Highly Multiplexed Antibody-Antigen Detection using Nanoscale Optofluidic Resonators

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Abstract:
Photonic crystal resonator based biosensors [1] have generated a lot of recent interest due to their ability to confine light within sub-wavelength modal volumes thus allowing for ultra-small detection sites. In addition, photonic crystal based architectures allow for a much larger degree of light-matter interaction making them significantly more sensitive as compared to evanescent field based sensing techniques. Our nanoscale optofluidic sensor array (NOSA) platform [2] consists of multiple evanescently coupled one dimensional (1D) photonic crystal resonators and incorporates both these advantages while allowing for highly multiplexed detections. We have characterized device behavior for biomolecular detections and our experimental results demonstrate the successful label-free detection of anti-streptavidin antibodies as well as the ability to perform real-time binding kinetics measurements.

Summary of Research:
Figure 1 shows a 3-D illustration of our sensor design. A central defect in the 1D photonic crystal [3] gives rise to a defect state in the photonic bandgap. By varying this defect cavity spacing, we can tune the resonant wavelength of this defect state across the bandgap of the side resonator. Analogous to ring resonators [4], light corresponding to the resonant wavelength couples evanescently into the side resonator and is sustained within it. This results in a dip in the output spectrum of the waveguide at the resonant wavelength. Since the resonant structures lie to the side of the waveguide the bandgap does not interfere with the light transmission outside of that which lies in the resonant peak. Thus our unique design allows multiplexing along a single waveguide by simple placement of a large number of side resonators along the waveguide, each of which is fabricated to have a slightly different resonant wavelength. By controlling the surface functionalization chemistry, one resonator can be utilized as a control while the other acts as a sensing element for the biomolecule of interest.

An SEM image of a typical nanoscale optofluidic sensor array (NOSA) device is shown in Figure 2. These resonators are extremely sensitive to refractive index changes in the innermost holes. When biomolecules bind to the surface of the sensor it causes a slight increase in the local refractive index around the sensor. This results in a red-shift in the resonant wavelength of the sensor. As a result one can make inferences regarding the binding of bio-molecules to the sensor surface by monitoring changes in the resonant wavelength of the sensor. With the $Q$-factor of these devices being between 2000 and 3000 and given the operational range of a standard...
1550 nm laser, we estimate that we could have at least 50 such resonators on a single bus waveguide, thus allowing the possibility of performing 50 detections in parallel on a single waveguide. The $Q$-factor of such resonators can be significantly improved so these devices could be used to perform more than a hundred parallel detections.

During the detection of streptavidin antibody binding at the NOSA sensor surface, it was possible to observe binding kinetics of the reaction by monitoring the transmitted power at a fixed wavelength as illustrated in Figure 3. To demonstrate the multiplexability of the NOSA device, monoclonal antibodies to interleukin 4, 6, and 8 were immobilized on adjacent resonators and tested for cross-reactivity (see methods). Glutaraldehyde functionalized resonators and streptavidin immobilized resonators served as controls for non-specific analyte adsorption. To assess multiplex capability, we tested concurrent detection of multiple interleukins. Figure 4a shows the resulting spectra after introducing 1 µg/ml of interleukin-8 along with 10 µg/ml of interleukin-6, followed by sequential association of secondary antibodies corresponding to each of these interleukins. In the figure, the resonant wavelengths numbered 1 through 5 correspond to control (glutaraldehyde functionalized), streptavidin-functionalized control, anti-interleukin-6, anti-interleukin-4, and anti-interleukin-8, respectively (Figure 4b). The test spectrum (red) is superimposed over the baseline spectrum (blue) to illustrate the lack of significant non-specific binding. We observe shifts in the resonance corresponding to immobilized monoclonal anti-interleukin-8 (0.58 nm) and -6 (0.68 nm), but no significant shift in the resonance corresponding to immobilized monoclonal anti-interleukin-4. This further supports the ability of the NOSA device to function as a multiplexed biosensor with little cross-reactivity or non-specific binding.

References: