Silicon Nitride Photonic Crystal Nanocavities for Biochip Applications

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ABSTRACT

We show that Silicon Nitride (Si_3N_4) photonic crystal (PhC) resonators are powerful building blocks to realize biocompatible photonic devices based on spontaneous emission engineering of nanoemitters in the visible spectral range. The versatility of this technological platform is demonstrated also in biological applications, where nanocavity modes are coupled to DNA strains marked with Cyanine 3 (Cy3) organic dyes and antibodies bounded to fluorescent proteins (TRITC).

Keywords: Photonic crystal nanocavities, Biochip, spectral detection, sensitivity, parallel detection.

1. INTRODUCTION

Moulding the flow of light by means of two dimensional (2D) photonic crystals (PhC) technology is a well-established milestone in optics since 1987 [1,2]. Two decades of research in this field have demonstrated the potential of such structures in guiding, filtering, concentrating and exploiting non-linear properties of photons, with a predominant interest towards telecommunication band [3-5].

More recently, 2D-PhC concepts have been also applied in the visible spectral range, aiming to improve performances of optical devices in different applications such as displays, LEDs, solar energy, biosensing etc. In this sense, still great debate is found in literature about an ultimate material suited for the development of visible-light guiding devices. Requirements such as transparency, reasonably high refractive index (n), low-cost, robustness limit material choice to Gallium Nitride (GaN) [6], Gallium Phosphide (GaP) [7], polymers [8], Silicon Dioxide (SiO_2) [9] and Silicon Nitride (Si_3N_4) [10]. Among these, Silicon Nitride is among the best choices for biological applications. In fact, besides the above-mentioned specifications, it is also biocompatible and allows the functionalization of several kinds of proteins [11-12].

Here we report the use of 2D-PhC Si_3N_4 nanocavities to improve sensitivity and efficiency in cutting-edge devices for biodiagnostics, such as DNA and protein biochips. Fluorophore-labeled bioanalyst are coupled to the cavity resonating modes; this interaction increases the emitters' detectability and, at the same time, peculiarly modifies their spectrum, thus allowing their identification through wavelength-resolved light detection.

2. DESIGN AND FABRICATION OF A BIOCHIP PROTOTYPE

2.1 Nanocavity layout

The investigated two-dimensional photonic crystal (PhC) pattern consists of a triangular lattice of air-holes obtained in a Si_3N_4 slab in air-bridge configuration. The photonic band gap (PBG) for transverse electric like modes (TE) has been designed by using a Plane Wave Expansion (PWE) algorithm and optimized as a function of the filling factor a/r (where a is the period of the lattice and r the radius of the holes) and of the slab thickness $t = \lambda/(2n)$. The electromagnetic behaviour of the H1 cavity, obtained by removing one element of the triangular lattice, and the following optimization of the cavity geometry have been investigated by exploiting 3D Finite Differences Time Domain (FDTD, in the commercial implementation FullWAVE, Rsoft Design Group) algorithm over a calculation domain of $50a \times 50a$ in xy plane. In order to compute the Q-factor of the resonant modes, the time decay of the energy stored in the PhC slab has been evaluated.

A careful modelling of the defect geometry was performed in order to optimize the Quality factor (Q-factor) and the Purcell factor of the resonant modes, obtaining maximum theoretical values of 810. Details on the optimization strategy based on the closed bandgap theory and on the modelling of the two degenerated dipole modes are reported in [10].

2.2 2D-PhC array fabrication

The two-dimensional photonic crystal (2D-PhC) nanocavities were fabricated into a 400-nm-thick silicon nitride (Si_3N_4) layer deposited on a silicon substrate by means of Plasma Enhanced Chemical Vapor Deposition

(PECVD) technique. Si_3N_4 refractive index was measured through spectrophotometric methods (performed with a Varian Cary 5000 spectrophotometer), giving a value of $n_{SiN} = 1.93$ @ $\lambda = 600$ nm.

The PhC geometry was defined using a Raith150 e-beam lithography tool. A thickness of 400 nm of ZEP520-A resist was chosen to ensure sufficient durability as a mask for pattern transfer into the underlying Si_3N_4 and, at the same time, to ensure good resolution of e-beam writing. The patterns defined in the ZEP were then transferred into the Si_3N_4 layer using inductive coupled plasma reactive ion etching (ICP-RIE) in fluorine chemistry until the silicon substrate surface was reached. The membrane structure was released by wet etching of the underlying Si substrate in a TetraMethylAmmonium Hydroxide (TMAH) solution.

Each sample consisted of an array of H1 nanocavities, whose dimensions were scaled according to the lattice period a (in the range $a =$ 257 nm – 277 nm) thus allowing spectral shifting of the resonant wavelength.

2.3 Si_3N_4 surface functionalization and hybridization experiments

Si_3N_4 surfaces containing 2D-PhC nanocavities were chemically modified as reported in [13]. Briefly, samples were preliminary washed with acetone, isopropanol, MilliQ water for 10 min each and then cleaned for 30 min by UV/Ozone (UV-tip Cleaner, BioForce Nanosciences, Inc.). Amino groups were chemisorbed on the surface by pouring the samples in a 0.5% v/v APTES (Sigma-Aldrich) in aqueous solution for 5 minutes, followed by rinsing in water and overnight storage in vacuum. Subsequently, solutions of gluteraldehyde (GTA) at a concentration of 2.5% (v/v) in 100 mM phosphate buffer were incubated onto the layer and reactions were carried out at 4 °C in the dark for 2 h. Afterward, substrates were abundantly washed with MilliQ water and dried in air. GTA functionalized 2D-PhC nanocavities were then covalently linked with two class of probe biomolecules, namely aminated-ssDNA and antibodies.

Hybridization experiments were carried out using complementary target DNA sequences modified with Cyanine 3 (Cy3), and Rhodamine (TRITC)-conjugated AffiniPure $F(ab')_2$ Fragment Goat Anti-Mouse IgG (H+L). Standard solutions of Cy3-modified ssDNA probes (1 μM) and antibodies (1:200) were spotted onto GTA functionalized 2D-PhC nanocavities.

2.4 Optical investigation

Confocal microscopy (Olympus Fluoview 1000) was performed in order to detect fluorophore-labeled bioanalytes emission. The excitation laser (CW @ $\lambda = 514$ nm) was focused on the sample by means of an objective lens and samples photoluminescence (PL) was detected through a 300 mm spectrometer equipped with a nitrogen cooled Charge Coupled Device (CCD). In order to increase signal-to-noise (S/N) ratio, a polarization cube and a half wavelength plate have been introduced in the optical path, thus enabling detection of the two cross-polarized resonant modes of the PhC cavity.

3. RESULTS AND DISCUSSION

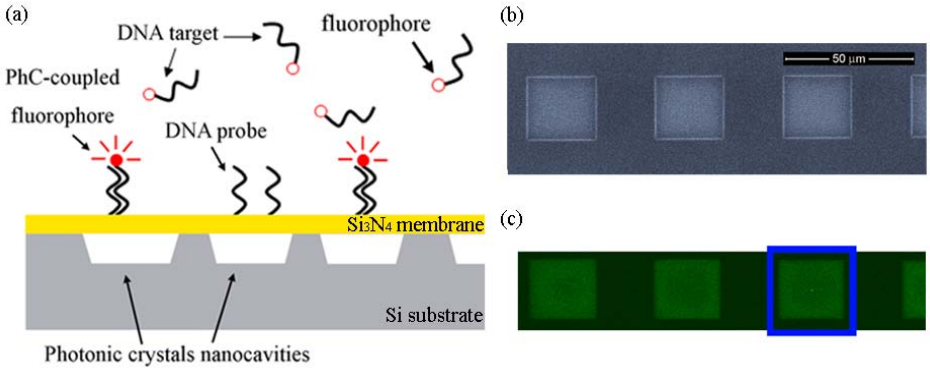

Figure 1: (a) sketch of the proposed architecture. ssDNA or antibodies probes are linked to 2D-PhC cavities realized on Si_3N_4 membranes. Complementary target molecules labelled with fluorophores are allowed to hybridize on the surface. Emission of surface-bound molecules is peculiarly modified by the underlying cavity modes. (b) Scanning Electron Micrograph of an array of 2D-PhC cavities. (c) Confocal image of the array, showing enhancement of fluorophores emission due to the cavity resonance (blue square)

Si_3N_4 biocompatibility and its possibility to be functionalized have been here exploited to develop an array of 2D-PhC nanocavities, able to enhance the emission properties of fluorescent markers bound to an unknown biological assay.

Figure 1(a) is a sketch of our PhC nanocavity biochip architecture. Si_3N_4 nanoresonators, each having a different resonant wavelength, are arranged in a matrix configuration. Specific biomolecules (probes) are immobilized through chemical surface modification. The as-prepared surface is allowed to selectively bind to complementary target species, or analytes, contained in the biological solution under investigation and

previously conjugated with fluorescent markers [14]. Two different prototypes have been developed for the detection of different biomolecules, namely DNA and proteins, conjugated with Cy3 organic dyes and TRITC fluorescent proteins, respectively.

Detection of the binding event can be performed by optical investigation of the device. According to already proposed DNA or protein microarrays [15], localization of bright spots in the detection area (also referred to as biochip readout area) allows the compositional recognition of the biological solution under investigation, since its spatial coordinates univocally identify each probe. Besides this, use of PhC resonators improves detection is two ways: 1) a significant increase in the luminescence intensity of the markers coupled to the PhC cavities is expected, thanks to the increased radiative emission rate induced by the Purcell effect [16]; 2) different bioprobes can be assigned to different resonators, thus an univocal correspondence between resonant wavelengths and labeled biomolecules is obtained. The spectral labeling enables the identification of the biological assay by means of a spectral scan of the readout area emission, therefore improving multiplexing capability of the system. Figure 1(b) shows a Scanning Electron Microscopy (SEM) image of the fabricated array of nanocavities after hybridization with ssDNA-Cy3 molecules, while Figure 1(c) reports its photoluminescence map collected at $\lambda = 592$ nm, corresponding to a resonant mode of the third cavity (marked in blue). Here, both the above-mentioned improvements are shown. In fact, the bright spot in the defect region witnesses a clear enhancement of Cy3 luminescence; moreover, emission of bioanalytes localized on neighboring cavities is not increased, being in out-of-resonance conditions.

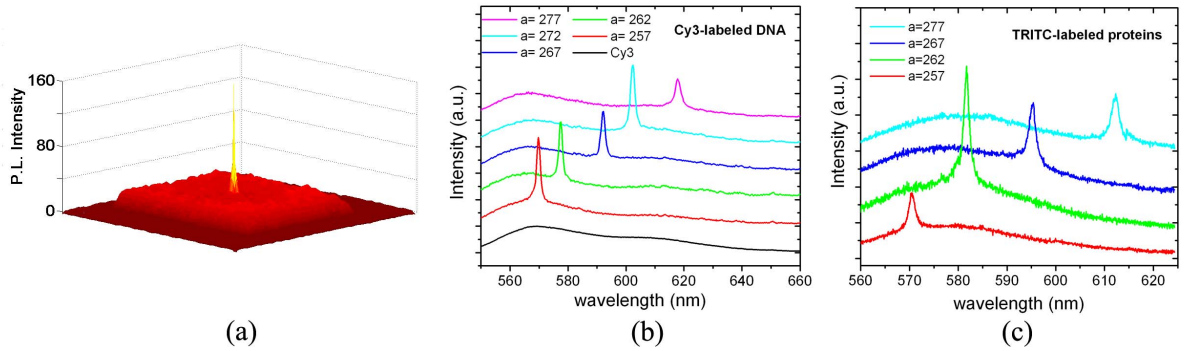

Figure 2: (a) Three dimensional intensity profile of photoluminescence collected from Cy3-labeled DNA captured by a functionalized nanocavity. Emission outside the PhC pattern has been normalized to unit; (b)-(c) PL spectra of the PhC nanocavities, collected from (b) Cy3-labeled DNA and (c) TRITC-labeled proteins.

A detailed quantification of the enhancement factor has been performed on the microarray functionalized with Cy3-labeled DNA. Figure 2(a) shows a three-dimensional intensity profile of a nanocavity in resonant conditions. Photoluminescence outside the PhC pattern has been normalized to unit. It is clear that the photonic crystal pattern itself significantly contributes to enhance the emission signal of Cy3, up to ~20 times as compared to the unpatterned region. Although Si3N4 surface has been uniformly functionalized, in the PhC region a larger surface area is exposed to probes immobilization due to the freestanding membrane configuration, partly contributing to the detected increase. Moreover, in 2D-PhC patterns an efficient transfer channel between externally radiated light and energy trapped in the membrane is represented by the so-called leaky modes [17]. The coupling of such modes with the absorption or emission bands of neighboring emitters may lead to a significant increase of their luminescence. In our PhC-NC biochip, the leaky modes localized on the PhC pattern are responsible for the further increase of the luminescence experimentally observed [13]. In the cavity defect region, the presence of a very intense resonant peak is also clear. Notably, a ~8-fold increase with respect to the photonic crystal pattern is reached in resonant conditions, which corresponds to an overall enhancement of ~160 as compared to unpatterned Si_3N_4 surfaces. Together with the previously discussed enhancement mechanisms, a major role of the Purcell effect [16,18] is here envisioned, by virtue of the strong optical quantum confinement performed by the H1-shifted nanocavities.

Spectral labeling is also shown in Figures 2(b) and 2(c) for samples functionalized with Cy3-labeled DNA and TRITC-labeled proteins, respectively. Here, the well-known scaling properties of photonic crystals have been exploited to tune the cavity resonant wavelength along the broad emission spectra of the two fluorophores. Each cavity shows its peculiar spectral shape, composed by the TRITC or Cy3 emission lineshape superimposed to a sharp resonant peak having a Q-factor as high as 725, corresponding to a full-width at half maximum (FWHM) of ~ 0.9 nm. Taking into account the spectral resolution limits, a conservative estimate suggests the possibility to distinguish up to 150 different spectral footprints (by considering a ~ 150 nm bandwidth of the Cy3 emission spectrum, for example). This means that up to 150 parallel analyses can be simultaneously performed

with one single spectral scan of the readout area of the biochip, thus drastically decreasing the time required for a complete compositional identification.

4. CONCLUSIONS

In this work, the application of PhC technology in biosensing devices has been demonstrated. The insertion of nanocavities in classical biochip architectures leads to a 160-fold increase of the emission intensity of fluorescent markers. The obtained higher sensitivity can be exploited to detect reduced amounts of target biomolecules in the investigated solution. Furthermore, peculiar spectral footprints are univocally associated to target analytes captured by their surface, thus allowing analysis of assay composition based both on spatial and spectral observation of the read-out optical response, with clear benefits on parallel detection of multiple elements. We have therefore evidenced the suitability of Si3N4 as a versatile material for the realization of opto-bio devices able to engineer the spontaneous emission of nanoemitters in the visible spectral range.

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