either the luminescent PSi matrix or the impregnated emitting molecules. In both cases, the emission spectrum is heavily narrowed and a substantial change in the emission directions with respect to the structure surface is observed. This kind of resonant structure consists of a spacer surrounded by two quarterwave dielectric multilayers (Bragg mirrors, also called Distributed Bragg Reflectors, DBR) made of PSi layers having different porosities.

Besides standard optical microcavities (spacer λ or λ/2 thick), characterized by a single resonant mode, silicon-based coupled cavities have also been fabricated and characterized [10,11]. In a coupled microcavity, two spacers are surrounded by adjacent DBRs. Such an arrangement provides the splitting of the cavity eigenmodes, which yields a double resonance within the stop band. Indeed, the weaker the coupling between the two spacers (i.e. larger total thickness of the central DBR), the larger the spectral splitting of the resonance modes is.

In this work, a PSi coupled microcavity (PSCM) is employed for enhancing the fluorescence of organic-dyes embedded within the porous matrix. The main advantage of a coupled microcavity is the resonant enhancement of both the excitation and the emission of the hosted dye. This effect can be obtained by tuning the two cavity resonances in correspondence to the maximum absorption wavelength and the maximum emission wavelength respectively. Thus, an efficient excitation as well as an efficient emission process can be performed, provided that a high intracavity enhancement of the light intensity is obtained.

2. Experimental

2.1. Fabrication of porous silicon coupled microcavity

PSCM are prepared from single polished (1 0 0)-oriented boron-doped p^+-type silicon wafer (<7 mΩ cm^{-1} resistivity) by

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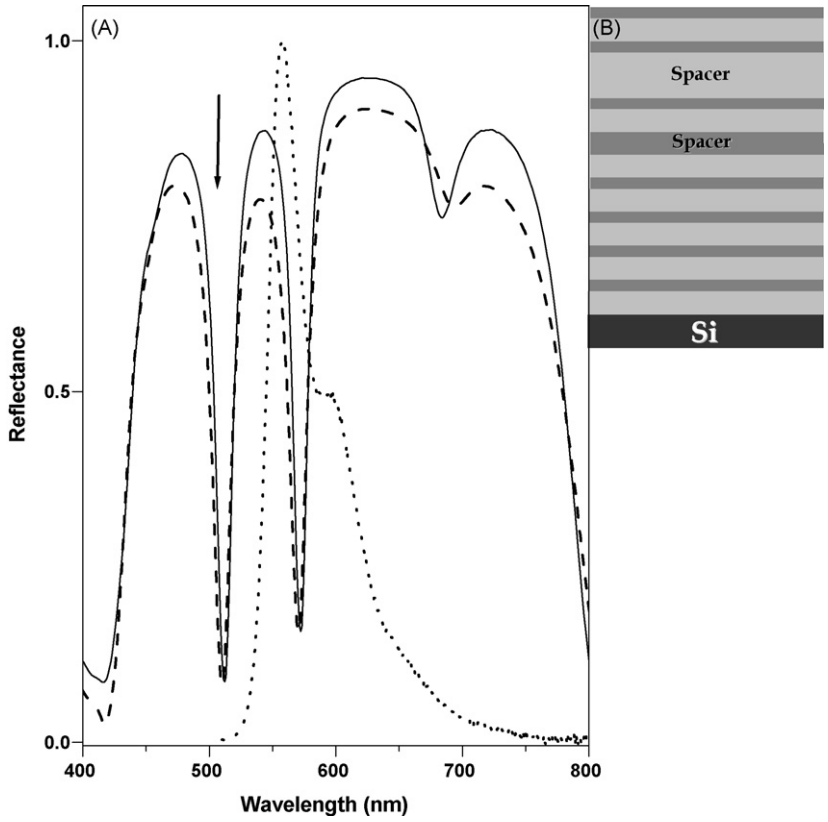

Fig. 1. (A) Reflectance spectrum of the PSi coupled microcavity. Full line: experimental results. Dashed line: calculation. Dotted line curve represents the emission spectrum of the Cy-3-H dye in ethanol solution; the arrow indicates the laser wavelength. (B) Layout of the PSCM.

electrochemical etching in a HF-based solution (35 wt% HF/35 wt% H_2O/30% EtOH). Etching process is performed in a HDPE cell with a platinum mesh electrode and a computer controlled galvanostat (Keithley 2425). The temperature of the etching process is fixed at −45 °C, in order to obtain abrupt interfaces within the Fabry–Pérot structure. The cell is placed in a thermostatic bath composed by ethylene glycol and water, in order to get rid of temperature fluctuations which would affect both the etching rate and the porosity (i.e. refractive index) during the stack synthesis.

The multilayer structure is obtained by managing a pseudo-periodical square-wave current varying between 2.8 and 22.6 mA cm^{-2}, applied for 2-2-5 cycles (126 s). An etch stop of 20 s is applied to ensure the best in-depth homogeneity of the porous layers. PSCM optimized for the fluorescence experiment are designed by using a standard calculations based on transfer matrix methods. The typical layout is as follows:

$$\underbrace{\mathrm{LHL}}_{1^{st}\ \text{mirror}} - \underbrace{\mathbf{HH}}_{1^{st}\ \text{spacer}} - \underbrace{\mathrm{LH}}_{2^{d}\ \text{mirror}} - \underbrace{\mathbf{LL}}_{2^{d}\ \text{spacer}} - \underbrace{\mathrm{HLHLHLHLH}}_{3^{d}\ \text{mirror}}$$

where **L** states for low porosity (high refractive index) layer and **H** for high porosity (low refractive index) layer. The halfwave spacers is designed with an high porosity first layer (HH, d_H = 95 nm, n_H = 1.37 @ λ = 500 nm, porosity 40%) coupled by a 1 period DBR with a low porosity second layer (LL, d_L = 63 nm, n_L = 2.5 @ λ = 500 nm, porosity 80%).

The thickness and the refractive index of the layers are properly optimized in order to have the above-mentioned excitation/emission resonant effect for a 1,3,3,1′,3′,3′-esamethylindocarbocyanine iodide (Cy-3-H), soluble in absolute ethanol and excited at 514.5 nm.

PSCM samples are passivated through a slight surface oxidation (250 °C in air for 30 min) in order to increase the hydrophilicity of the spongiform matrix (improving the diffusion efficiency of the dye in the PSi multilayer structures) as well as to avoid reactions between the solution and the hydride covered surface.

2.2. Fluorescent analyte synthesis

A Cyanine dye 1,3,3,1′,3′,3′-esamethylindocarbocyanine iodide (Cy-3-H), is synthesized by reaction of indolenine and a polymethinic chain obtained through condensation of aniline with propane. The solid powder is dissolved in absolute ethanol. The Cy-3-H molecule is chosen since the excitation laser frequency of the fluorescence experimental setup (514.5 nm) is close to the dye maximum absorption (530 nm) and because cyanines are widely used as fluorescent bio-markers.

The fluorescein isothiocyanate (FITC)-labelled protein A, lyophilized powder (from Sigma), is dissolved in phosphate buffered saline (PBS) 1× solution (from GIBCO). This starting solution is then further diluted using a water/ethanol (50%/50% by volume) solution, aimed to improve the diffusivity inside the porous matrix as compared to a pure water solution. We point out that the increased dilution of the protein solution, may lead to an eventual variation of the emission efficiency of the fluorescein conjugated protein.

2.3. Fluorescence measurements

Emission spectra are collected in backscattering configuration using a Renishaw micro-Raman spectrometer equipped with a cooled CCD detector. Samples are mounted under a microscope equipped with a 20× objective (0.4 N.A.). The samples are illuminated by means of a focused Ar–Kr laser beam (wavelength 514.5 nm).

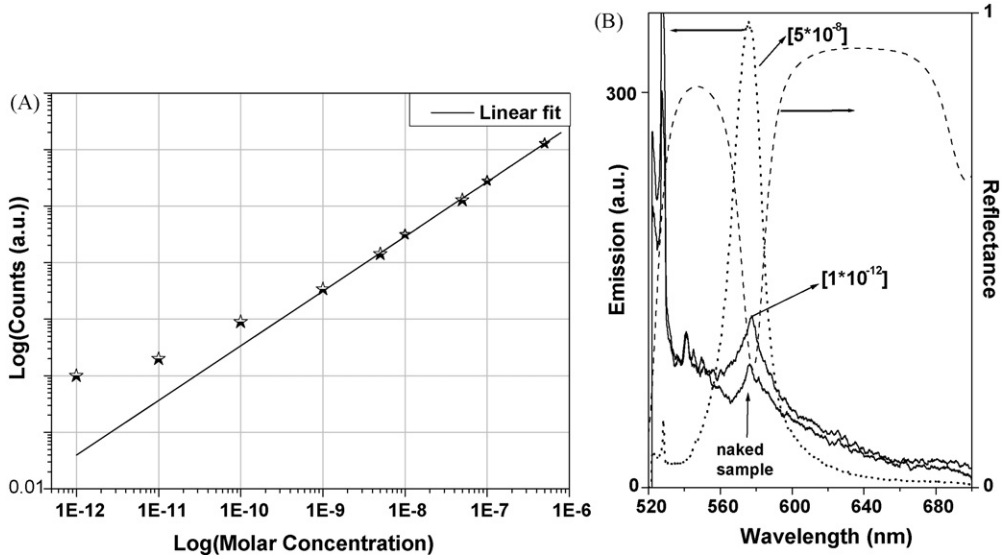

Fig. 2. (A) Fluorescence intensity of Cy-3-H impregnated PSCM samples as a function of the dye molar concentration in ethanol solution. (B) Fluorescence spectra of the naked PSCM (solid line) magnified by a factor of 100, impregnated with Cy-3-H [10^{-12}] (dashed line) magnified by a factor of 100 and with Cy-3-H [5×10^{-8}] (dotted line). The resonant spectrum of the naked cavity is due to the weak fluorescence of the mesoporous silicon matrix.

Samples impregnated with the cyanine dye are immersed in the ethanolic solution with different molar concentrations ranging from 10^{-12} to 5×10^{-7} for 3 min. Samples used for the fluorescence analysis of the protein A are impregnated for 10 min in order to compensate the lower diffusivity of such bulky molecule. For each concentration, a fresh PSCM is employed. The estimation of fluorescence intensity is obtained after subtraction of the background luminescence emission of the bare p-Si PSCM.

Fig. 1 shows the quasi-normal-incidence reflectance spectrum of a coupled cavity tuned at 514 and 570 nm. The cyanine emission spectrum is also shown. Cy-3-H is a plane organic molecule with a large absorption spectrum in the 430–550 nm range and a maximum at 530 nm. The emission spectrum shows a tailored band broadened within 530–650 nm range, with a maximum at 560 nm.

3. Result and discussion

In Fig. 2(A) we plot the measured fluorescence intensity of impregnated PSCM samples as a function of the corresponding dispersed dye concentration. Some of the detected fluorescence spectra of Cy-3-H impregnated PSCMs are presented in Fig. 2(B). Fluorescent emission is narrowed around the resonance frequency, as expected for a fluorescent analyte embedded in an optical cavity. A concentration as small as 10^{-12} M is detected.

In order to check the PSCM efficiency as a fluorescence enhancer, the same set of measurements are performed after impregnating a PSi single layer (SL) having the same thickness L of the PSCM and a porosity close to the average of the considered microcavity (65% porosity). In this case, a molar concentration of [10^{-7}] indicates the detection threshold (i.e. *five* order of magnitude larger as compared to the case of the coupled microcavity).

The impressively low concentration limit obtained by means of the PSCM can be explained by considering several physical and chemical effects. Nevertheless, the enhancement due to the resonant optical cavity, together with the complex interaction between the dye and the PSi surface can be considered as the dominant contributions.

In order to provide a more quantitative estimation of the role played by the photonic structure, we measured the photoluminescence intensity of the PSCM and the PSi SL dye-impregnated at identical molar concentration [10^{-7}]. Measurements are performed at the (fixed) emission wavelength of the dye. The ratio between the fluorescence yield of the PSCM and the single layer is about 50. Such experimental result is well matching results expected from rigorous calculations. We estimate the intensity enhancement factor by calculating the following parameter |

$$\eta = \frac{\left[\int_0^L |E_p(x)^2| \times |E_f(x)^2| dx\right]_{PSCM}}{\left[\int_0^L |E_p|(x)^2 \times |E_f|(x)^2 dx\right]_{SL}}$$

defined as the ratio between the superposition integrals of the excitation $|E_p(x)^2|$ and the fluorescence intensity $|E_f(x)^2|$ spatial distributions in the cavity and the single layer respectively.

The above experimental and numerical analyses clearly show that the fluorescence gain due to the photonic structure cannot fully explain the remarkably low detection threshold of the dye-impregnated PSCM. We guess a possible mechanism that might give an account of our observations, might be represented by a higher dispersion of dye molecules at very low concentration, thus leading to an increased fluorescence as compared to higher molar concentrations. Furthermore, we cannot exclude an increase in the emission yield due to the immobilisation of the cyanine dye after the solvent evaporation. A similar effect has been already reported in the case of silica spheres [12]. Moreover, since the matrix provides an effective physical barrier, the typical photobleaching and photodegradation observed in conventional dyes dispersed in aqueous/ethanolic solutions is minimized [13]. Thus, the stratified mesoporous silicon matrix greatly contributes to the total fluorescence boosting. Moreover, it is worth to underline that the molar concentration of Cy-3-H reported in Fig. 2(A), is referred to the ethanol solution used for the PSCM impregnation and reasonably differs from the effective concentration of dye within the PSi. In particular, for small dye quantities, where the fluorescence yield is no more linear with respect to the molar concentration, the diffusion of the molecules inside the porous matrix could yield a pre-concentration effect. Such a behaviour, which does not reduce the potentiality of the PSCM for high sensitive detection, cannot be appreciated in PSi single layers since the absence of the cavity enhancement impedes the analysis at very low concentration.

The previous results represent a proof-of-principle for high-sensitivity detection of fluorescent molecules provided by the

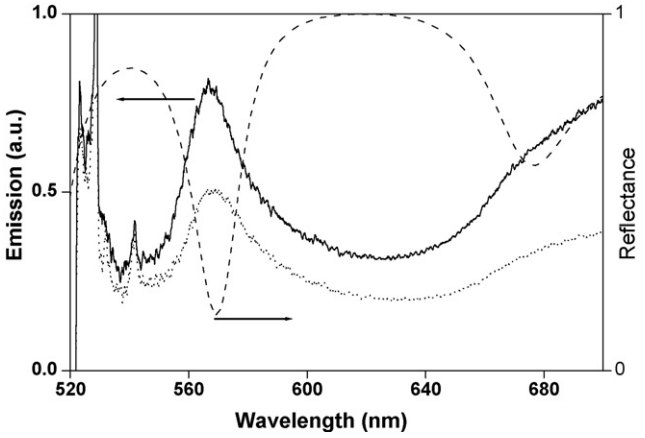

Fig. 3. Fluorescence spectrum of the PSCM impregnated with Fluorescein-labelled protein A [10^{-9}] (solid line) compared with the emission of the naked cavity (dotted line). The reflectance spectrum of the PSCM is shown for sake of comparison (dashed line).

PSCM structure. In order to check the real possibility of using PSi coupled microcavities in biomolecules analysis, we conduct a fluorescence-based detection of low concentrations of a commercial fluorescein-labelled protein A. The experimental conditions are the same, except that the impregnation time increases up to 10 min, in order to assure a good penetration of protein A within spacers. With such biological assay, a molar concentration [10^{-9}] is still detected. The detection threshold is three orders of magnitude smaller as compared to the Cy-3-H dye. Nevertheless, it is worth to remark that the fluorescent marker is not tailored for the measurement setup: the absorption peak of fluorescein (480 nm) is quite far in respect to the laser line (514.5 nm) and the large wavelength cavity mode (570 nm) falls within the tail of the emission spectrum peaked around 530 nm. Fig. 3 shows that the fluorescence peak of the PSCM impregnated with the marked-protein overlaps with the reflectance resonance dip: a clear fingerprint of an efficient biomolecule diffusion in the porous matrix despite the large steric hindrance of protein A as compared to the mean pores diameters. Hence, notwithstanding the above-mentioned limitations, this result strongly supports the future applications of PSCM as a very simple platform for the optical detection of marked biomolecules.

4. Conclusions

In conclusion, we developed a PSi photonic structure consisting of a coupled microcavity which allows the optical detection of very low amounts of fluorescent molecules, and merging the advantages of an efficient hosting matrix with the optical enhancement due to the cavity double resonance. For an optimized structure, a molar concentration of Cy-3-H dye as low as [10^{-12}] is measured. The structure is also employed with a commercially available fluorescein-labelled protein A. Despite its large steric hindrance, the experiments demonstrate an efficient diffusion in the mesoporous stratified structure, which foresee promising applications in bio and life science.

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Biographies

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