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An optofluidic metasurface for lateral flow-through detection of breast cancer biomarker

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ABSTRACT

The rapid growth of point-of-care tests demands for biosensors with high sensitivity and small size. This paper demonstrates an optofluidic metasurface that combines silicon-on-insulator (SOI) nanophotonics and nanofluidics to realize a high-performance, lateral flow-through biosensor. The metasurface is made of a periodic array of silicon nanoposts on an SOI substrate, and functionalized with specific receptor molecules. Bonding of a polydimethylsiloxane slab directly onto the surface results in an ultracompact biosensor, where analyte solutions are restricted to flow only in the space between the nanoposts. No flow exists above the nanoposts. This sensor design overcomes the issue with diffusion-limited detection of many other biosensors. The lateral flow-through feature, in conjunction with high-Q resonance modes associated with optical bound states of the metasurface, offers an improved sensitivity to subtle molecule-bonding induced changes in refractive index. The device exhibits a resonance mode around 1550 nm wavelength and provides an index sensitivity of 720 nm/RIU. Biosensing is conducted to detect the epidermal growth factor receptor 2 (*ErbB2*), a protein biomarker for early-stage breast cancer screening, by monitoring resonance wavelength shifts in response to specific analyte-ligand binding events at the metasurface. The limit of detection of the device is 0.7 ng mL^{-1} for *ErbB2*.

1. Introduction

Optical label-free biosensors can detect biomolecules based on their intrinsic physical properties, such as Raman scattering, refractive index, and second harmonic generation (Celebrano et al., 2015; Choi et al., 2010b; Salafsky, 2006; Wu et al., 2012). In particular, many refractive index-based biosensors have been implemented to study analyte-ligand interactions without using labels (Im et al., 2014; Liu et al., 2014; Zhang et al., 2008). In contrast to binding assays that require fluorescent or enzymatic tags, label-free assays often eliminate the need for time-consuming labeling processes and can monitor binding kinetics in real time (Ali et al., 2015; Ali et al., 2017b; Diaz-Diestra et al., 2017). Therefore, label-free biosensors are gaining increasing attention in the fields of life sciences, pharmaceutics, and clinic diagnosis (Bergwerff and Van Knapen, 2006; de Mol, 2012; Fivash et al., 1998; McDonnell, 2001; Navratilova and Hopkins, 2011; Rich and Myszka, 2003; Sridharamurthy et al., 2007; Thillaivinayagalingam et al., 2010; Xue et al., 2014a, 2014b). Recently, optical resonators using surface plasmon resonance, photonic crystal, and whispering gallery mode (Arnold et al., 2003; Cunningham et al., 2004; Fan et al., 2008; Heeres et al., 2009; Sun and Fan, 2011; Vollmer and Arnold, 2008) have been extensively studied and exploited for label-free biosensors. Owing to their strong ability to confine resonating fields, these biosensors are sensitive to the presence of biomaterials immobilized in the close vicinity of their surfaces (Sun et al., 2016b).

While significant progress has been made to develop optical label-free biosensors, how to efficiently delivery samples to the sensor surface remains challenging. To address the issue with the mass transfer limitation, microfluidic systems have been developed and applied to label-free biosensors (Choi and Cunningham, 2006, 2007). To improve the integration between the sensing and fluidic elements, several optofluidic biosensors (e.g., liquid-core ring resonators (White et al., 2006) and anti-resonant reflecting optical waveguides (Yin et al., 2004)) have been used to facilitate the transport of analyte. Both the microfluidic and optofluidic approaches rely on a flow-over scheme, where liquid samples flow through a channel, during which the analytes diffuse from the sample stream onto the surface of the biosensor (Brolo et al., 2004; Lindquist et al., 2009; Sinton et al., 2008; Wang et al., 2014). Recently, a vertical flow-through sensor design was implemented to label-free optical biosensing, where liquid samples flow through a horizontally

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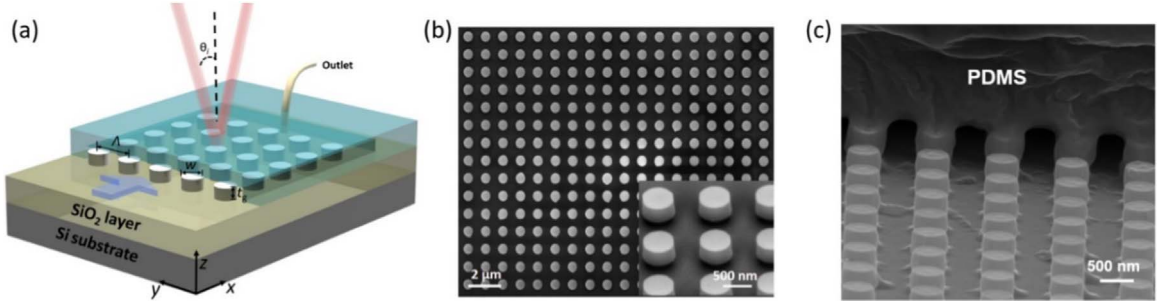

Fig. 1. (a) Schematic illustration of the optofluidic metasurface with a 2D array of SNPs. (b) SEM image of the fabricated SNPs. The inset shows a perspective view of the SNPs. (c) SEM images of the nanofluidic channel where the SNPs are sandwiched between the buried oxide of an SOI substrate and a capped PDMS cover. A sample can laterally flow through the SNP region and be captured by the recognition biomolecules onto the metasurface.

placed, nanopatterned dielectric or metallic diaphragm with nanoholes that functions as both the sensing element and conduits (Cetin et al., 2014; Eftekhari et al., 2009; Escobedo et al., 2010; Yanik et al., 2010). This sensor has enhanced the interaction between the sensing surface and analytes, thus reducing detection time.

This paper reports on a novel lateral flow-through biosensor, consisting of a metasurface with a two-dimensional (2D) periodic array of silicon nanoposts (SNPs), for the detection of cancer biomarker. The structure is manufactured in the thin top silicon layer of a silicon-on-insulator (SOI) substrate, coated with graphene oxide (GO) nanosheets, and biofunctionalized with specific antibody molecules. A polydimethylsiloxane (PDMS) slab with an inlet and an outlet is bonded to the top surface of the SNPs, thus restricting the flow of liquid analytes in between the PDMS and the buried oxide layer of the SOI substrate (Fig. 1). It is worth noting that silicon or SOI-based metasurfaces have attracted increasing attention due to the flexibility in tuning of their optical properties (Sun et al., 2016a; Zhu et al., 2015, 2013a), and the fabrication compatibility with complementary metal-oxide-semiconductor (CMOS) process. The high refractive index of silicon is favourable for light modulation (Cheung et al., 2012; Fang et al., 2016; Ferrara et al., 2015; Taillaert et al., 2006; Van Laere et al., 2007), e.g., to enhance optical fields. Our biosensor is featured with the lateral flow-through design for improved analyte-ligand interactions at the metasurface. Owing to a reduced diffusion length, the biosensor design will overcome the issue of mass transfer limit that occurs in many existing label-free biosensors (Choi et al., 2010a). Therefore, the biosensor will have an improved sensitivity and a reduced assay time. In addition, the metasurface supports different optical resonance modes, such as the bound states in the continuum (BIC) mode, and leaky waveguide mode (Chang-Hasnain and Yang, 2012; Wang et al., 2016a, 2016b), to detect biomolecule absorptions. In this work, the guided mode resonance (GMR) mode, whose linewidth depends on the coupling angle, is utilized and exhibits a high sensitivity to a change in refractive index at the surface of SNPs. The sensor design emphasizes both analyte delivery and sensitivity. The key figure of merit of the device and its ability to detect cancer biomarkers are demonstrated.

2. Experimental section

2.1. Fabrication of the optofluidic metasurface

An SOI wafer is used to fabricate the SNPs. First, 200 nm-thick PMMA is coated onto the substrate at 2000 rpm for 45 s. Subsequently, e-beam lithography is used to pattern the nanoholes array in the PMMA. Next, a 15 nm-thick Al_2O_3 layer is evaporated using electron-beam evaporation, and then patterned using lift-off process, to form a protection layer during the following deep reactive-ion etching of Si (Fig. 1(b)). After the SNPs are formed, the Al_2O_3 layer is removed via wet chemical etching. The overall size of the device is $1 \times 1\ mm^2$. To enable laterally flowing liquid analytes through the SNP area, a 2 mm-thick PDMS slab with the pre-drilled inlet and outlet is bonded directly onto the top surface of the SNPs via oxygen plasma treatment. Fig. 1(c) shows the formed nanofluidic channels embedded with the SNPs.

2.2. Setup for optical reflection measurement

A tunable laser (ANDO, AQ4321) is used as a light source providing a wavelength range from 1520 nm and 1620 nm with a central wavelength of 1570 nm. The light is collimated and incident onto the metasurface through a 50/50 beam splitter cube. The biosensor is mounted on a rotation stage to adjust the angle of incidence. The reflection spectrum is measured in real time using an InGaAs photodetector synchronized through an oscilloscope.

2.3. ErbB2 detection assay

The biosensor is used to quantify a well-established breast cancer biomarker, *ErbB2* (Ali et al., 2017a). The biofunctionalization of the surface begins with introducing an intermediate layer of GO to the surface. The GO layer allows enhancing the loading capability of *anti-ErbB2* molecules. In this step, the metasurface is treated with oxygen plasma for 50 s to make the SNPs hydrophilic. Next, a well-dispersed solution of single-layer GO nanosheets (0.4 mg/mL) is prepared in DI water, followed by thorough sonication for 1 h. 50 μL of this solution is drop-cast onto the metasurface and then dried at room temperature (25 °C) for 2 h. 20 μL of PBS (pH = 7.4) solution containing *anti-ErbB2* molecules (0.24 μM) is drop-cast onto the GO-coated metasurface, followed by treating a mixed solution of EDC (1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride, 0.2 M) and NHS (N-hydroxysuccinimide, 0.05 M) at a 1:1 ratio (Ali et al., 2016a). The abundant oxygenated groups such as –COOH and –CHO at GO are activated and utilized to make covalent binding with *anti-ErbB2* using the EDC-NHS coupling chemistry (Ali et al., 2016b, 2017a). To immobilize antibody molecules, the metasurface is kept in a humidity chamber for 12 h, and then is washed with PBS to remove unbound antibody molecules. The resulting primary amine groups present at *anti-ErbB2* bind with carboxyl groups at GO to form strong CH-NH amide bonds. Finally, 2.0 mg/mL of bovine serum albumin molecules is used to block non-specific sites of anti-*ErbB2* on the metasurface.

3. Results and discussion

3.1. Nanophotonic and nanofluidic simulations

The SNP array (350 nm thickness) is designed on the top of a 3 μm-thick oxide layer to provide an optical resonance around 1550 nm wavelength. The silicon device layer is transparent around this wavelength and has a negligible extinction coefficient $\kappa < 0.001$ and a large refractive index $n = 3.477$. The geometric parameters of the SNPs include the array period (Λ), nanopost width (w), device layer thickness