

Short communication

Nanoscale porous silicon waveguide for label-free DNA sensing

Guoguang Rong^a, Ali Najmaie^b, John E. Sipe^b, Sharon M. Weiss^{a,*}^a *Department of Electrical Engineering and Computer Science, Vanderbilt University, Nashville, TN 37212, USA*^b *Department of Physics and Institute for Optical Sciences, University of Toronto, Toronto, ON M1S5A7 Canada*

Received 25 September 2007; received in revised form 6 December 2007; accepted 14 January 2008

Available online 24 January 2008

Abstract

Porous silicon (PSi) is an excellent material for biosensing due to its large surface area and its capability for molecular size selectivity. In this work, we report the experimental demonstration of a label-free nanoscale PSi resonant waveguide biosensor. The PSi waveguide consists of pores with an average diameter of 20 nm. DNA is attached inside the pores using standard amino-silane and glutaraldehyde chemistry. Molecular binding in the PSi is detected optically based on a shift of the waveguide resonance angle. The magnitude of the resonance shift is directly related to the quantity of biomolecules attached to the pore walls. The PSi waveguide sensor can selectively discriminate between complementary and non-complementary DNA. The advantages of the PSi waveguide biosensor include strong field confinement and a sharp resonance feature, which allow for high sensitivity measurements with a low detection limit. Simulations indicate that the sensor has a detection limit of 50 nM DNA concentration or equivalently, 5 pg/mm².

© 2008 Elsevier B.V. All rights reserved.

Keywords: Porous silicon; Resonant waveguide; DNA biosensor; High sensitivity**1. Introduction**

The detection of biomolecules is important for applications including medical diagnostics, food safety, and anti-bioterrorism. Label-free biosensors can directly measure unmodified samples as they do not require the use of reporter molecules to generate a signal (Haake et al., 2000). Optical label-free biosensors operate based on a change in refractive index due to affinity binding events of biomolecules. For example, biomolecules immobilized on the surface of SPR (Homola, 2003), fiber optic (Ferguson et al., 1996), and planar waveguide (Rowe-Taitt et al., 2000) sensors interact with the evanescent field of either the surface plasmon or waveguide mode and cause a refractive index change near the surface of these sensors. However, these evanescent wave sensors are limited in sensitivity, especially for small molecule detection, since the surface area is small and the interaction between biomolecules and the electromagnetic field is not optimal. In order to increase the available surface area and enhance the field-molecule interaction strength, porous media have been

proposed as an extension to existing SPR and waveguide sensor technology (Oh et al., 2006; Qi et al., 2007; Awazu et al., 2007).

Biosensors-based entirely on porous materials, such as porous silicon (PSi), have also been investigated (De Stefano et al., 2004; Stewart and Buriak, 2000). PSi is an attractive material for biosensing. Like other porous materials, it has a large surface area, offering the possibility of immobilizing a large number of biomolecules, which significantly increases the probability of capturing target species. However, PSi has the additional advantages of compatibility with semiconductor processing and a widely tunable pore size (nanometers to microns), which allows infiltration of appropriate-sized target biomolecules while excluding larger-sized non-specific species (Pacholski et al., 2005; Ouyang et al., 2005). PSi optical biosensors, including PSi rugate filters (Chapron et al., 2007), single layer interferometers (Dancil et al., 1999; De Stefano et al., 2007; Lin et al., 1997), and resonant microcavities (Ouyang et al., 2005; Chan et al., 2001), have been demonstrated. PSi waveguides have been used to detect the presence of liquids (Arrand et al., 1998), but no demonstration of biological detection has been realized. Recently, the theoretical demonstration of PSi waveguide biosensors with superior sensitivity to SPR sensors was reported (Saarinen et al., 2005).

* Corresponding author. Tel.: +1 615 343 8311; fax: +1 615 343 6702.
E-mail address: sharon.weiss@vanderbilt.edu (S.M. Weiss).

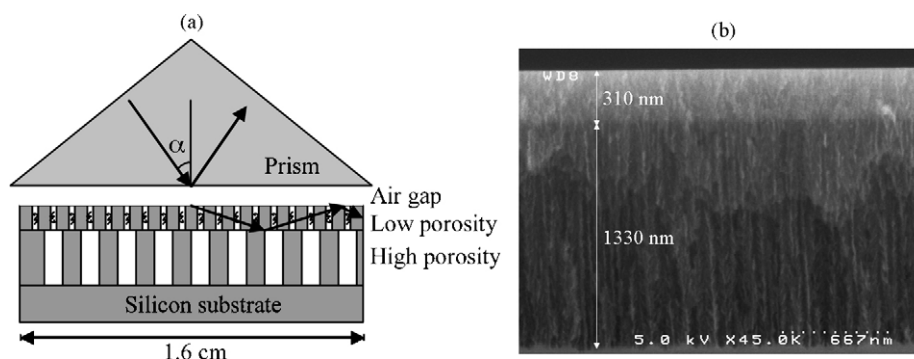


Fig. 1. (a) PSi waveguide consisting of a low porosity (high index) layer, a high porosity (low index) layer, and air gap. Total internal reflection enables waveguiding in the low porosity layer. A prism is used to couple light at a specific angle α into the waveguide through an evanescent wave. Biomolecules infiltrated into the PSi waveguide increase its effective refractive index and change the angle at which light is coupled into the waveguide. (b) Cross-sectional SEM of PSi waveguide with 310 nm low porosity layer and 1330 nm high porosity layer.

This paper reports the experimental demonstration of the proposed PSi waveguide biosensor (Saarinen et al., 2005). With a thinner active sensing layer, and comparable or stronger field confinement than other porous silicon-based biosensors, the PSi waveguide is well suited for fast-response, high sensitivity nanoscale biosensing.

2. Materials and methods

2.1. PSi waveguide structure

As shown in Fig. 1(a), the PSi waveguide consists of two PSi layers: a low porosity (high index) layer, and a high porosity (low index) layer. The air gap above the low porosity layer provides mode confinement at the top waveguide interface. In this work, the 2D Maxwell–Garnett effective medium theory is used to model the relationship between refractive index and porosity (Lugo et al., 1997). A prism is used to evanescently couple a laser beam into the waveguide at a specific angle, at which the horizontal component of the incident beam's wavevector matches that of the waveguide mode (Taylor and Yariv, 1974), and launch the TE₀ mode. A detector placed at the output face of the prism detects a minimum in reflectance at this coupling angle due to the coupling of light into the waveguide. Since the coupling angle depends on the refractive index of the waveguide and effective index of the waveguide mode (Lukosz, 1991), the angle changes when biomolecules are infiltrated into the pores of the waveguide.

2.2. Fabrication

Mesoporous silicon with average pore diameter of 20 nm is fabricated by electrochemical etching of p⁺ (0.01 Ω cm) silicon wafers in a 15% HF electrolyte (75 mL 50% aqueous HF + 175 mL 99% ethanol). By applying different current densities during etching, it is possible to control the porosity and thickness of distinct PSi layers (Lehmann, 2002). Details regarding the porosity and thickness optimization of our waveguide to achieve strong field confinement and a narrow resonance can be found elsewhere (Rong et al., 2006; Saarinen et al., 2005). After optimization, the parameters of the two PSi layers are:

310 nm PSi layer with 56% porosity ($n = 2.08$ at 1550 nm for TE-polarization) on top of a 1330 nm PSi layer with 84% porosity ($n = 1.41$ at 1550 nm for TE-polarization). The design wavelength was chosen as 1550 nm to minimize silicon absorption losses in the waveguide. To fabricate the two-layered PSi waveguide, the silicon wafer was first cleaned with ethanol and DI water and then etched. The top low porosity PSi layer was etched at 5 mA/cm² for 62 s and then the applied current density was changed to 48 mA/cm² for 53 s to form the bottom, high porosity PSi layer. The resulting PSi active area is 0.8 cm in radius. After etching, the sample was rinsed with ethanol and dried with N₂. A cross-sectional SEM image of the PSi waveguide is given in Fig. 1(b).

After anodization, the PSi waveguide was oxidized at 900 °C for 10 min in O₂ in order to lower the waveguide loss (Amato et al., 2000) and to prepare the surface for subsequent chemical functionalization.

2.3. Functionalization

We mixed 40 μ L of 3-aminopropyltriethoxysilane (99%, Aldrich) with 500 μ L of DI water and 480 μ L of methanol. We then dropped 100 μ L of the resulting 4% silane solution onto the PSi waveguide to completely cover the 2 cm² surface. After incubation in a humid environment for 20 min, the sample was rinsed with DI water, dried with N₂, and baked at 100 °C for 10 min.

Next, we mixed 50 μ L of glutaraldehyde (50%, Sigma) with 950 μ L of HEPES buffer (20 mM HEPES, 150 mM NaCl, pH 7.4) and dropped 100 μ L of the resulting 2.5% glutaraldehyde solution onto the PSi waveguide. We then added 1 μ L of 5 M sodium cyanoborohydride in 1 M NaOH (Aldrich) to stabilize the Schiff base formed during reaction of the aldehyde group with the amine group. After 2 h of incubation, the PSi waveguide was rinsed with buffer and then soaked in buffer for 1 h, followed by an additional buffer rinse to remove unreacted glutaraldehyde from the pores. Then the sample was dried with N₂. The latter four steps, namely, rinsing with buffer, soaking in buffer, additional rinsing with buffer, and drying with N₂ will be collectively noted as “post-process cleaning”.

DNA cross-linking was initiated within 30 min of drying because the functionality of glutaraldehyde degrades upon long-time exposure to air. The DNA was purchased from MWG-Biotech, with HPLC purification. The sequences are: probe DNA, 5'-TAGCTATGGAATTCCTCGTAGGCC-3'; complementary DNA, 5'-GGCCTACGAGGAATTCATAGCTA-3'; and non-complementary DNA, 5'-AGCTAGCTAGCTCATGATGCTGTC-3'.

We applied 100 μ M of amino-modified probe in buffer to the PSi waveguide and incubated for 2 h, followed by post-process cleaning. To close any unreacted aldehyde groups for minimizing non-specific binding, 3 M ethanolamine (>99% ethanolamine hydrochloride, Aldrich) in buffer, with pH 9.0, was dropped onto the PSi waveguide and the waveguide sample was soaked for 2 h, followed by post-process cleaning. We note that the Schiff base was stabilized in all reactions of an aldehyde group with an amine group.

We spotted separately 100 μ L of 50 μ M complementary DNA in buffer, 50 μ M non-complementary DNA in buffer, as well as buffer solution alone, onto three PSi waveguide samples and incubated the samples in a humid environment for 1 h. Afterwards, all samples were rinsed with buffer, soaked in buffer for 20 min, rinsed again vigorously with buffer to remove any

remaining non-bounded species from the pores, and dried with N_2 . All processes were carried out at room temperature (22 $^{\circ}$ C), which is well below the melting temperature of the DNA (63 $^{\circ}$ C).

3. Results and discussion

Prism coupler (Metricon 2010) measurements were taken after each functionalization step to confirm chemical attachment. Fig. 2(a) shows the waveguide resonance after each step, from left to right: after oxidation, after silanization, and after glutaraldehyde + probe + ethanolamine (GA + probe + EA). The latter 3 steps are combined together due to the instability of glutaraldehyde in air. The amplitude change is attributed to different gain values of the prism coupler detector used for each measurement and has no effect on the absolute position of the resonance angle. The resonance shifts suggest that the functionalization was carried out successfully, and this was confirmed when complementary DNA binding was observed. Fig. 2(b) demonstrates the detection of DNA in the PSi waveguide. The waveguide resonance shifts 0.046 $^{\circ}$ after the PSi waveguide was exposed to 50 μ M of complementary DNA. To demonstrate specificity, Fig. 2(c) and (d) show the resonance after exposure to 50 μ M of non-complementary

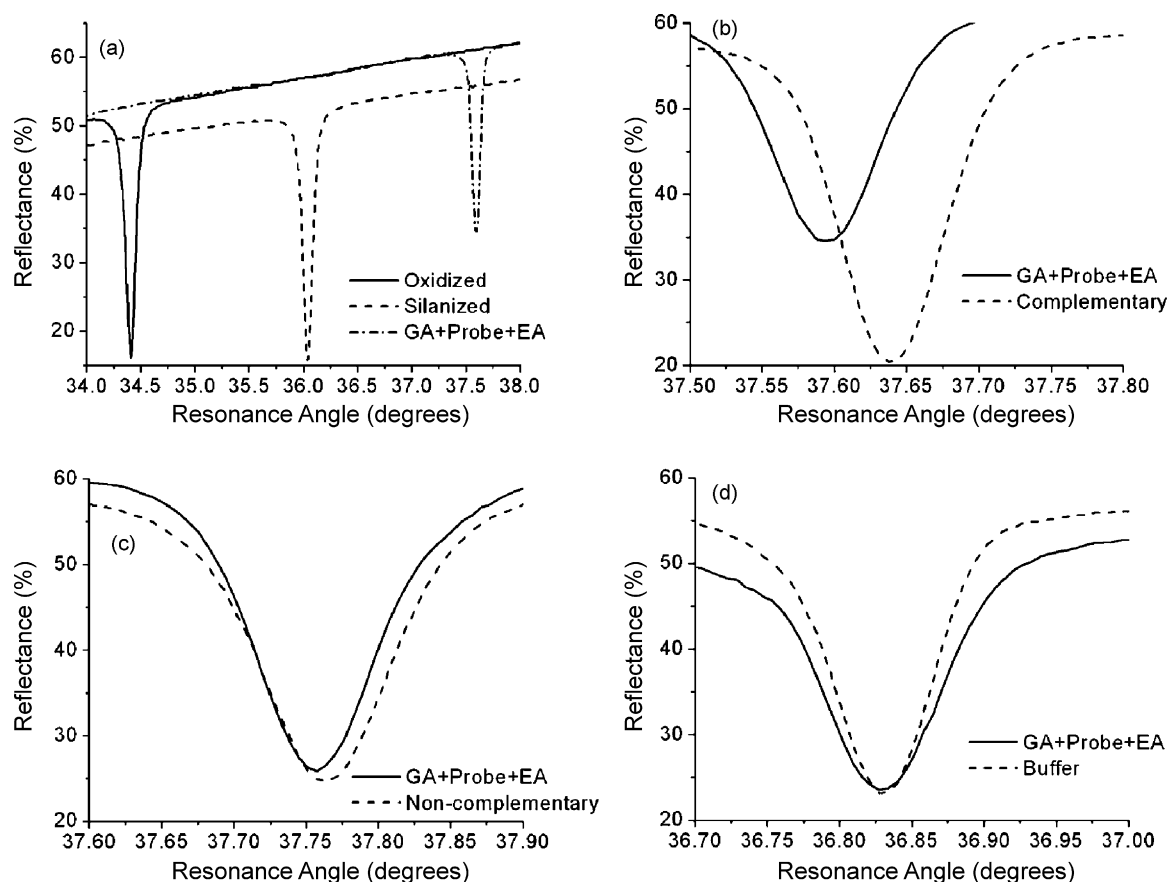


Fig. 2. (a) PSi waveguide resonance after each functionalization step: after oxidation, after silanization, and after glutaraldehyde + probe + ethanolamine (GA + probe + EA). (b) PSi waveguide resonance shift for complementary DNA, demonstrating the recognition of DNA binding inside the PSi waveguide. (c) Negligible PSi waveguide resonance shift for non-complementary DNA, demonstrating the PSi waveguide can distinguish complementary and non-complementary DNA. (d) No waveguide resonance shift upon exposure of PSi waveguide to buffer solution. The resolution of the prism coupler is 0.002 $^{\circ}$, and random angular variations of the rotary table of the prism coupler ($\pm 0.004^{\circ}$) are averaged out by taking multiple measurements.

DNA and after exposure to buffer solution, respectively, with negligible shift. Hence, the PSi waveguide sensor is able to discriminate between complimentary and non-complimentary DNA.

The magnitude of the resonance shift quantifies the DNA concentration with larger shifts indicating higher concentrations. Since the angular resolution of the prism coupler is 0.002° and the full width at half maximum (FWHM) of the PSi waveguide resonance is 0.07° , we expect the ultimate detection limit is much smaller than $50\text{ }\mu\text{M}$. We note that experiments with non-complimentary DNA have also shown that the magnitude of the resonance shift corresponds to its concentration. In order to determine the detection limit of the PSi waveguide, the following calculation was performed. Using the Maxwell–Garnett model and assuming the pore refractive index is a linearly weighted average of air and biomolecules by volume, the refractive index of the top PSi layer was calculated for arbitrary DNA coverage on the pore walls. We assume a DNA refractive index of 1.5 (Samoc et al., 2007); a maximum probe DNA density of $8 \times 10^{13}/\text{cm}^2$ where there is no spacing between probes and 50% coverage results in a probe-to-probe distance just large enough to allow binding of the complementary strand (Steel et al., 2000); and a single-strand DNA length of $2.2\text{ }\text{\AA}/\text{base}$ and double-stranded DNA length of $3.4\text{ }\text{\AA}/\text{base}$ (Rekesh et al., 1996). With the refractive index calculated, the wavevector of the waveguide mode can be obtained, and the angle at which light is coupled into the waveguide can be deduced (Taylor and Yariv, 1974; Lukosz, 1991). Fig. 3 shows the simulated resonance shift for 24-mer complementary DNA at different probe and complementary DNA coverage. The bottom x -axis indicates the percent of probe DNA hybridized by complimentary DNA and the top x -axis gives the complimentary DNA concentration needed for each corresponding percent probe hybridization, given that $100\text{ }\mu\text{L}$ of solution was used to completely cover the

2 cm^2 PSi waveguide active area. As expected, the resonance shift after exposing the PSi waveguide to a given concentration of complementary DNA increases in accordance with the probe coverage in the waveguide. The effective index of the waveguide mode, and hence the confinement of the electric field inside the waveguide, increases for larger probe coverage; the stronger field makes the waveguide more sensitive to small changes in refractive index from DNA hybridization. The inset of Fig. 3 shows the detection limit of the PSi waveguide biosensor for different probe DNA coverage. The detection limit is found by checking the concentration corresponding to a resonance shift of 0.002° , the resolution of the prism coupler. At the optimal 50% probe coverage, the detection limit is 50 nM , which corresponds to $5\text{ pg}/\text{mm}^2$ (given the DNA molecular weight of 7 kDa and total pore surface area of 64 cm^2). As the probe coverage decreases, the detection limit increases.

Based on the comparison of our experimental results with the simulations, it appears that there is low probe DNA coverage and hybridization efficiency inside the porous silicon. Our current functionalization protocol relies on an unstable Schiff base that can compromise probe immobilization, and it is likely that only partial hybridization is taking place inside the pores. We are currently investigating stronger stabilizing agents for the Schiff base and alternative cross-linking protocols that do not rely on the formation of Schiff bases (Hermanson, 1996) in order to increase our probe coverage. In addition, preliminary experiments suggest that the use of smaller DNA strands improves the DNA diffusion into the pores, which will lead to improved hybridization efficiency and detection sensitivity. We note that hybridization is taking place inside the pores, and not simply on the surface of the PSi waveguide. Even if we assume an optimal 50% probe coverage on the surface and 100% hybridization efficiency (which is not realistic), the weak evanescent field of the waveguide mode interacting with DNA on the surface would lead to a 0.04° resonance shift, which is smaller than our experimentally measured value.

In addition to non-optimal probe coverage and hybridization efficiency, we believe there are other factors that are compromising the PSi waveguide sensor sensitivity. PSi corrosion due to interaction with negatively charged DNA causes a decrease in refractive index, which reduces the magnitude of the resonance shift upon DNA hybridization (Steinem et al., 2004). The incorporation of Mg^{2+} into buffer solution reportedly inhibits the corrosion process (Steinem et al., 2004); we are conducting experiments to verify this process. In addition, we believe some DNA infiltrates into the lower PSi layer. Since the electromagnetic field magnitude in the lower layer is very small compared to the top PSi layer, it is advantageous to have DNA bind in the top layer. We believe reducing the incubation time will minimize diffusion of DNA into the lower PSi layer. Experiments are in progress to determine and optimize the distribution of DNA in the two layers of the PSi waveguide. These ongoing research efforts will facilitate the transition from our current μM experimental detection to the theoretically predicted nM concentration.

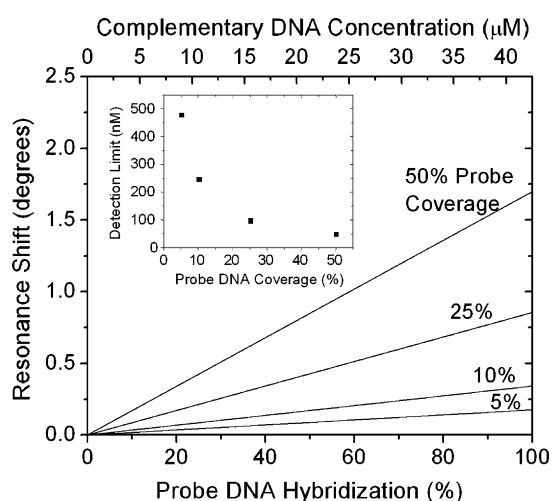


Fig. 3. Simulated PSi waveguide resonance shift as a function of probe DNA hybridization percentage (bottom x -axis) and corresponding complementary DNA concentration (top x -axis), given for different probe DNA coverage on the pore walls. Larger resonance shifts occur for larger probe coverage and when a greater fraction of the probe molecules are hybridized. The inset shows the detection limit at different probe DNA coverage. At the optimal probe coverage of 50%, the detection limit is 50 nM .

4. Conclusion

We have experimentally demonstrated a sensitive label-free optical biosensor based on a resonant PSi waveguide. DNA hybridization was detected by measuring an angular resonance shift with no shift measured for exposure to non-complementary DNA. The large surface area available for biomolecular attachment and strong field confinement in the region where biomolecules are immobilized make it possible for the PSi waveguide to detect DNA hybridization with high sensitivity. Theoretical calculations indicate that the sensor has a detection limit of 50 nM, or 5 pg/mm².

Acknowledgements

The authors acknowledge the National Science Foundation for funding (ECCS-0722143), the Vanderbilt Institute of Nanoscale Science and Engineering for financial and technical support, the Center for Nanophase Materials Sciences of Oak Ridge National Laboratory where a portion of this research was conducted, and the Natural Sciences and Engineering Research Council of Canada.

References

- Amato, G., Boarino, L., Borini, S., Rossi, A.M., 2000. *Phys. Status Solidi A* 182, 425–430.
- Arrand, H.F., Benson, T.M., Loni, A., Arens-Fischer, R., Kruger, M., Thonissen, M., Luth, H., Kershaw, S., 1998. *IEEE Photonics Technol. Lett.* 10, 1467–1469.
- Awazu, K., Rockstuhl, C., Fujimaki, M., Fukuda, N., Tominaga, J., Komatsubara, T., Ikeda, T., Ohki, Y., 2007. *Opt. Express* 15, 2592–2597.
- Chan, S., Horner, S.R., Fauchet, P.M., Miller, B.L., 2001. *J. Am. Chem. Soc.* 123, 11797–11798.
- Chapron, J., Alekseev, S.A., Lysenko, V., Zaitsev, V.N., Barbier, D., 2007. *Sens. Actuators B* 120, 706–711.
- Dancil, K.P.S., Greiner, D.P., Sailor, M.J., 1999. *J. Am. Chem. Soc.* 121, 7925–7930.
- De Stefano, L., Moretti, L., Lamberti, A., Longo, O., Rocchia, M., Rossi, A.M., Arcari, P., Rendina, I., 2004. *IEEE Trans. Nanotechnol.* 3, 49–54.
- De Stefano, L., Arcari, P., Lamberti, A., Sanges, C., Rotiroli, L., Rea, I., Rendina, I., 2007. *Sensors* 7, 214–221.
- Ferguson, J.A., Boles, T.C., Adams, C.P., Walt, D.R., 1996. *Nat. Biotechnol.* 14, 1681–1684.
- Haake, H.M., Schutz, A., Gauglitz, G., 2000. *Fresen. J. Anal. Chem.* 366, 576–585.
- Hermanson, G., 1996. *Bioconjugate Techniques*. Academic Press, New York.
- Homola, J., 2003. *Anal. Bioanal. Chem.* 377, 528–539.
- Lehmann, V., 2002. *Electrochemistry of Silicon: Instrumentation, Science, Materials and Applications*. Wiley-VCH, Weinheim, Germany.
- Lin, V.S.Y., Motesharei, K., Dancil, K.P.S., Sailor, M.J., Ghadiri, M.R., 1997. *Science* 278, 840–843.
- Lugo, J.E., Delrio, J.A., Taguenamartinez, J., 1997. *J. Appl. Phys.* 81, 1923–1928.
- Lukosz, W., 1991. *Biosens. Bioelectron.* 6, 215–225.
- Oh, S., Moon, J., Kang, T., Hong, S., Yi, J., 2006. *Sens. Actuators B* 114, 1096–1099.
- Ouyang, H., Christophersen, M., Viard, R., Miller, B.L., Fauchet, P.M., 2005. *Adv. Funct. Mater.* 15, 1851–1859.
- Pacholski, C., Sartor, M., Sailor, M.J., Cunin, F., Miskelly, G.M., 2005. *J. Am. Chem. Soc.* 127, 11636–11645.
- Qi, Z.M., Honma, I., Zhou, H.S., 2007. *Appl. Phys. Lett.* 90, 1–2, 011102.
- Rekesh, D., Lyubchenko, Y., Shlyakhtenko, L.S., Lindsay, S.M., 1996. *Biophys. J.* 71, 1079–1086.
- Rong, G., Saarinen, J.J., Sipe, J.E., Weiss, S.M., 2006. In: Brongersma, M., Gardner, D.S., Lipson, M., Shin, J.H. (Eds.), *Silicon-Based Microphotonics*, Mater. Res. Soc. Symp. Proc. 934E, Warrendale, PA, 0934-I10-04.
- Rowe-Taitt, C.A., Hazzard, J.W., Hoffman, K.E., Cras, J.J., Golden, J.P., Ligler, F.S., 2000. *Biosens. Bioelectron.* 15, 579–589.
- Saarinen, J.J., Weiss, S.M., Fauchet, P.M., Sipe, J.E., 2005. *Opt. Express* 13, 3754–3764.
- Samoc, A., Miniewicz, A., Samoc, M., Grote, J.G., 2007. *J. Appl. Polym. Sci.* 105, 236–245.
- Steel, A.B., Levicky, R.L., Herne, T.M., Tarlov, M.J., 2000. *Biophys. J.* 79, 975–981.
- Steinem, C., Janshoff, A., Lin, V.S.Y., Volcker, N.H., Ghadiri, M.R., 2004. *Tetrahedron* 60, 11259–11267.
- Stewart, M.P., Buriak, J.M., 2000. *Adv. Mater.* 12, 859–869.
- Taylor, H.F., Yariv, A., 1974. *Proc. IEEE* 62, 1044–1060.