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Spectral tagging by integrated photonic crystal resonators for highly sensitive and parallel detection in biochips

Luigi Martiradonna,^{1,a)} Ferruccio Pisanello,^{2,3} Tiziana Stomeo,² Antonio Qualtieri,^{1,2} Giuseppe Vecchio,¹ Stefania Sabella,¹ Roberto Cingolani,¹ Massimo De Vittorio,^{1,2} and Pier Paolo Pompa¹

¹*Istituto Italiano di Tecnologia (IIT), Center for Bio-Molecular Nanotechnology, Via Barsanti 1, Arnesano, Lecce 73010, Italy*

²*National Nanotechnology Laboratory, CNR/INFM, Scuola superiore ISUFI, Università del Salento, 16 Via Arnesano, Lecce 73100, Italy*

³*Laboratoire Kastler Brossel, Université Pierre et Marie Curie, CNRS UMR 8552, Ecole Normale Supérieure, 4 place Jussieu, 75252 Paris Cedex 05, France*

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We propose a technological approach aimed at improving biochips performances, based on an efficient spectral modeling and enhancement of markers fluorescence through the insertion of photonic crystal nanocavities (PhC-NCs) in the readout area of biochips. This strategy univocally associates a specific emission wavelength to a specific bioprobe immobilized on a nanocavity, therefore guaranteeing parallel detection of multiple elements and faster analysis time. Moreover, PhC-NCs significantly enhance the markers fluorescence, thus improving the detection sensitivity. © 2010 American Institute of Physics. [doi:10.1063/1.3360810]

Biodiagnostics is a rapidly developing field, where the use of micro- and nanotechnologies enables major advancements in sensitivity and sample volume requirements.^{1–5} The light molding properties of photonic crystals⁶ (PhC), intended as periodic dielectric perturbations at wavelength scale, are considered a fundamental backbone for photonic applications^{7–12} and, recently, have been profitably exploited for the enhancement of optical sensors and transducers for biochemical analyses.^{13–15}

Here we propose the idea of exploiting the sharp resonances of PhC nanocavities to assign unique spectral features to fluorophore-labeled bioanalytes, thus allowing their identification through wavelength-resolved light detection. Spectral tagging of organic dyes through photonic crystal nanocavities is experimentally proved to bring important benefits to cutting-edge devices for biodiagnostics, such as DNA and protein biochips, in terms of improved sensitivity, efficiency and multiplexing capability.

Figure 1(a) reports a three-dimensional sketch of our PhC NanoCavity biochip (PhC-NC biochip) architecture. An array of two-dimensional PhC (2D-PhC) nanocavities, each having a different resonant wavelength, is realized on a flat substrate. The patterned surface is part of a miniaturized assay for genomic and proteomic analyses, usually referred to as DNA or protein microarray. In such devices, specific biomolecules (probes) are immobilized through chemical surface modification or *in situ* synthesis and allowed to selectively bind to complementary target species, or analytes, contained in the biological solution under investigation.¹⁶ Since fluorescent markers are typically conjugated to the target analytes, the binding events can be revealed through optical inspection of the biochip readout area, thus allowing a complete compositional analysis of the assay.¹⁷ In inset of Fig. 1(a), an example of probe molecules immobilized on the

PhC patterns is reported, showing biorecognition and capturing of specific fluorophore-labeled target sequences. Microarray configurations, based on the localization of different probes in different regions of the readout area, permit a certain degree of parallelism in the analysis thanks to spatial discrimination.^{17,18} In our approach, different bioprobes are assigned to different resonators, thus establishing a one to one correspondence between resonant wavelengths and labeled biomolecules.¹⁹ In this way, despite all target analytes are labeled with the same marker, upon specific binding to the complementary bioprobes, they acquire a peculiar spectral signature, thanks to the interaction of the fluorophore with the confined optical modes resonating in the cavity. A

FIG. 1. (Color online) Sketch of the proposed strategy for PhC- NC biochip. (a) Schematic of the array of PhC nanocavities patterned on the readout area. Inset: Detail showing examples of PhC nanocavities. The cavities are functionalized with different probes molecules, that specifically interact with complementary target analytes labeled with fluorescent markers. The signal is collected from this area and spectrally discriminated in order to identify the different spectral tags univocally associated to each nanocavity and thus to each bioprobe. (b) Example of a possible luminescence detected from the whole readout area (black line) as compared to the unmodified broad marker luminescence (red dotted line). The presence of each peak in the spectrum reveals the presence of the corresponding analyte in the investigated assay.

^{a)}Author to whom correspondence should be addressed. Electronic mail: luigi.martiradonna@iit.it. Tel.: +39832295726. FAX: +39832298386.

spectral scan of the signal collected from the whole readout area resembles the profile sketched in Fig. 1(b): The broad emission spectrum of the fluorescent marker is superimposed to distinct peaks, each tagging the presence of a specific target analyte in the investigated assay. Besides the spatial discrimination implemented in microarray configurations, in this case the spectral distinction contributes substantially to the parallelization of the device. Moreover, thanks to the increased radiative emission rate induced by the Purcell effect,^{20,21} a significant increase in the luminescence intensity of the markers coupled to the PhC cavities is also expected, thus significantly improving the signal-to-noise ratio and the overall sensitivity of the biochip detection.

In order to verify the feasibility and wide applicability of this approach, we realized two prototypes of PhC-NC biochips for the detection of different biomolecules, namely DNA and proteins. PhC nanocavities resonating in the visible spectral range were fabricated in silicon nitride (Si_3N_4) membranes on a Si substrate, exploiting a modified single defect H1 nanocavity.^{22,23} This consists of a triangular air-holes lattice pattern, with a local periodicity variation around a missing air hole.²⁴ A careful modeling 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 and 90, respectively.²⁵ Each fabricated prototype consists of arrays of optimized H1 resonators with variable lattice constant a , corresponding to different resonant wavelengths.

The two samples were prepared by covalently immobilizing single-stranded DNA (ss-DNA) or antibody probes on the Si_3N_4 surface, respectively. Complementary DNA targets or specific secondary antibodies, labeled with cyanine 3 (Cy3) and rodhamine (TRITC) fluorophores, respectively, were then allowed to recognize the immobilized probes, thus obtaining a uniform fluorescent monolayer of the biomolecular species.²⁵

Spatially-resolved spectral mapping of the PhC-NC biochips surface was performed through a confocal microscope.²⁵ In Fig. 2(a) we display four examples of the emission spectra from TRITC-labeled proteins localized onto the PhC nanocavities; the influence of the H1 resonators underneath the molecules is clearly evidenced by the presence of intense resonant peaks superimposed to the broad TRITC emission lineshape. Similarly, Fig. 2(b) shows the emission spectra collected from DNA-functionalized nanocavities (five uppermost lines), compared to the emission of Cy3-DNA strands without photonic resonators (lowest spectrum). In both cases it is evident that, by simply varying the lattice period a of the photonic crystal resonator, it is possible to assign a specific spectral feature to the emission band of the target analytes captured by the probes onto the different cavities, even though they are all labeled with the same broad emitting organic dye. The best measured Q-factor obtained in the PhC-nanocavities DNA-chip prototype is ~ 725 , corresponding to a full-width at half maximum of ~ 0.9 nm. Taking into account the spectral resolution limits, a conservative estimate suggests the possibility to distinguish up to 150 different resonant peaks within the ~ 150 nm bandwidth of the Cy3 emission spectrum. 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 drasti-

FIG. 2. (Color online) Optical characterization of a PhC-nanocavities biochip prototype. (a) PL spectra collected from TRITC-labeled proteins captured onto the PhC nanocavities; (b) PL spectra collected from Cy3-labeled DNA (colored lines) onto the PhC nanocavities as compared to Cy3 emission spectrum collected on a PhC pattern (black line); (c) PL maps of an array of five Cy3-labeled DNA-PhC nanocavities, collected at different wavelengths [also indicated (b)]. For each spectrum and PL map, the reported a value indicates the lattice period of the measured PhC nanocavity (enhanced online). [URL: <http://dx.doi.org/10.1063/1.3360810.1>]

cally decreasing the time required for a complete compositional identification.

Figure 2(c) displays the photoluminescence maps collected from an array of five different nanocavities, functionalized with ss-DNA and hybridized with Cy3-labeled complementary DNA sequences. Each map is acquired by collecting a 2 nm wide spectral signal centered at one of the five resonating wavelengths of the nanocavities, marked from λ_1 to λ_5 in Fig. 2(b). By comparing the different images, it is possible to distinguish a bright spot in the center of each nanocavity when the detection wavelength matches the resonant frequency. The wavelength-dependent ON-OFF switching of the cavity regions can be better visualized in the spectral scan video provided in the Supplementary material, obtained by collecting photoluminescence maps at different wavelengths in the 552–638 nm range.

Besides the resonant effect of the H1 nanocavity, it is noteworthy that the photonic crystal pattern significantly contributes to enhancement of the emission signal of Cy3 and TRITC. The squared areas occupied by each photonic crystal pattern are appreciably brighter than the surrounding unpatterned Si_3N_4 layer, although the immobilization and hybridization processes have been homogeneously performed on the whole sample surface. Figure 3 represents a three-dimensional (3D) intensity profile collected on a H1 nanocavity upon hybridization with Cy3-labeled DNA. The profile has been taken at the resonant wavelength of the

FIG. 3. (Color online) 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. A ~ 20 -fold luminescence enhancement due to the PhC pattern, as compared to the unpatterned Si_3N_4 surface, has been measured. The cavity confinement further enhances Cy3 emission up to ~ 160 -fold.

analyzed cavity. The photonic crystal pattern enhances the Cy3 luminescence up to ~ 20 times as compared to the unpatterned region. This behavior may be ascribed to the combination of two effects. First, the free standing membrane layer makes available a larger surface area to the probes immobilization (about a factor of 4 compared to the unpatterned layer), resulting in a higher number of immobilized Cy3-labeled analytes in the PhC regions. Second, 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.^{13,26,27} The coupling of such modes with the absorption or emission bands of neighboring emitters may lead to a significant increase of their luminescence.^{12,13} In our PhC-NC biochip, the leaky modes localized on the PhC pattern²⁵ are responsible for the further increase of the luminescence experimentally observed. Nevertheless, at this stage our PhC pattern has been designed to improve the resonant modes localized in the H1 defect region, while a specific optimization of leaky modes effects has not been performed. Further theoretical and experimental efforts aimed to combine both enhancing effects will be the subject of forthcoming investigation.

In Fig. 3, the presence of a very intense resonant peak is also clear. Notably, a approximately eightfold 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^{20,21} is here envisioned, by virtue of the strong optical quantum confinement performed by the H1-shifted nanocavities.

The insertion of PhC cavities in classical biochip architectures leads, therefore, to a huge increase of the emission intensity of fluorescent markers, thus providing higher sensitivity, and allowing detection of very small amounts of target biomolecules in the investigated solution. In addition, the nanocavities attribute peculiar spectral features to the target analytes captured by their surface, so that the presence of specific species in the solution can be inferred by a simple spectral analysis of the optical response of the read-out region. This enables parallel detection of multiple elements, thus accelerating the analysis time. In preliminary demonstrations, emission enhancements as high as 160-fold along with high multiplexing capability and fast detection have been obtained by engineering PhC H1 resonators. Further

significant improvements in sensitivity can be foreseen by combining the enhancing effects of leaky modes with resonant cavity modes. Importantly, the proposed detection system based on the spectral identification of different analytes can be complementarily implemented in traditional systems based on spatial recognition to further increase the degree of parallelization. The interesting achievements here obtained may constitute a further step toward the next generation of PhC-assisted biochips, whose potential as fast, highly sensitive devices is far from being completely expressed yet.

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