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Characterization of the evanescent field profile and bound mass sensitivity of a label-free silicon photonic microring resonator biosensing platform☆

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ABSTRACT

Silicon photonic microring resonators have emerged as a sensitive and highly multiplexed platform for real-time biomolecule detection. Herein, we profile the evanescent decay of device sensitivity towards molecular binding as a function of distance from the microring surface. By growing multilayers of electrostatically bound polymers extending from the sensor surface, we are able to empirically determine that the evanescent field intensity is characterized by a 1/e response decay distance of 63 nm. We then applied this knowledge to study the growth of biomolecular assemblies consisting of alternating layers of biotinylated antibody and streptavidin, which follow a more complex growth pattern. Additionally, by monitoring the shift in microring resonance wavelength upon the deposition of a radioactively labeled protein, the mass sensitivity of the ring resonator platform was determined to be 14.7 ± 6.7 [pg/mm^2]/Δpm. By extrapolating to the instrument noise baseline, the mass/area limit of detection is found to be 1.5 ± 0.7 pg/mm^2. Taking the small surface area of the microring sensor into consideration, this value corresponds to an absolute mass detection limit of 125 ag (i.e. 0.8 zmol of IgG), demonstrating the remarkable sensitivity of this promising label-free biomolecular sensing platform.

1. Introduction

Waveguides, including fiber optics, have become valuable tools for detecting chemical and biological species through a range of optical transduction mechanisms, including absorbance, fluorescence, and refractive index-induced phase or transmission intensity modulation (Fan et al., 2008; Mukundan et al., 2009; Passaro et al., 2007; Wolfbeis, 2002, 2006, 2008). For all waveguide materials and geometries, light propagates through the waveguide on account of total internal reflection that occurs due to the contrast in refractive index between the core and cladding materials. At the core-cladding interface, an evanescent optical field extends from the surface and decays exponentially with distance (Tsai et al., 1990). Regardless of the particular transduction mechanism utilized for sensing, the measured signal is elicited through light-matter interactions between this evanescent field and proximal target molecules.

Most relevant to this report are label-free transduction methods whereby the presence of a particular analyte causes a fundamental change in the optical transmission properties of the waveguide, thus eliminating requirements for chromophoric or fluorescent labels (Qavi et al., 2009). In most examples, the waveguide is chemically modified to present a target-specific capture element, and localization of the analyte at the core–cladding interface leads to an attenuation in the power of the transmitted light.

Recently there have been reports of chemical and biomolecular sensors based upon waveguides composed from a range of different materials, many of which leverage advances in semiconductor processing for micro- or nano-scale device fabrication (Passaro et al., 2007). A particularly promising waveguide material is silicon-on-insulator (SOI), a feedstock of the microelectronics industry (Jokerst et al., 2009). SOI waveguides are patterned into the top layer of silicon, and light is effectively guided due to the high refractive index contrast between Si and the cladding layers, which include the buried oxide and the top/side cladding layers. In addition to the obvious advantages of SOI in terms of scalability and potential for mass production, silicon photonic devices also feature good modal overlap between the guided optical mode and an analyte recognition layer (Densmore et al., 2006). The high refractive index of the waveguide core layer leads to a high sensitivity towards surface-confined binding events.

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Several groups have demonstrated silicon photonic waveguide configurations for chemical or biological sensing applications, including Mach–Zehnder interferometers (Densmore et al., 2006, 2008, 2009), resonant gratings (Schmid et al., 2009), and microcavity resonators (Carlborg et al., 2010; De Vos et al., 2007, 2009; Ramachandran et al., 2008; Xu et al., 2008). Using arrays of SOI microring resonators, we previously reported a bulk refractive index sensitivity of 7.6×10^{-7} refractive index units (RIU) (Iqbal et al., 2010) and have demonstrated the sensitive, label-free detection of multiple proteins and nucleic acid sequences in both single and multiparameter analytical applications (Luchansky and Bailey, 2010; Qavi and Bailey, 2010; Washburn et al., 2009, 2010).

Another label-free optical method that is particularly well-suited to analyzing biomolecular binding, thus serving as a basis for comparison, is surface plasmon resonance (SPR). SPR measures the interaction of an evanescent field extending from a metallic film, usually gold, in contact with the analyte-containing solution. Binding events that change the refractive index at the gold–solution interface are measured either as a change in the angle or wavelength of light which is maximally coupled into the metal film. SPR has been actively investigated as a biosensing technique over the past several decades with a number of reported sensor geometries and modes of operation, several of which are available commercially (Homola, 2008; Piliarik and Homola, 2006; Scarano et al., 2010). For SPR sensors, the evanescent field penetration depth, which is a function of wavelength and dielectric environment, is on the order of hundreds of nanometers (Homola, 2006) with bulk refractive index sensitivities usually varying between 10^{-5} and 10^{-7} RIU (Fu et al., 2008; Homola, 2008). For reference, a bulk index sensitivity of 10^{-6} RIU corresponds to a surface coverage resolution of 0.91 pg/mm^2 (Homola, 2008).

In this paper, we present a simulation of the waveguide optical intensity profile as well as a direct, experimental interrogation of the evanescent intensity decay profile of a silicon photonic microring resonator. We empirically measure the distance dependence of the sensing platform utilizing layer-by-layer electrostatic polymer deposition (Ganesan et al., 2006; Mandal et al., 2009). We also directly determine the bound mass sensitivity and limit of detection (LOD) of our system by correlating the resonance wavelength shifts with the amount of bound ^{125}I-labeled streptavidin, measured via radiometric methods. Importantly, this paper presents key, experimentally determined sensitivity metrics for an emerging silicon photonic biomolecular sensing platform. In addition to providing a benchmark for comparison, the dependence of device sensitivity on distance from the sensor surface will be important when designing coatings for optimal analyte recognition and localization within the evanescent intensity profile of the guided optical mode.

2. Materials and methods

2.1. Chemicals and materials

N-hydroxysuccinimidobiotin (NHS-Biotin), streptavidin, Zeba spin filter columns, and pre-coated iodination tubes were obtained from Pierce (Rockford, IL). 3-Aminopropyltriethoxysilane (APTES) was purchased from Gelest (Morrisville, PA). Succinimidyl 4-formylbenzoate (S-4FB) and 3-N-((6-(N′-isopropylidenehydrazino))nicotinamide)propyltriethoxysilane (HyNic-silane), were purchased from SoluLink (San Diego, CA). Shipley 1813 photoresist, MF 319 developer, and 1165 photoresist stripper were purchased from MicroChem Corp. (Newton, MA). Poly(sodium 4-styrene-sulfonate) (PSS, MW ~ 70,000 Da), polyethyleneimine (PEI, 50% (w/w) in H_2O, MW ~ 750,000 Da), and poly(allylamine hydrochloride) (PAH, MW ~ 56,000 Da) were obtained from Sigma–Aldrich (St. Louis, MO). Biotinylated, monoclonal mouse anti-human IL-2 antibody (catalog# 555040, clone B33-2) was purchased from BD Biosciences (San Jose, CA). All other chemicals were used as received from Sigma–Aldrich (St. Louis, MO), and all buffers were made with purified water (ELGA PURELAB filtration system; Lane End, UK), and the pH was adjusted using 1 M HCl or 1 M NaOH. Tris buffer consisted of 0.5 mM Tris and 100 mM NaCl adjusted to pH 7.1. Tris/EDTA buffer was made by dissolving 1.21 g Tris base, 0.558 g disodium EDTA, and 0.2 g sodium azide in 1 L water and adjusting to pH 7.4. PBS was made by dissolving 9.6 g Dulbecco's phosphate buffered saline mixture into 1 L water and adjusting to pH 7.4. Antibody immobilization buffer consisted of 100 mM PBS with 150 mM NaCl adjusted to pH 6.0. BSA–PBS buffer consisted of 0.1 mg/mL BSA in PBS. For blocking, StartingBlock blocking buffer (Pierce) was used.

2.2. Device: microring resonator sensors and instrumentation

The design and fabrication of microring resonator array chips, the accompanying instrumentation, and the fluidics have been described previously (Iqbal et al., 2010; Washburn et al., 2009). Briefly, chips having 32 individually addressable microrings were used for the experiments. The entire substrate was coated with a fluoropolymer cladding layer that was removed from annular openings over 24 of the sensors. The remaining eight microrings were left occluded by the cladding and were utilized as thermal controls, since they were not exposed to the solution. Microring resonance frequencies were measured as described previously. Briefly, the beam of a 1560-nm center wavelength tunable, external cavity diode laser is focused onto an input grating coupler on the chip surface to couple light into the linear waveguide adjacent to a given microring. The laser output is then rapidly swept through a 12-nm spectral window, and the intensity of light projected from the output grating coupler is monitored as a function of laser wavelength. Resonance wavelengths are determined as minima in output coupler intensity. This process is repeated for each interrogated microring sensor by rastering the laser across all 32 input grating couplers, allowing resonance determination with ~250-ms time resolution. Thermal control ring responses are used to control for ambient thermal drift.

2.3. Layer-by-layer electrostatic polymer deposition for evanescent decay profiling

For the layer-by-layer electrostatic deposition of polymers, PEI, PSS, and PAH were dissolved in Tris buffer to 5 mg/mL. After cleaning the microring surface with Piranha solution (3:1 H_2SO_4:H_2O_2)[2] and loading the chip into a previously described microfluidic flow cell (Washburn et al., 2009), chips are exposed to PEI for 5 min at 30 μL/min. Maintaining constant flow conditions with a P625 peristaltic pump from Instech Laboratories (Plymouth Meeting, PA), the surface is then rinsed with Tris buffer and exposed to PSS for 10 min followed by a buffer rinse. The surface is then exposed to PAH for 4 min with a subsequent 5 min buffer rinse. The PSS and PAH deposition cycles (with the PSS time reduced to 6 min) and buffer rinses were repeated until a total of 72 bilayers had been grown. To facilitate automated solution switching, we constructed a robot using the LEGO (Billund, Denmark) MINDSTORMS NXT 2.0 system that automatically moved the inlet tubing between solutions.

[2] Caution! Piranha solutions are extraordinarily dangerous, reacting explosively with traces quantities of organics.