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Label-Free Detection of *Escherichia coli* using Silicon Nanophotonic Biosensors

Emily Thompson
Biomedical Engineering, Arizona State University

NNIN REU Site: Washington Nanofabrication Facility & Molecular Analysis Facility, University of Washington, Seattle, WA
NNIN REU Principal Investigator: Daniel M. Ratner, Ph.D., Bioengineering, University of Washington
NNIN REU Mentors: Shon Schmidt and Pakapreud Khumwan, Bioengineering, University of Washington
Contact: erthomp2@asu.edu, dratner@uw.edu, shons@uw.edu, pakapreud@gmail.com

Abstract:

Medical diagnostics influence 60-70% of patient treatment decisions [1], yet sophisticated, sensitive diagnostics are still largely confined to hospitals and laboratories, limiting their impact in point-of-care settings. The growing field of nanophotonic biosensors has the potential to bring clinically relevant and sensitive medical diagnostic capabilities to the patient bedside. These silicon-based sensors utilize light to detect biomolecular interactions and are compatible with today's established complementary metal-oxide-semiconductor (CMOS) foundry processes for high-volume, low-cost fabrication. The goal of this project was to demonstrate the potential application of silicon photonic biosensors to bacterial detection and compare their performance with that of a competitive technology, surface plasmon resonance (SPR). We first verified and optimized binding of *Escherichia coli* (*E. coli*) with SPR, developing an assay suitable for use on two different silicon photonic systems (transverse electric and transverse magnetic mode ring resonators). The results were compared to show the viability of bacterial detection using silicon photonic biosensors, and binding was characterized with scanning electron microscopy (SEM).

Introduction:

We employed SPR imaging to validate our bacterial binding methodology due to its well-established reputation as an optical biosensing platform [2] and its similarity to silicon photonics. In SPR, a beam of visible-spectrum light is guided through a prism onto a gold chip, and the intensity of the reflected beam is detected. Light directed at a certain resonant angle, dependent upon the refractive index of the chip, excites the surface electrons, or plasmons, causing them to oscillate. In biosensing applications, the chip is functionalized with ligands that bind to a target analyte, which shifts the resonant angle of the light [2]. This shift can be measured, enabling direct detection of analyte binding.

In silicon photonics, light is directed through a linear silicon wire known as a waveguide, which allows for coupling of the light into a resonator. Binding of bacteria at the resonator's functionalized surface changes the local refractive index and shifts the resonant wavelength, the wavelength of input light at which signal intensity is minimal due to interference [4].

The polarization of light traveling through the ring resonators determines the sensing region at the rings' surface, where binding occurs. We studied two types of ring resonators: rings using transverse electric (TE) mode light and rings using transverse magnetic (TM) mode light. The TM mode rings have a larger sensing region extending beyond their surface, so we hypothesized that TM mode rings would be better suited than TE mode rings for detection of large molecules like bacteria.

Experimental Procedure:

In our SPR experiment, a gold chip was spotted with RNase B, a ligand to which *E. coli* fimbriae bind [3]. The rest of the chip was blocked in bovine serum albumin (BSA), which served as a negative control. *E. coli* were flowed across the chip using a fluidic channel. To validate specific bacterial binding, we mixed the *E. coli* with alpha-phenyl mannoside, which contains the D-mannose moiety and inhibits the bacteria FimH receptors, preventing binding to RNase B.

The TE mode ring resonators were tested using the Maverick Detection System (Genalyte, San Diego, CA). Bacteria were flowed across a chip functionalized with RNase B and no shift in resonant wavelength was observed, indicating that there was no binding. We developed a custom test platform and software in order to test bacterial binding on TM mode ring resonators.

First, a phosphate buffered saline (PBS) baseline was established, after which RNase B was flowed across the chip for functionalization. Bacteria were then flowed across the chip.

Results and Conclusions:

The SPR experiment shows a shift in intensity for the RNase B regions of the chip as more bacteria are flowed across, indicating bacterial binding (Figure 1). The PBS wash at 1100 seconds removed any weakly bound molecules. Additionally, our experiment to validate specific bacterial binding was successful (Figure 2). Bacteria mixed with FimH inhibitor did

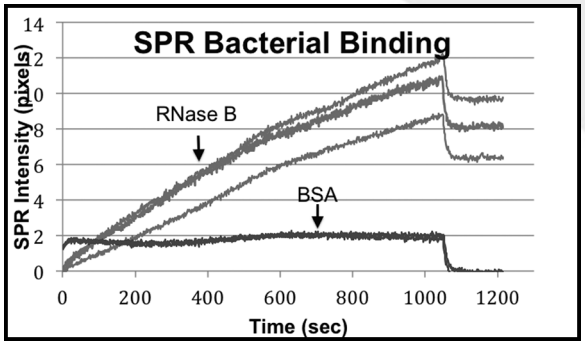

Figure 1: Bacterial binding curve using SPR.

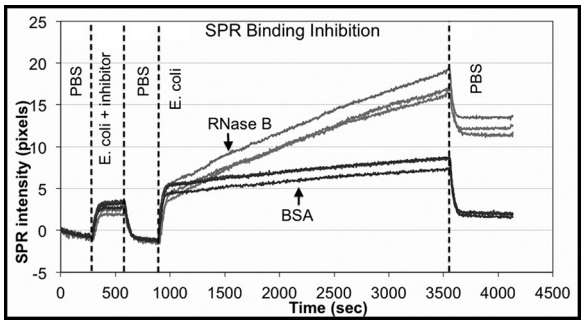

Figure 2: SPR bacterial binding curve using alpha-phenyl mannoside inhibitor.

not bind to the RNase B, while bacteria without the inhibitor did bind. This verifies that the signal response detected was specific binding of *E. coli*.

The TE ring resonators showed no discernible binding, in part because of their lower level of sensitivity and their smaller resonator size relative to the large cell bacteria. This size disparity can be seen by SEM imaging of a ring resonator chip spotted directly with bacteria (Figure 3). We hypothesize that the protective fluoropolymer cladding makes binding of the bacteria to the smaller resonator difficult when the bacteria are flowed across the chip.

By comparison, the TM ring resonators did show bacterial binding (Figure 4). The shift in resonant wavelength when bacteria were flowed across the resonators indicates bacterial binding.

Future Work:

Future work with silicon photonic devices will focus on repeating and validating our results with the TM mode chips using various on-chip controls, as well as improving the devices' biocompatibility so that they can process undiluted clinical samples. Silicon photonic sensors will likely be expanded into many other diagnostic applications, especially at the point-of-care.

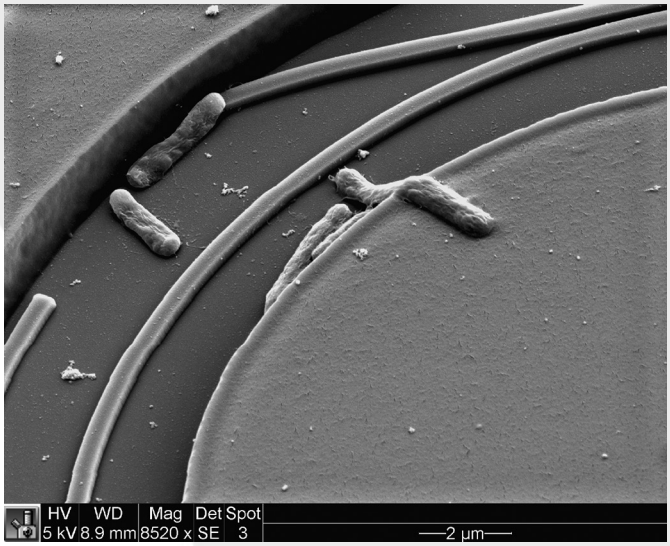

Figure 3: SEM of a Genalyte TE ring resonator spotted with E. coli.

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References:

[1] The Lewin Group, I. The Value of Diagnostics Innovation, Adoption and Diffusion into Health Care. (2005)

[2] Fan, Xudong, et al. "Sensitive optical biosensors for unlabeled targets: A review." analytica chimica acta 620.1 (2008): 8-26.

[3] Nilsson, Lina M., et al. "Catch Bond-mediated Adhesion without a Shear Threshold: Trimannose Versus Monomannose Interactions with the FimH Adhesion of *Escherichia coli*." Journal of Biological Chemistry 281.24 (2006): 16656-16663.

[4] Washburn, Adam L., and Ryan C. Bailey. "Photonics-on-a-chip: recent advances in integrated waveguides as enabling detection elements for real-world, lab-on-a-chip biosensing applications." Analyst 136.2 (2011): 227-236.

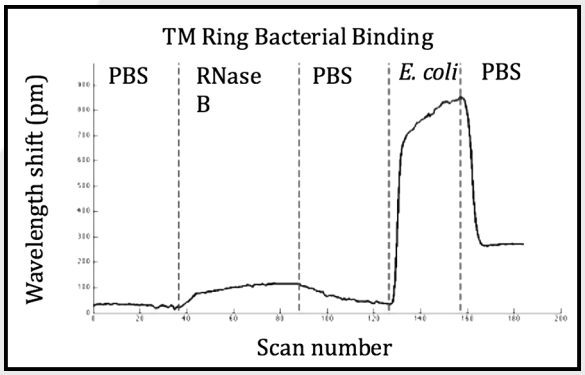

Figure 4: Bacterial binding curve using TM ring resonator.