VCSEL Optoelectronic Biosensor for Detection of Infectious Diseases

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***Abstract*—We report on the detection of human anti-dengue antibodies from serum samples using a compact optoelectronic label-free biosensor. The system consists of a tunable vertical-cavity surface-emitting laser, guided-mode resonant sensor surface, and two silicon pin detectors. This novel biosensor demonstrated sufficient sensitivity in a clinical-relevant assay, making it a potential new platform for simplified and rapid point-of-care diagnostic tests for the detection of infectious diseases.**

***Index Terms*—Biomedical transducer, medical diagnosis, vertical-cavity surface-emitting lasers (VCSELs).**

I. Introduction

RAPID detection of infectious diseases is indispensable for diagnosing patients and directing public health decisions, especially for developing countries. It often requires sensitive tools that can detect and quantify interactions of various biomolecular pathogens. The priorities for point-of-care diagnostics are rapidity, ease of use and low cost, while maintaining the required clinical sensitivity [1]. Enzyme-linked immunosorbent assay (ELISA) is the most predominant immunological diagnostic assay technology used in today's clinical settings [2]. Typical ELISA performs sufficiently to address a broad range of clinical diagnostic needs. However, the ELISA platform requires laboratory facilities and equipment that are restrictive for point-of-care diagnostics. Also, the standard ELISA procedures require tedious sample preparation from the numerous incubation and liquid dispensing-washing steps, and the data quantification process requires a spectrophotometer that is not available in a low-cost, mobile platform. Furthermore, a colorimetric label is required to be attached to the molecules under study, which may substantially increase the assay complexity and potentially alter the functionality of the molecules.

Label-free optical biosensors are among the most desirable technologies for bioassay because they utilize light as their detection mechanism [3], [4]. Assays using this method are much faster than those that require the attachment of labels, since no additional incubation and activation steps are required. Thus, the reduction in assay complexity results in faster screening or diagnosing time. In addition, they have several advantages, such as *in situ* real-time monitoring and high sensitivity to surface modifications.

Recently, we have demonstrated a compact label-free optoelectronic biosensor system utilizing a surface photonic crystal as the sensing surface [5] and a tunable vertical-cavity surface-emitting laser (VCSEL)-based detection platform [6], [7]. The surface photonic crystal is made from plastic material hence it is disposable and can be bonded to a standard 96-wells microtiter plate. Light incident in the normal direction is strongly reflected at a particular wavelength by the guided-mode resonance effect [8]. The resonant wavelength $(\lambda_{\mathrm{peak}})$ is strongly dependent on the optical thickness variation of the layer immediately above the sensor surface. Hence, the wavelength shift can be used to detect minute changes in the optical thickness resulting from the bimolecular interactions. The measurement system consists of a wavelength-tunable VCSEL and two silicon *pin* detectors. To scan the spectral resonance position, the VCSEL wavelength is varied by sweeping its bias current, causing a rapid thermal effect that shifts the lasing wavelength (2–3 nm). While the wavelength is tuned, the ratio of the two detector electrical signals is used to map out the spectral response of the resonant sensor surface and hence its resonant wavelength can be monitored. If required, the utilization of a microelectromechanical system (MEMS) tunable VCSEL can further increase the dynamic range of wavelength scanning to monitor interactions resulting larger wavelength shifts [9].

II. Dengue Virus Immunoassay

Dengue infection is a viral disease spread by mosquitoes very common in the tropics. It is considered as one of the most important infectious diseases in terms of morbidity and morality. Annual estimates shows more than one hundred million cases of dengue fever, several hundred thousand cases of dengue hemorrhagic, and about 2.5 billion people at risk for the infection [10]. Since dengue has a short incubation period in the human body—often days—a sensitive and high-throughput diagnosis tool is required to detect the epidemic disease for early treatment. Here, we investigated the application of the VCSEL optoelectronic biosensor for the detection of this infectious disease,

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specifically the biomolecular interactions of human antibodies against dengue viral antigens. This is a realistic test for the performance of the label-free biosensor in a clinical relevant assay, since human serum contains various interfering proteins such as albumin and other immunoglobulins to various pathogens, which can significantly lower the detection sensitivity and result in false-positive errors.

The experimental protocol followed the standard immunoglobulin G (IgG) capture immunoassay. Dengue viral antigens, prepared from lysed dengue virus-infected mosquito cells, were diluted in carbonate coating buffer [11]. For the first layer antigen coating, 50 μl of diluted dengue virus antigen was incubated for 12 hours at 4 °C in the microtiter wells, to allow for sufficient time for the dengue viral antigen proteins to be absorbed to the sensor surface. After the incubation period, all wells were washed with phosphate buffered saline-tween (PBS-T). Then the wells were blocked with commercial Starting Block solution for 2 h at room temperature. This step was required to minimize the nonspecific binding of the antibody in the subsequent step to the sensor surface, rather than to the initially deposited viral antigen proteins. Following the removal of block solution and the PBS-T wash procedure, the spectral response of the resonant sensor was measured with PBS as the background solution. The measured peak resonant wavelength was used as the initial reference for the subsequent measurement.

The testing samples in the experiment were human serum from two individuals: dengue-positive (serum with prior dengue infection) and dengue-negative (serum without prior infection). The dengue-negative serum was used to serve as the negative control. Both serum samples were diluted in PBS with the following serial concentrations (1 : 50, 1 : 100, 1 : 200, 1 : 400, and 1 : 800). The concentration was varied so we could determine the threshold concentration at which a patient's anti-dengue IgG antibody becomes detectable. Clearly the lower the detectable threshold, the more sensitive the sensor is and the earlier a patient can be treated. After the removal of the block solution, we dispensed 50 μl the diluted serum solution and incubated for 4 h at room temperature. Optical measurements utilizing the VCSEL biosensor were taken after the serum incubation.

ELISA was also performed in parallel in the dengue virus immunoassay, as a measure of comparison. In addition to the same procedure outlined above, after the 4 h of serum incubation the excess solution was removed and the wells were washed with PBS-T. The protocol for ELISA had additional incubation and wash steps for the secondary biotinylated goat anti-human antibody and streptavidin-conjugated Alkaline Phosphate enzyme. Lastly, 100 μl/well of 1-mg/ml pNPP in 0.2-M Tris-HCl was added and the plates were incubated. The AP enzyme would elicit a colorimetric change in background solution and the result was quantified as optical density measured by an ELISA spectrophotometer.

III. Experimental Results

The spectral shift from the optical response of the resonant sensor surface before and after dispensing the dengue-positive serum (at the concentration of 1 : 50 dilution) is shown in Fig. 1. The magnitude of the shift in the resonant wavelength ($\Delta\lambda$) quantifies the optical thickness change induced by the interactions between the first-layer coated dengue viral antigen and the human anti-dengue antibody from the dengue-positive serum sample. The raw experimental data contains periodic oscillations originates from the optical reflections within the measurement system, which can be minimized with proper antireflection coatings. In our case, the periodic oscillation in the data is removed by numerical methods, and the peak resonant wavelength can be more accurately obtained.

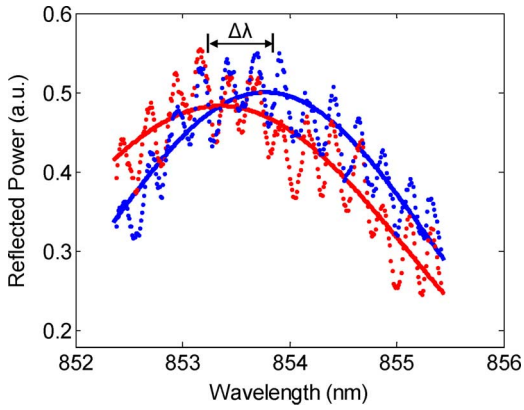

Fig. 1. Spectral shift from the guided-mode resonance of the surface photonic crystal sensing surface before and after dispensing the dengue-positive serum. The magnitude of the shift ($\Delta\lambda$) quantifies the optical thickness change induced by the biomolecular interactions of dengue virus antigen and human anti-dengue antibody proteins.

To determine if a person was previously infected by dengue virus, we performed an assay to measure the biomolecular interaction of dengue viral antigen with both the dengue-positive and dengue-negative serum. The measurement was performed both using the VCSEL optoelectronic biosensor and ELISA, as shown in Fig. 2(a). The proteins' interactions were quantified by the wavelength shifts for the VCSEL biosensor, while they were characterized by the change in optical density for ELISA. For the dengue-positive serum, the magnitude of wavelength shift measured in the biosensor platform is proportional to the serum dilution, as the wavelength shift ranges from 0.15–0.35 nm for the dilution concentration of 1 : 800 to 1 : 50. Meanwhile, the wavelength shift for the same dilution concentration of dengue-negative serum ranges from 0.1–0.2 nm. The small wavelength shift measured from the dengue-negative serum is mainly due to the nonspecific protein bindings. However, the measurement result indicates that even with large background of interfering proteins in the human serum, the VCSEL optoelectronic biosensor demonstrated its capability for detecting the human antibodies against dengue viral antigen, with sufficient differentiation from the negative control—particularly in the 1 : 50 to 1 : 200 dilutions which would be of most practical for clinical assay use. In comparison, the performance of ELISA exhibits similar characteristic between the optical intensity and dengue-positive serum dilution.

To further investigate the issue of nonspecific binding, we performed an assay using only the dengue-positive serum but varying the first-layer coated receptor: dengue viral antigen or mock proteins (without dengue antigen). The mock proteins are extracted from lysed uninfected mosquito cells and consisted

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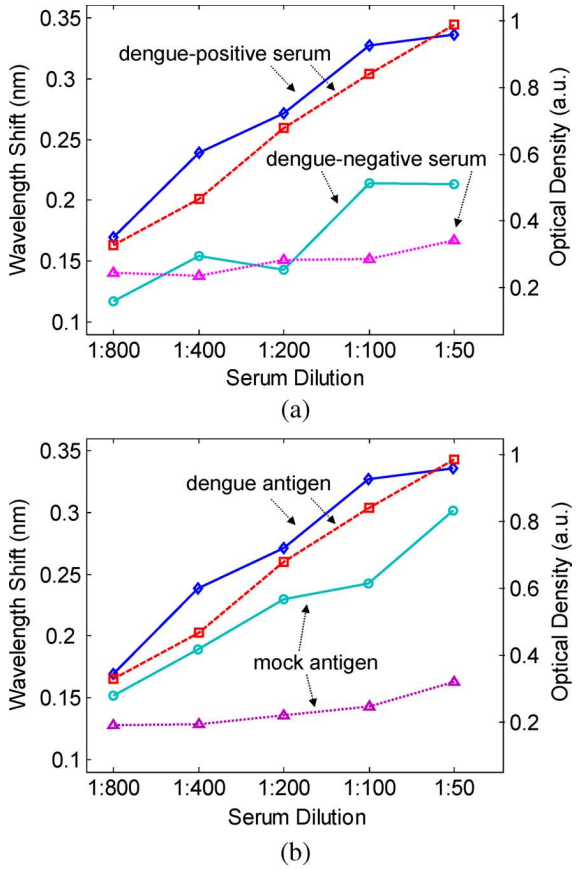

Fig 2. The dengue IgG antibody capture bioassay measurement was performed both using the VCSEL optoelectronic biosensor (solid lines) and the predominant technique ELISA (dotted lines). Wavelength shift is measured in the VCSEL biosensor, while optical density is measured in the ELISA. (a) Diagnosis by measuring interactions between dengue viral antigen and dengue-positive/dengue-negative serum samples. (b) The negative control experiment by measuring interactions between dengue viral antigen/mock proteins and dengue-positive human serum.

of a wide variety of proteins. Fig. 2(b) shows the protein interactions measured both by the VCSEL biosensor and ELISA. In this scenario, ELISA has a much better signal differentiation since the addition of secondary antibody increases the assay specificity, and hence the measured signal from the interaction of mock proteins and dengue-positive serum is much lower. On the contrary, the same measurement using the label-free biosensor illustrated the consequence of the insufficient binding specificity. Smaller signal differentiation was observed from the interaction of mock proteins and dengue-positive serum, as compared to that measured from dengue viral antigen and the dengue-positive serum. This is attributed to the nonspecific bindings from variety of proteins in human serum to mock proteins as well as to the sensor surface. Although the lack of specificity is an intrinsic limitation of any label-free biosensing system, we anticipate a better signal-to-noise could be obtain with a longer blocking step and by performing the PBS-T washing after serum incubation and right before the optical measurement.

IV. Conclusion

A compact label-free optoelectronic biosensor utilizing a surface photonic crystal sensing surface and tunable VCSEL-based measurement system is presented for the detection of human anti-dengue IgG antibodies. It has the potential to serve as a versatile tool for clinical diagnostics in resource-poor environments, where infectious disease monitoring is most critical. Experimentally, the biosensor has shown to be highly sensitive to surface modifications, with the ability to detect biomolecular interactions of human antibodies against dengue virus from serum samples. In this clinical-relevant assay, the biosensor demonstrated comparable detection sensitivity as the predominant technology ELISA and with sufficient signal differentiation for diagnosis, but with shorter and simpler assay preparation. However, the results from our negative control experiment suggest a better surface-blocking protocol is required to improve the proteins binding specificity to minimize false-positive errors. While the present work shows the proof-of-concept demonstration, for early infectious disease one should detect immunoglobulin M (IgM) in serum or saliva specimens [11]. But it is difficult to distinguish IgG and IgM bindings to determine past or present infection due to the lack of specificity in a direct assay, unless a secondary anti-human IgG or anti-human IgM antibody is used to differentiate between the two. Lastly, the sensor surface presented here utilizes the guided-mode resonance effect, but the tunable VCSEL measurement system in principle can serve as a compact and mobile replacement of bulky spectrometer or optical setup used in other optical biosensor technologies.

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