Multiplexed protein detection with an array of silicon-on-insulator microring resonators

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Abstract: We have demonstrated simultaneous detection of multiple antibodies in a highly specific way with a novel integrated lab-on-a-chip, featuring silicon-on-insulator ring resonators, non-aggressive microfluidics packaging, receptor binding through a thin PEG layer, and camera based parallel readout.

OCIS codes: (130.0130) Integrated optics; (230.0230) Optical devices; (280.1415) Biological sensing and sensors

1. Introduction

Label-free monitoring of biomolecular interactions has become of key importance for the emerging proteomics field. Optical cavities are considered promising devices to satisfy the high demands for real-time screening of complex fluids [1,2]. The shift of resonance wavelength when biomolecular interaction takes place at the cavity's surface, is a direct measure for the number of binding events and can be used for sensing. We have demonstrated simultaneous detection of several proteins (anti-HuIgG, anti-HSA, BSA…) in complex body fluid with clinically relevant concentrations in a highly specific way. For that we developed a novel platform consisting of an array of three by four silicon-on-insulator ring resonators, low-temperature PDMS microfluidics packaging, and camera based parallel readout. Using silicon-on-insulator (SOI) as material platform offers high sensitivity through miniaturization and low cost fabrication by means of standard technology steps as used in CMOS fabs, in particular 193nm deep-UV lithography[3]. Multiplexed and low noise detection requires uniform fluid delivery. Our non-aggressive microfluidic integration approach enables excellent compatibility with spotted receptor molecules. The quality of a biosensor critically depends on the quality of the interfacial layer. The chemical modification of the surface has to allow immobilization of receptor molecules and at the same time effectively block non-specific interactions with the macromolecular components of the analyzed sample. We obtain extremely selective biomolecular interaction by grafting a 2.5nm poly (ethylene glycol) (PEG) polymer layer to the silicon.

2. Platform fabrication: SOI micro ring resonators, microfluidics and surface modification.

The device is illustrated in Fig. 1A. Four rings are connected to one bus waveguide, each of them having its own drop signal port. Three of these are placed independently next to each other. The input waveguides are addressed simultaneously with a collimated bundle from a tunable laser source. The output signals are vertically coupled to free space by means of integrated grating couplers and are imaged with an infra-red camera. Hence the optical setup allows for extremely high alignment tolerances. Dedicated software stores the maximum intensity per output spot for each wavelength sweep. A Lorentzian is fitted to all signals and the resonance wavelengths are tracked over time (1 measurement/min). Waveguide dimensions are 450x220nm^2, the racetracks have 5µm radius and 2µm straight section. The resonators in series differ by 30nm in circumference to avoid spectral overlap of resonances. Each resonator having a free spectral range of 15nm, about 100 ring resonators could be placed in series. Q-factors are up to 20,000. A high internal Q-factor corresponds to small peaks and hence to a high detection resolution. Moreover the higher the Q-factor, the greater the fitting accuracy. However, high Q-factors set high demands to the equipment resolution and the Q-factor is related to the physical size of the resonator. The smaller the ring, the smaller the amount of molecules needed for full coverage. A Q-factor of 20,000 holds a good compromise between small surface area, small peak width and maximal extinction.

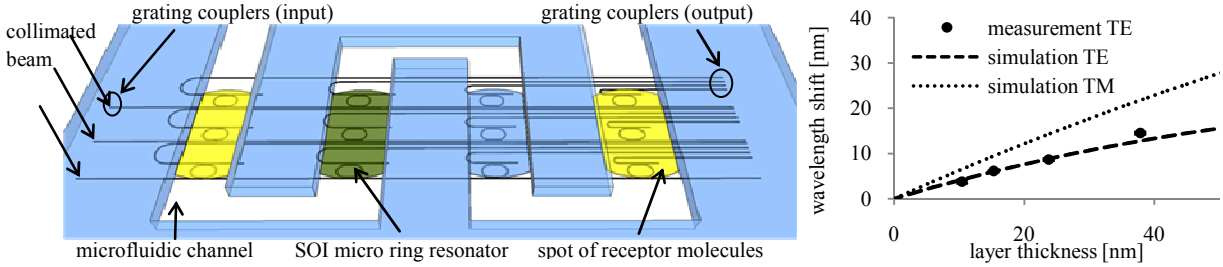

Fig. 1. A) illustration of the disposable lab-on-a-chip (not to scale), B) simulation and measurement of SiO2 adlayers with increasing thickness.

A H_2N-PEG-NH-Boc layer was grafted to the SOI chip as described in [4]. We obtain 2.5nm coatings with 0.59 amino groups per nm^2. The amino groups anchored to the PEG serve to couple the receptor molecules. After deprotection of the PEG layer and dissuccinimidyl carbonate (DSC) coupling, we spotted small drops of HuIgG, HSA, BSA and Biotin-NH to the surface. 10% of glycerin was added to prevent fast evaporation of the spot, so coupling of the proteins to the surface could happen over several hours.
We use a stamp-and-stick method to transfer the channels on the SOI chip without damaging the fragile receptor molecules. 200x50μm^2 microfluidic channels are fabricated in PDMS by soft lithography. The PDMS is given a short O_2-plasma treatment to enhance adhesion to the glue. After that the PDMS microfluidics chip is stamped in a 7μm SU-8 glue layer, after which a thin layer of glue surrounds the channels. The PDMS channels are aligned to the sensor array with a flip-chip bonder and the SU-8 glue is UV-cured for 2.5minutes.

3. Experimental results

Figure 2A and 2B are results of two sensing experiments. In experiment 2A, three different proteins were spotted on the resonators: HuIgG, HSA and BSA. The fourth column of resonators was left untouched. Two different diluted body fluids are successively pumped through the channels; goat serum with 128μg/ml anti-HuIgG and 1.9mg/ml protein and goat serum with 82.6μg/ml anti-HSA and 1.5mg/ml protein (dilutions with PBS+0.1% Tween). Notice the high concentration of non-specific proteins versus the low concentration of the specific antibodies in the serum. The non-specific background signal is extremely low versus the specific signal, ratios go up to 20/1. In [4] we proved to have 10ng/ml avidin detection limit. This concentration detection limit depends on protein affinity and working conditions, a reference table for various antibodies can be made. The wavelength shifts are transformed into biolayer thicknesses based on simulations (refractive index biolayer ~ 1.42). To verify the validity of the simulation method we measured the resonance shift after deposition of SiO_2 layers of various thicknesses on the micro ring resonators (see fig. 1B). Sensing with TM polarized light is 1.5 times more sensitive, but the grating coupler design for these experiments was optimized for TE polarization. Experiment 2B gives similar results with an extra spot of biotin-NH. The concentration of avidin was 102μg/ml in 37x diluted goat serum. Comparison with results of [3,4] shows that the saturation levels are not maximal (0.6nm shift). This is probably due to partial hydrolysis of surface NHS-ester groups during the coupling step. Adjusting temperature or molecular concentration and control of the water content during coupling can further optimize the receptor coverage.

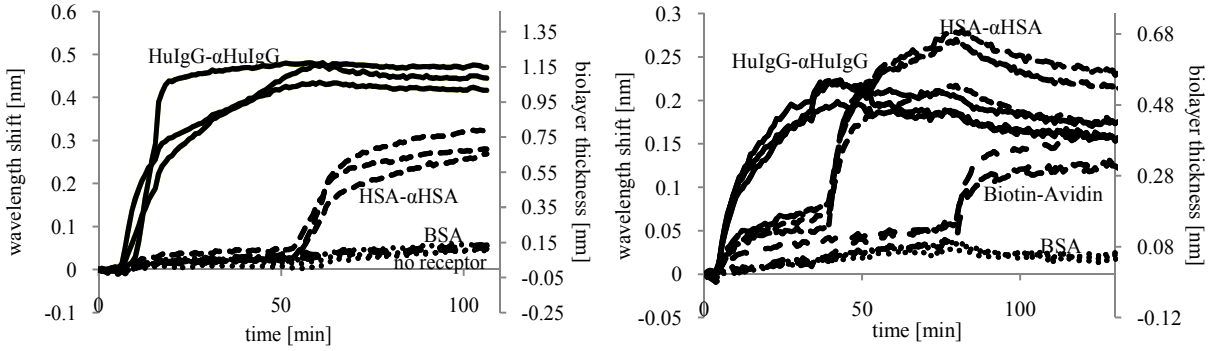

Fig. 2. A) experiment with 3 different molecules spotted on the SOI chip, B) experiment with 4 different molecules spotted on the SOI chip.

4. Conclusions

We developed a novel lab-on-a-chip using silicon photonics integrated chips with 3x4 micro ring resonator sensors. With this platform we demonstrated simultanous detection of several antibodies. Fabrication, chemistry, readout and packaging are scalable to detect hundreds of interactions within minutes.

5. References

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