Label-free porous silicon membrane waveguide for DNA sensing

Guoguang Rong,[1] Judson D. Ryckman,[1] Raymond L. Mernaugh,[2] and Sharon M. Weiss[1,a)]
[1]*Department of Electrical Engineering and Computer Science, Vanderbilt University, Nashville, Tennessee 37235, USA*
[2]*Department of Biochemistry, Vanderbilt University, Nashville, Tennessee 37235, USA*

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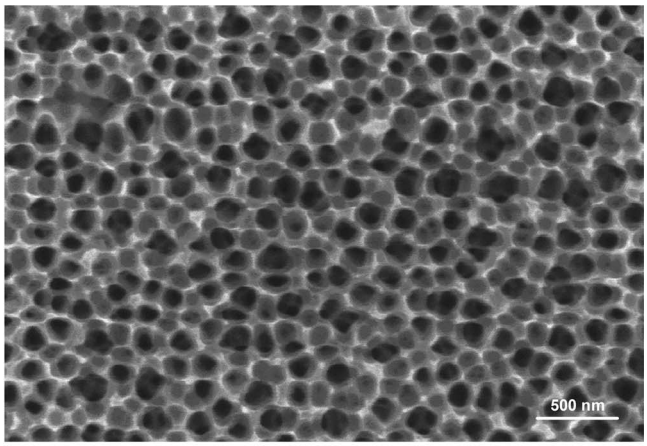
We report a label-free porous silicon membrane waveguide biosensor based on a 1 μm thick freestanding porous silicon film with 100 nm diameter pores. The sensor operates in the Kretschmann configuration. A formvar polymer film provides robust adhesion of the porous silicon membrane to a rutile prism and enables confinement of guided modes in the porous silicon membrane. Attenuated total reflectance measurements are performed, along with theoretical calculations, to fully characterize the waveguide. The sensitivity of the sensor is investigated through DNA hybridization in the porous silicon membrane. A detection limit of 42 nM was demonstrated for 24-base pair DNA oligonucleotides. © *2008 American Institute of Physics*. [DOI: 10.1063/1.3005620]

Porous materials offer several advantages for biosensing applications due to their large available surface area for molecular detection and size-selective filtering of contaminant species. The limited surface area of many traditional sensors is one of the main factors inhibiting efficient detection of small molecules.[1] Consequently, porous materials have recently been incorporated into the design of several different sensors in order to enable improved detection sensitivity. For example, porous alumina have been used to increase the surface area and sensitivity of surface plasmon resonance (SPR) sensors in the traditional Kretschmann configuration.[2,3] As a variation of the SPR sensor, waveguides based on porous materials have also been fabricated using the Kretschmann setup; instead of detecting biomolecules using the exponentially decaying field of the surface plasmon at the metal-dielectric interface, the low refractive index metal layer serves as a cladding for the porous waveguide core. Waveguide modes, as opposed to SPR modes, are interrogated for biosensing. Such metal cladded waveguides in the Kretschmann configuration allow field localization inside the porous waveguide core where biomolecules are immobilized, leading to improve detection sensitivity. A leaky porous TiO_2 waveguide with gold cladding was recently demonstrated for aqueous sucrose detection,[4] and a gold-cladded porous SiO_2 waveguide with a hole density of 10^8 cm^{-2}, formed by ion bombardment and subsequent etching, was shown for detection of biotin-streptavidin binding.[5] In this letter, we report a high pore density, porous silicon (PSi) waveguide in the Kretschmann configuration that uses a low-loss polymer cladding. The PSi membrane waveguide is fabricated in a straightforward manner by electrochemical etching, and functionalization for biomolecule detection follows from the well-studied procedures on Si-SiO_2-based systems.[6–9] We demonstrate the sensitivity of our guided mode sensor through the investigation of DNA hybridization in the PSi waveguide core.

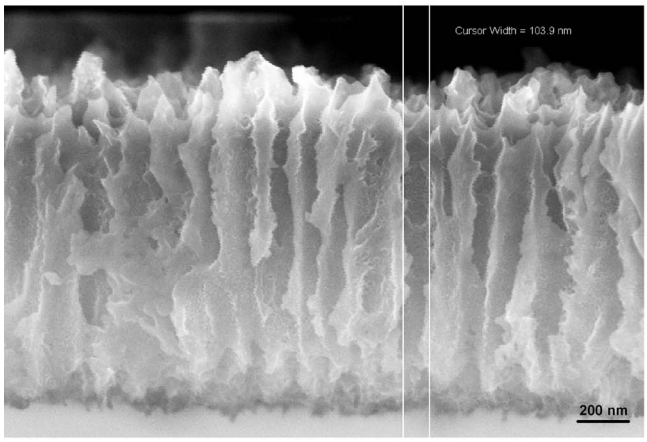
The fabrication of the PSi membrane waveguide starts with electrochemical etching of a *n*-type silicon wafer ($\langle 100 \rangle$, 0.01 Ω cm) in 5.5% aqueous hydrofluoric acid (200 ml of de-ionized water, 25 ml of 50% aqueous hydrofluoric acid, and five to six drops of NCW-1001 surfactant). A current density of 40 mA/cm^2 was applied for 35 s without back side illumination. The PSi film was then removed from the silicon substrate by applying a series of five high current pulses (200 mA/cm^2 for 4 s with 50% duty cycle). This procedure caused electropolishing and subsequent detachment of the porous film from the substrate.[10] During the electropolishing, a slight widening of the pore diameter at the bottom of the PSi film occurs. Figure 1 shows scanning electron microscopy (SEM) images of the PSi membrane, evidencing pores with an average diameter of 100 nm. Since the pore size is much smaller than the wavelength of light used in our experiments (1550 nm), the pore size distribution does not adversely affect the optical properties of the PSi membrane.[11] We note that eight to ten PSi membranes can be fabricated from the same silicon substrate without significantly degrading the PSi film quality. The typical membrane size was 0.2 cm^2. After fabrication, a PSi membrane was placed on a BK7 glass slide for ease of handling during oxidation, although the membrane could be held by tweezers. The membrane was oxidized at 500 °C for 5 min in an Omegalux LMF-3550 oven, after insertion at 300 °C. To build the waveguide structure, 0.25% formvar polymer in ethylene dichloride (Ernest F. Fullam, Inc.) was dropped onto the surface of a rutile prism (Metricon, $n=2.1252$). Ethylene dichloride evaporates quickly to leave behind a thin film of formvar ($n \sim 1.5$, transparent in infrared region). In order to ensure strong adhesion of the PSi membrane to the polymer film, the membrane was placed at the thinner edge of the ethylene dichloride solution drop before the solution completely dried. Using this method, no air gap was formed between the polymer film and the PSi membrane, or between the polymer film and the prism. The PSi membrane was placed such that the larger pore openings were at the air interface to facilitate molecule infiltration. The PSi membrane waveguide structure is shown in Fig. 2(a).

The PSi membrane waveguide was characterized by SEM and by optical measurements in the Kretschmann configuration shown in Fig. 2(a). SEM analysis (Fig. 1) revealed a PSi membrane thickness of approximately 1.1 μm with a

a)Electronic mail: sharon.weiss@vanderbilt.edu.



(a)



(b)

FIG. 1. SEM images of PSi membrane in (a) plan view and (b) cross section. The plan view image shows the slightly widened pores at the bottom of fabricated PSi layer after the electropolishing procedure separated the PSi layer from the silicon substrate. The cross-sectional image confirms the ~100 nm pore openings and reveals the roughness of the electropolished interface.

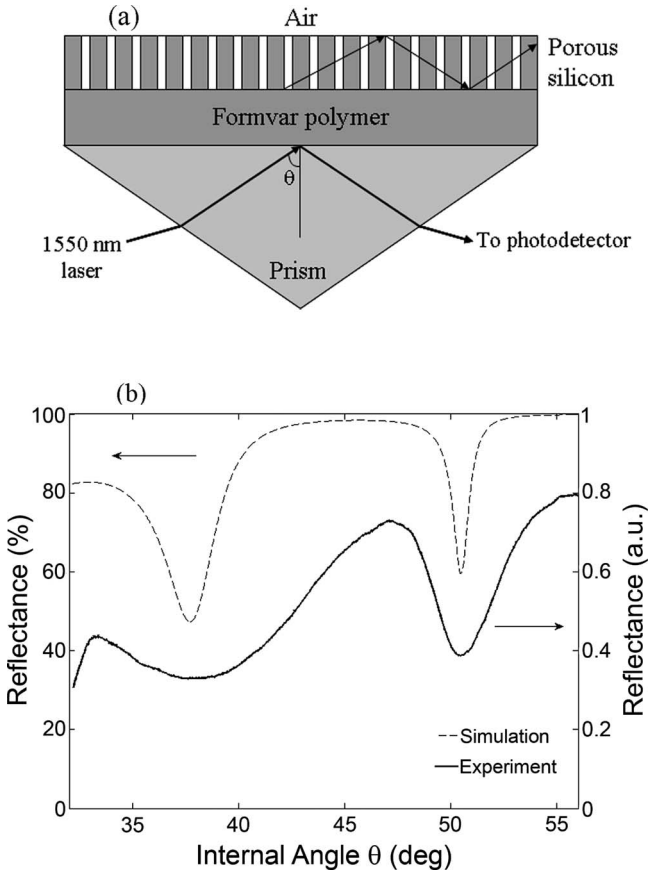


FIG. 2. (a) Schematic of PSi membrane waveguide biosensor. (b) Experimental (solid line) and theoretical (dotted line) angle-resolved attenuated total reflectance spectrum of PSi membrane waveguide showing the guided mode (50.5°) and substrate mode (37.9°). The simulation assumes the structure shown in (a) with a 1.1 μm PSi membrane having a refractive index of 1.94 and a 752 nm thick polymer layer of index 1.5.

pore density of approximately 5×10^9 cm^{-2}. Figure 2(b) shows the attenuated total reflectance measurement of the waveguide mode. Using a Metricon 2010 Prism Coupler, transverse-electric (TE) polarized light from a 1550 nm diode laser with a spot size of approximately 1 mm^2 was incident on a rutile prism at variable angle. The reflected light was detected using a germanium photodetector. A waveguide mode is observed at the angle for which the component of the incident light wavevector in the prism parallel to the interface matches that of a guided mode. At this angle, light is coupled into the waveguide and not directly reflected back to the photodetector, giving rise to the resonance dip in the measured spectrum [Fig. 2(b)]. Given the PSi membrane thickness and the polymer refractive index, the PSi refractive index was calculated to be 1.94 and the polymer thickness was determined to be 752 nm by fitting the waveguide mode and substrate mode angles.[12] Figure 2(b) shows good agreement between calculation and experiment. The larger width of the experimental resonance is attributed to scattering losses, which were not taken into account in the calculation. We note that the waveguide mode measured is the first order TE mode. Reducing the thickness of the PSi membrane or using a higher index prism would be necessary to measure the zeroth order mode.

The sensing operation of the PSi membrane waveguide is demonstrated by detection of DNA hybridization. In order to enable specific detection of complementary DNA oligonucleotides, the PSi waveguide was functionalized in a manner similar to what we have reported previously.[13,14] Briefly, the PSi waveguide was first silanized by 4% 3-aminopropyltriethoxysilane, followed by attachment of a monolayer of the cross-linking chemical, Sulfo-SMCC (Pierce), using water and ethanol as the solvent. Before attachment in the PSi membrane, 24-base pair thiol modified probe DNA was reduced for 30 min by mixing 1:1 by volume DNA in HEPES buffer with TCEP (Pierce) in water and ethanol. In order to screen the negative charges of DNA, which has been reported to cause oxidation and corrosion of PSi upon hybridization,[15] and to enhance DNA infiltration and surface immobilization,[16] 3*M* NaCl was added to the probe DNA solution. Finally, the functionalized PSi membrane waveguide sensor was tested through exposure to 24-base pair complementary and noncomplementary DNA (all solutions included water, ethanol, and 3*M* NaCl).

Figure 3 shows the experimental resonance shifts after the PSi membrane waveguide was exposed to different concentrations of complementary DNA. When the complementary DNA is exposed to the probe DNA immobilized in the pores, the two DNA strands bind. This hybridization in-

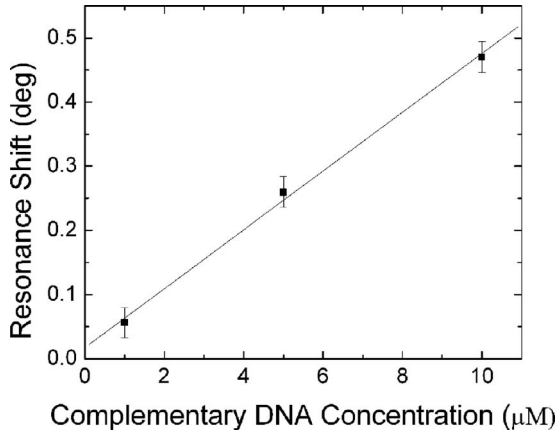


FIG. 3. Measured angular shift of guided mode after exposure to various concentrations of 24-base pair DNA oligonucleotides. Three measurements were taken at each data point and the error bars are also shown. A linear fit of the data points suggests a sensitivity of 0.048 deg/μM and a detection limit of 42 nM.

creases the effective refractive index of the PSi, which changes the waveguide mode dispersion and hence the waveguide resonance angle measured using the prism coupler. Larger resonance shifts indicate that a greater number of DNA oligonucleotides are hybridized in the PSi waveguide. We note that the resonance shifts to higher angle for all experiments, suggesting that the previously reported DNA hybridization-enhanced corrosion problem for *p*-type PSi DNA biosensors[15] is not present for our conditions. A linear fit of the experimental data in Fig. 3 gives the sensitivity of the sensor, which is found to be 0.048°/μM. Given that the angular resolution of the Metricon prism coupler is 0.002°, the ultimate detection limit of the PSi membrane waveguide biosensor is 0.002 deg/(0.048 deg/μM)=42 nM. We stress here that while traditional SPR sensors are capable of nanomolar detection of proteins, the PSi membrane waveguide sensor is capable of nanomolar detection of small molecules. We anticipate that lower detection limits are possible after optimization of the PSi membrane and polymer layer thicknesses. The thickness optimization will enable stronger field confinement in the PSi, stronger field-molecule interaction, and thus a larger resonance shift for the same level of molecular attachment.[17] Control experiments were performed on the PSi membrane waveguides to demonstrate selectivity. No resonance shift was observed upon exposure to 1 μM noncomplementary DNA, suggesting that no binding occurred. Additionally, no resonance shifts were observed upon exposure to solutions containing only HEPES buffer, TCEP and 3*M* NaCl (data not shown).

While the present study was limited to 24-base pair DNA, the 100 nm pores of the PSi membrane waveguide are expected to allow detection of a large size range of molecules, from small toxins to proteins. A modified design of the PSi membrane waveguide sensor is also proposed here for direct compatibility with commercial SPR instruments offering integrated microfluidics and real-time analysis. Instead of depositing the formvar polymer layer directly on the prism, a standalone sensor chip can be fabricated. The polymer layer and PSi membrane are deposited onto a glass slide having the same refractive index as the prism (n=2.1252, Ohara Corp.). Index matching fluid of the same refractive index (Cargille Laboratorie) is used to fill the space between the prism and the glass slide. Thus, the prism, index matching layer, and glass slide form a homogeneous medium, giving a structure exactly the same as in Fig. 2(a). The sensor chip, consisting of the glass slide, polymer, and PSi waveguide membrane, is then an independent module that can be removed from the prism.

In summary, a high sensitivity label-free PSi membrane waveguide biosensor in the Kretschmann configuration with a polymer cladding has been shown. The resonance angles of the substrate and guided modes allowed characterization of the PSi membrane refractive index and polymer layer thickness. The detection sensitivity of the PSi membrane waveguide sensor was demonstrated through the investigation of DNA hybridization in the pores, with a detection limit of 42 nM reported. The high sensitivity derives from a large overlap of the modal volume and the biomolecules in the nanoscale porous waveguide. The ease of fabrication, small molecule detection capability, low cost design, and potential for direct integration with existing instrumentation make the PSi membrane waveguide attractive for widespread biosensing applications.

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