Improvement of antigen detection efficiency with the use of two-dimensional photonic crystal as a substrate

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Abstract. Multiplex detection of different antigens in human serum in order to reveal diseases at the early stage is of interest nowadays. There are a lot of biosensors, which use the fluorescent labels for specific detection of analytes. For instance, common method for detection of antigens in human serum samples is enzyme-linked immunosorbent assay (ELISA). One of the most effective ways to improve the sensitivity of this detection method is the use of a substrate that could enhance the fluorescent signal and make it easier to collect. Two-dimensional (2D) photonic crystals are very suitable structures for these purposes because of the ability to enhance the luminescent signal, control the light propagation and perform the analysis directly on its surface. In our study we have calculated optimal parameters for 2D-dimensional photonic crystal consisting of the array of silicon nano-rods, fabricated such photonic crystal on a silicon substrate using reactive ion etching and showed the possibility of its efficient application as a substrate for ELISA detection of human cancer antigens.

1. Introduction

Photonic crystals (PhC) are widely used in many scientific areas nowadays including optoelectronics, photonics [1], gas sensing [2] and biosensing [3–5]. The numbers of directions in which the PhC have refraction index periodicity determines their classification within the three principle groups: one- (1D), two- (2D) and three- (3D) dimensional PhC. Each group of PhC has its own advantages and disadvantages in applications, depending on the physical properties of PhC material and fabrication technique. For instance, 1D PhC may be easily fabricated by electrochemical etching of silicon wafers and have large surface area, what makes them suitable for fabrication of different sensors. However, by using 1D PhC it is difficult to achieve a very low mode volume and high Q-factor in order to observe strong coupling effect. 3D photonic crystals, on the other hand, have great confinement in all three dimensions, but their fabrication is much more sophisticated, and the need for periodicity in all dimensions makes it almost impossible to change refractive index inside the structure and thus vary their photonic properties. 2D PhC could be fabricated using a well-developed electron beam lithography technique from a silicon wafer, which is a conventional material for semiconductor industry. Rather high refractive index of silicon (around 4 in the visible range) leads to a great refractive index contrast in air. The presence of one “free” dimension in a 2D photonic crystal, in
which the confinement of electromagnetic waves is only due to the index waveguiding, opens up an opportunity to alternate refractive index of the media inside the PhC by introduction of analyte, and hence to affect on photonic properties of the system. Moreover, 2D PhC allow to control the propagation of light in plane of periodicity, also, the very high Q-factors along with low mode volumes could be achieved. Thereby, weak coupling regime between the cavity of 2D photonic crystal and the luminophores embedded inside the PhC structure can be possible, and the luminescence inside the cavity could be enhanced [6]. These properties make 2D PhCs appear to be the best compromise for application in biosensing, for example as a substrate for improvement of luminescence detection techniques. In this study we used 2D PhC in form a of an ordered array of silicon nanorods on a silicon wafer substrate for detection of antigens in human serum samples in enzyme-linked immunosorbent assay (ELISA). We have used quantum dots (QDs) as the fluorescent markers because of their high photostability, fluorescence quantum yield almost reaching 100 % and ability to be conjugated with the detection antibodies using well developed approaches [7]. We have also made a finite difference time-domain (FDTD) calculation of the optimal parameters for 2D PhC array made of silicon nanorods, fabricated photonic crystal with desired properties on a silicon substrate using electron beam lithography and reactive ion etching (BOSCH process) and have demonstrated the possibility of its efficient application as a substrate for ELISA detection of human cancer antigens.

2. Materials and methods

2.1 Materials
Cadmium oxide (powder, 99.5%), zinc oxide (powder, 99.99% trace metals basis), 1-octadecene (ODE, technical grade, 90%), oleic acid (OA, technical grade, 90%), selenium (powder, 100 mesh, 99.5%), oleylamine (OLA, technical grade, 70%), thiourea (ACS reagent, ≥99.0%), tri-n-octylphosphine (TOP, technical grade, 97%), tri-n-octylphosphine oxide (TOPO, reagent grade, 99%), triethylene glycol dimethyl ether (TEGDME, ReagentPlus, 99%), anhydrous solvents (chloroform, hexane, ethanol, methanol and 2-propanol) and hydrogen peroxide solution (30 wt% in H2O) were purchased from Sigma-Aldrich; n-hexadecylphosphonic acid (97%) was purchased from PlasmaChem GmbH. All chemicals were used as received without additional purification. P-type <100> Si wafers were purchased from Telecom-STV.

2.2 Calculation method
For simulation of electromagnetic wave propagation in a 2D photonic crystal and further determination of photonic structure properties we used FDTD approach. Software from MIT (MEEP) was used for calculations. Different types of structures, cavities, lattice periods and rod morphology for rod-type 2D photonic crystal have been tested in order to achieve a good mode confinement and wide enough photonic bandgap for TM polarization with the cavity eigenmode at 565 nm wavelength corresponding to the maximum of QD photoluminescence that have been used as fluorescent markers.

2.3 Photonic crystal fabrication and characterization technique
Monocrystalline silicon wafers with 380 µm thickness have been used as an initial substrate. Electron beam lithography has been used to create a Ni pattern mask for subsequent reactive ion etching with the use of BOSCH process with the following parameters: chamber pressure – 5 mTorr, etching gas – SF6 10 sccm, passivation gas – C4F8 100 sccm, O2 10 sccm, etch coil power 450 W and cathode power – 20 W. Scanning electron microscope (SEM) studies have been performed using RAITH 150 TWO in order to estimate the resulting sample morphology after the fabrication.

2.4 Surface modification of the photonic crystal substrate
Surface functionalization of the previously fabricated silicon substrates included three stages: (i) chemical oxidation of the surface of nanorod array, (ii) modification of the oxidized surface with the amine groups and (iii) modification of the exposed amine functional groups by succinic anhydride. Surface oxidation was carried out with using Piranha solution – the mixture of sulfuric acid, hydrogen
peroxide and deionized water in a volume ratio of 3:1:8, respectively. Silicon substrate was placed in the cool oxidative solution with the temperature of 4º C and held there for 2 hours under gentle stirring. Excess of the oxidative solution was washed away from the surface of the silicon wafer by the deionized water. Surface modification with the amine groups was carried out with using (3-aminopropyl) triethoxysilane (APTES). 5 µl of APTES was dissolved in 15 ml of ethanol, and oxidized silicon substrate was placed into the obtained solution for 12 hours under slight agitation. In order to remove unreacted APTES, silicon substrate was placed for three times into a fresh portion of pure chloroform for 15 minutes. Then the substrates were dipped in a solution of succinic anhydride in N, N-dimethylformamide for 30 minutes under continuous stirring, and resultant photonic crystal substrates with carboxyl surface functionality were finally washed with chloroform according to the procedure used to remove unreacted APTES.

2.5 Preparation of water-soluble core-shell CdSe/ZnS quantum dots.
Core CdSe QDs used in this study were synthesized from CdO and TOPSe by injection technique, and further coated by a ZnS shell using the SILAR technique. The details of the procedure is described in details in [8]. After the synthesis, QDs were modified with DL-cysteine to render them water-soluble, and further treated using thiol-PEG-carboxylate to achieve high stability of colloidal solution and fluorescence properties.

3. Results and discussion
In our study we have investigated the possibility of improvement of antigen detection efficiency with the use of photonic crystal as a surface for immunocomplex formation. Two candidate types of 2D photonic crystals were considered, one of which represents a crystal slab with holes forming a PhC, and an array of nanorods on a substrate. In order to achieve good embedding of the liquid samples inside PhC structure and to have an ability to regenerate the surface for multiple detections, we have chosen the structure consisting of nanorods with line of nanorods missing in the center forming a defect. This type of structure is schematically shown in Figure 1.

![Figure 1](image)

**Figure 1.** Schematic representation of a 2D photonic crystal structure used in this work.

The following parameters were found to be optimal to achieve photonic bandgap big enough to cover the photoluminescence spectrum of QDs by the FDTD calculation: lattice period 280 nm, diameter of individual nanorod around 100 nm and nanorod height of 600 nm. SEM image of a 2D PhC fabricated using such parameters is demonstrated in Figure 2.
The next step was to perform a modification of the surface for further antigen immobilization using the procedure described in materials and methods section. After the surface modification and immunocomplex formation, we measured the luminescent signal of quantum dots using TECAN Infinite® 200 or TECAN Spark® 10M fluorescence readers. The fabricated arrays demonstrated a full compatibility with this standard model of fluorescent readers by and good repeatability of the obtained signal. Typical luminescence spectrum measured from QDs immobilized on the 2D PhC substrate is shown on Figure 3.

4. Conclusions
In this study we have calculated optimal parameters for two-dimensional photonic crystal consisting of the array of silicon nanorods in order to enhance the photoluminescence of embedded QDs, fabricated photonic crystal with such properties from a silicon wafer using BOSCH process. Surface modification technique has been developed for efficient immobilization of antibodies on photonic
crystal surface. Finally we have demonstrated the possibility of its application for the immunodetection of human cancer antigens using a standard fluorescent reader.

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