Photonic crystal Microarray Nanoplatform for High Throughput Detection of Biomolecules

Swapnajit Chakravarty*[a], Wei-Cheng Lai[b], Kathryn Moncivais[c], Xiaolong Wang[a] , Che-Yun Lin[b], Zhiwen J. Zhang[c], Ray T. Chen[b],

[a]Omega Optics Inc., 10306 Sausalito Drive, Austin, TX, USA 78759; [b]Dept. of Electrical and Computer Engineering, University of Texas, 10100 Burnet Road Bldg. 160, Austin, TX, USA 78758; [c]College of Pharmacy, University of Texas, 2515 Speedway, Austin, TX, USA 78712

ABSTRACT

We present preliminary designs and experimental results for creating a microarray nanoplatform based on two-dimensional photonic crystal devices in silicon. Multiple photonic crystal microcavities are coupled along the length of a single photonic crystal waveguide that undergo resonance wavelength shifts when an antibody-antibody binding event occurs in the immediate vicinity of the corresponding photonic crystal microcavity. The microarray nanoplatform enables high throuput measurements of multiple antibody-antibody interactions via a single optical waveguide transmission measurement.

Keywords: photonic crystal waveguide, photonic crystal microcavity, nanophotonic microarray, multiple analyte detection.

1. INTRODUCTION

Microarrays provide an unprecedented opportunity for comprehensive concurrent analysis of thousands of biomolecules such as proteins [1], genes [2], DNA molecules [3], small molecules [4] or nucleic acids [5]. The global analysis of the response to a toxic agent, as opposed to the historical method of examining a few select biomolecules, provides a more complete picture of toxicologically significant events. Array based expression profiling is useful for differentiating compounds that interact directly with the species from those compounds that are toxic via a secondary mechanism. Microarrays are consequently finding numerous applications in pathogen detection and biodefense [6].

In recent years, lab-on-a-chip systems for detection of biomolecules at very low concentrations have become very important for high throughput biomolecular analyses, such as protein assays for basic molecular biology research [1], disease marker identification [7] and pharmaceutical drug screening [8]. At present, the most frequently used technique for detection of target-probe binding is fluorescence detection [3, 9]. Other techniques include wavelength absorbance, ELISA (enzyme-linked immunosorbent assay), and radiometric methods. A *major drawback of these methods is the need to perform target labeling* and the potential to cause changes in target-probe interactions due to structural or functional changes or steric hindrance induced by the label. These assays are functional and rely on inhibition of particular reactions. However, not all binding events of a probe molecule to a target result in functional inhibition. At the same time, arrays of immobilized target biomolecular species must be fabricated and their functionality preserved in aqueous phase. Hence, alternative technologies to affinity- tagged detection that preserve biomolecule functionality in aqueous phase are being actively sought.

In this paper, the authors propose a label-free microarray based on multiple photonic crystal microcavity devices coupled to a photonic crystal waveguide that preserves the biomolecule functionality in aqueous phase. The proposed program will develop a portable, diagnostic device suitable for highly parallel, label-free biomolecule detection and identification for cancers and allergies.

2. PRINCIPLE OF OPERATION

The device consists of a triangular lattice of air holes etched into silicon in a silicon-on-insulator (SOI) substrate. Photonic crystal waveguides are defined and photonic crystal microcavities with different geometries are fabricated at

*swapnajit.chakravarty@omegaoptics.com;   phone 1 512 996-8833; fax 1 512-873-7744; omegaoptics.com

specific intervals along the length of the photonic crystal waveguide. The resonant wavelength of a photonic crystal microcavity is dependent on the geometry of the microcavity. Light propagating in a photonic crystal waveguide couples to a photonic crystal microcavity at the resonant wavelength of the microcavity. The transmission spectrum of the photonic crystal waveguides consequently shows minima corresponding to the resonant wavelength of each photonic crystal microcavity. Target proteins for bio-agents and pathogens, each specific to a unique probe molecule for diagnostic distinction, are patterned on a unique microcavity using a fabrication technique that preserves the protein functionality. When a probe molecule binds with its specific target, the resonant wavelength of that microcavity shifts which consequently shifts the corresponding minimum in the waveguide transmission spectrum. The shift in the minimum in the transmission spectrum identifies the occurrence of a binding event. The magnitude of binding is determined by De Feijter's formula [10] that relates the absolute amount of adsorbed molecules M with the change in refractive index as:

$$M = d_A \frac{n_A - n_C}{dn/dc}$$

where d_A is the thickness of adsorbed layer, n_A is the refractive index of adsorbed molecules, n_C is the refractive index of cover solution and dn/dc is the change in refractive index of molecules which is proportional to the shift $d\lambda$ in position of the resonance peak [11]. The magnitude of resonant wavelength shift is proportional to the amount of adsorbed biomolecules and hence provides a label-free means to quantitatively determine biomolecules of interest.

The *goal of the research is multiple analyte sensing with a single measurement*. In spite of significant research performed in semiconductor optofluidic detection and integration by various groups [12] to achieve sensing of individual species, sometimes to single molecule levels [13], limited research has been performed to achieve multiplexed label-free miniaturized lab-on-a-chip optofluidic protein microarrays. It must be noted that the detection principle of our device is significantly different from fluid sensing techniques demonstrated using photonic crystal waveguides only [14-16], which rely on shifts of photonic crystal band edge upon sensing and thereby can identify only one analyte at a time (since there is only one stop gap), or research performed that couples light straight from a standard ridge waveguide into a single photonic crystal microcavity [17-19] which can again identify only one analyte at a time. Multiplexed structures in two-dimensional photonic crystals have demonstrated modest quality-factors of the resonances [20]. Recently, ring resonators coupled to waveguides are being designed for multiplexed antibody-antibody binding detection [21], with one ring being measured per waveguide; ring resonator devices however, have significantly narrow free spectral range (~5nm) than photonic crystal microcavity devices (~60nm) that limits the number of rings that can be multiplexed along a single waveguide with a narrow-band wavelength source.

3. DEVICE DESIGN FOR ON-CHIP SPECTROSCOPY

Fig. 1 shows a typical microcavity resonance spectrum of a L3 photonic crystal microcavity. A L3 microcavity is formed by removing a row of three air holes as shown in the top inset of Fig. 1. The mode profile of the L3 microcavity is shown in the bottom inset. As noted from the experimental spectrum of the L3 microcavity, the free spectral range of the photonic crystal microcavity is ~57nm when designed around the center wavelength of 1550nm.
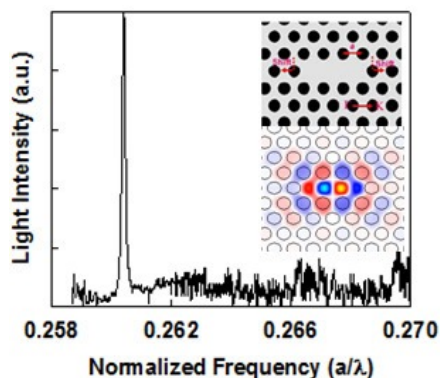

Fig. 1: Experimental transmission of a L3 photonic crystal microcavity. (top inset) Schematic of a L3 photonic crystal microcavity. (bottom inset) TE mode profile of the photonic crystal microcavity showing strong confinement in the dielectric volume of the L3 photonic crystal microcavity.

Several L3 microcavities are fabricated along the length of a single photonic crystal waveguide. Fig. 2(a) shows a three-dimensional finite difference time domain (FDTD) simulation where 8 resonances from 8 individual photonic crystal microcavities are coupled to a single photonic crystal waveguide. Schematic of our silicon PC slot waveguide device with geometry parameters is shown in Fig. 2(a). The photonic crystal waveguide is a W1 line defect with uniform lattice constant a, and width $\sqrt{3}a$. A photonic crystal impedance taper is designed, as in ref. [22] to enable efficient coupling of light into the slow light region where coupling efficiency into photonic crystal microcavities is enhanced.
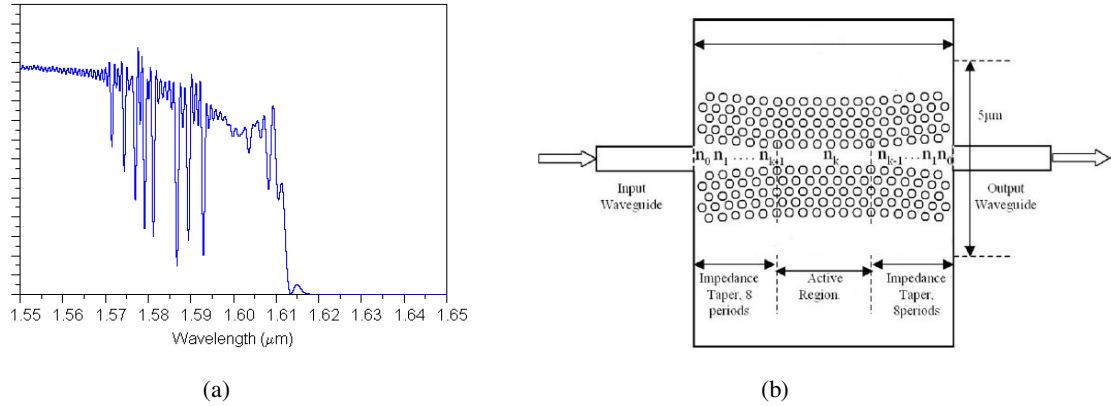

(a)

(b)

Fig. 2: (a) Finite Difference Time Domain (FDTD) simulation for a set of 8 photonic crystal microcavities coupled to a single photonic crystal waveguide. (b) Schematic showing the input-output coupling to the photonic crystal waveguide from ridge waveguides using impedance tapers [22]

4. DEVICE FABRICATION

Devices were fabricated on silicon-on-insulator (SOI) wafer with 230nm top silicon layer and 3μm buried oxide. 45nm thermal oxide was grown on top of silicon as etch mask for pattern transfer. PC waveguides, PC impedance tapers, and strip waveguides are patterned in one step with e-beam lithography followed by reactive ion etching. Scanning electron micrograph (SEM) of fabricated structure is shown in Fig 3. Fig. 4 shows scanning electron micrograph images of the devices fabricated.

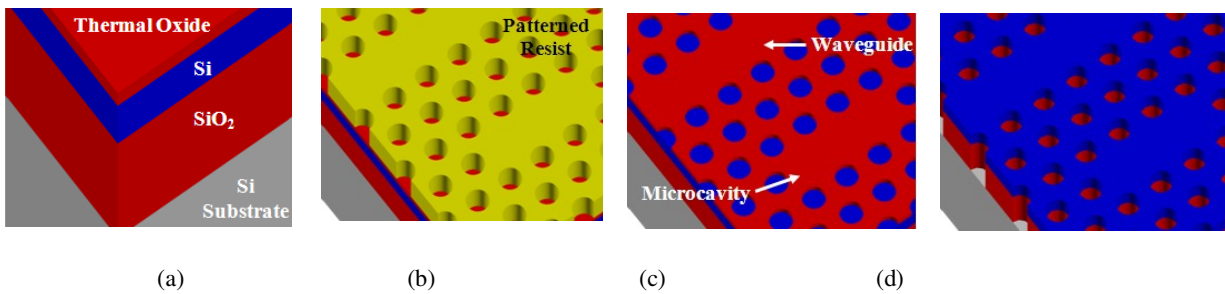(a)   (b)   (c)   (d)

Fig. 3: Fabrication steps of a photonic crystal (a) Growth of thermal oxide (b) E-beam Resist (ZEP-520A) patterning (c) Transfer resist pattern to thermal oxide by RIE using CHF_3 followed by resist strip (d) Transfer pattern from SiO_2 to Si by RIE in HBr and Cl_2. [**Silicon (blue), Silicon Dioxide, SiO_2 (red)**].
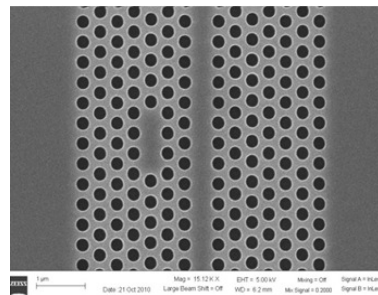

Fig. 4: Scanning electron micrograph (SEM) images of photonic crystal microcavity coupled into a single photonic crystal waveguide.

5. EXPERIMENTAL SETUP

The devices were tested on a Newport six-axis auto-aligning station. Input light from a broadband source was TE-polarized and butt-coupled to / from the device with polarization maintaining single mode tapered lensed fiber with mode field diameter ~3μm. Preliminary experiments have been performed with a single photonic crystal microcavity coupled to a single photonic crystal waveguide. The wafers need to be functionalized for protein binding prior to bio-sensing. Wafers were treated with 1mg/mL poly-L-lysine in water, followed by washing 3 times in PBS. The wafers are then incubated in 1% glutaraldehyde in PBS for 5 minutes and then treated with Goat anti-rabbit Alexa Fluor 594 antibodies in 30% glycerol. The wafers are then washed 3 times in PBS again. Our device is intended for label-free operation; we have used labeled biomolecules for our research to enable correlation of the binding experiments with fluorescence binding experiments.

6. EXPERIMENTAL RESULTS

In Fig. 5(a), the photonic crystal waveguide transmission spectrum is shown. The single photonic crystal microcavity resonance is dropped from the waveguide transmission spectrum. The linewidth of the resonance spectrum translates to a quality factor (Q-factor) of 3430.
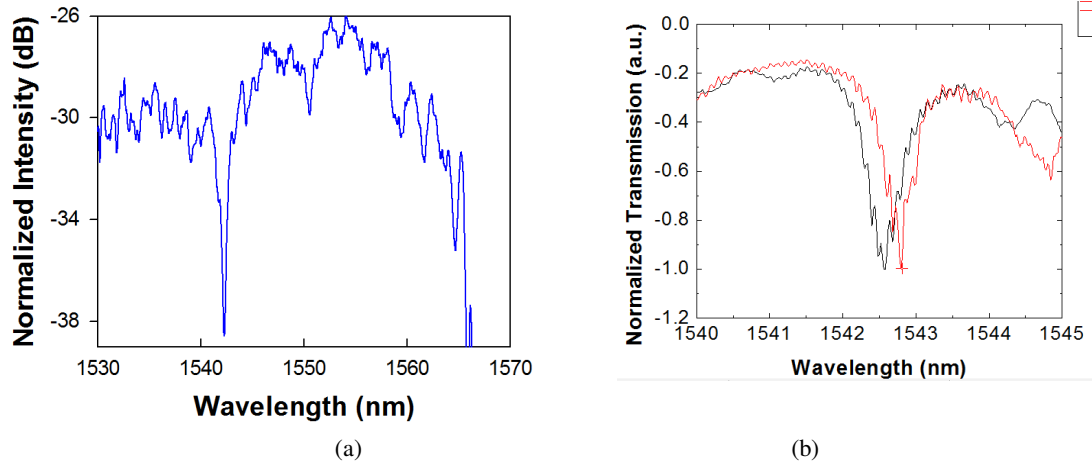
| (a) | (b) |

Fig. 5: (a) Experimental photonic crystal waveguide transmission spectrum showing the photonic crystal microcavity resonance at ~1542nm with 8dB extinction ratio and 3dB linewidth ~0.45nm. (b) Experimental transmission spectrum showing the shift of photonic crystal microcavity resonance shift with probe protein binding (to red) when the probe protein solution is introduced to the sample that has been functionalized and bound with target protein. Target Protein: BioRad HRP-conjugated goat-anti-rabbit antibody. Probe Protein: Invitrogen Rabbit anti-goat Alexa Fluor

Fig. 5(b) shows how the microcavity resonance shifts (to red curve) when the probe protein binds to the target protein. The target protein in this measurement is BioRad HRP-conjugated goat-anti-rabbit antibody while the probe protein is Invitrogen Rabbit anti-goat Alexa Fluor.
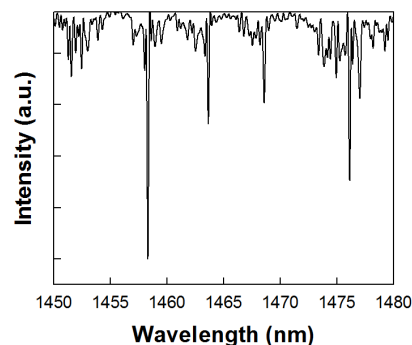
Fig. 6: Photonic crystal waveguide transmission spectrum showing multiple photonic crystal microcavity resonances from individual photonic crystal microcavities that are arrayed along the length of the photonic crystal waveguide.

In Fig. 6, another multi-cavity device with multiple resonances (four in this case) was characterized through optical measurements. Further measurements are in progress for biosensor characterization.

7. SUMMARY

In summary, we have done preliminary measurements of our device with multiple photonic crystal microcavities coupled along the length of a single photonic crystal waveguide. When each of these microcavities are coupled with a unique target biomolecule(s), a probe biomolecule(s) that is/are a unique conjugate of the target biomolecule(s) will bind above the resonant microcavity and lead to multiple biomolecule detection with a single measurement, thereby leading to high throughput measurements.

ACKNOWLEDGEMENTS

REFERENCES

[1] Templin, M.F., Stoll,D., Schwenk, J.M., Potz, O., Kramer, S., Joos, T.O., "Protein microarrays: Promising tools for proteomic research", Proteomics **3** (11), 2155 (2003).

[2] Schena, M., Shalon, D., Davis, R.W., Brown, P.O., "Quantitative monitoring of gene-expression patterns with a complementary DNA microarray", Science **270** (5235), 467 (1995).

[3] Shalon, D., Smith, S.J., Brown, P.O., "A DNA microarray system for analyzing complex DNA samples using two-color fluorescent probe hybridization", Genome Research **6** (7), 639 (1996).

[4] Falsey, J.R., Renil, M., Park, S., Lam, S.J., Lam, K.S., "Peptide and small molecule microarray for high throughput cell adhesion and functional assays", Bioconjugate Chemistry **12** (3), 346 (2001).

[5] Wang, L., Li, P.C.H., Yu, H.Z., Parameswaran, A.M., "Fungal pathogenic nucleic acid detection achieved with a microfluidic microarray device", Analytica Chimica Acta **610** (1), 97 (2008).

[6] Uttamchandani, M., Neo, J.L., Ong, N.N.Z., Moochhala, S., "Applications of microarrays in pathogen detection and biodefence", Trends in Biotechnology **27** (1), 53 (2008).

[7] Hanash, S., "Disease Proteomics", Nature **422** (6928) 226 (2003).

[8] Kumble, K.D., "Protein microarrays: new tools for pharmaceutical development", Analytical and Bioanalytical Chemistry **377** (5), 812 (2003).

[9] Bertone, P., Snyder, M., "Advances in functional protein microarray technology", FEBS Journal **272**, 5400 (2005).

[10] Voros,J., "The density and refractive index of adsorbing protein layers", Biophysical Journal **87** (1), 553 (2004).

[11] Chow, E., Grot, A., Mirkarimi, L.W., Sigalas, M., Girolami, G., "Ultracompact biochemical sensor built with two-dimensional photonic crystal microcavity", Optics Letters **29** (10), 1093 (2004).

[12] Skivesen, N., Tetu, A., Kristensen, M., Kjems, J., Frandsen, L.H., Borel, P.I., "Photonic crystal waveguide biosensor", Optics Express **15** (6), 3169 (2007).

[13] Zlatanovic, S., Mirkarimi, L.W., Sigalas, M.M., Bynum, M.A., Chow, E., Robotti, K.M., Burr, G.W., Esener, S., Grot, A., "Photonic crystal microcavity sensor for ultracompact monitoring of reaction kinetics and protein concentration", Sensors and Actuators B: Chemical **141** (1), 13 (2009).

[14] Akahane, Y., Asano, T., Song, B.S., Noda, S., "High-Q photonic nanocavity in a two-dimensional photonic crystal", Nature **425** (6961), 944 (2003).

[15] Buswell, S.C., Wright, V.A., Buriak, J.M., Van, V., Evoy, S., "Specific detection of proteins using photonic crystal waveguides", Optics Express **16** (20), 15949 (2008).

[16] Topolancik, J., Bhattacharya, P., Sabarinathan, J., Yu, P.C., "Fluid detection with photonic crystal-based multichannel waveguides", Appl. Phys. Lett. 82, 1143-1145 (2003).

[17] Lee, M., Fauchet, P.M., "Two-dimensional silicon photonic crystal based biosensing platform for protein detection", Opt. Express **15**, 4530-4535 (2007).

[18] Mirkarimi, L.W., Zlatanovic, S., Sigalas, S., Bynum, M.A., Robotti, K., Chow, E., Grot, A., "Toward single molecule detection with photonic crystal microcavity biosensors", Digest of the LEOS Summer Topical Meetings pp. 29-30 (2006).

[19] Lee, M., Fauchet, P.M., "Two-dimensional Si photonic crystal microcavity for single particle detection", Proceedings of the 4th IEEE International Conference Group IV Photonics pp. 1-3 (2007).

[20] Guillermain, E., Fauchet, P.M., "Resonant microcavities coupled to a photonic crystal waveguide for multi-channel biodetection", Mater. Res. Soc. Symp. Proc. 1191, 1191-OO11-06 (2009).

[21] Iqbal, M., Gleeson, M.A., Spaugh, B, Tybor, F., Gunn, W.G., Hochberg, M., Baehr-Jones, T., Bailey, R.C., Gunn, L.C., "Label-Free Biosensor Arrays based on silicon ring resonators and high-speed optical scanning instrumentation", IEEE Journal of Selected Topics in Quantum Electronics **16**(3), 654 (2010).

[22] Wang, X., Chakravarty, S., Lee, B-S., Lin, C., Chen, R.T., "Ultra-efficient control of light transmission through photonic potential barrier modulation", Opt. Lett. 34(20), 3202 (2009).