A Self-Referencing Biosensor Based upon a Dual-Mode External Cavity Laser

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Abstract: We demonstrated a dual-mode external cavity laser biosensor, and developed a self-referencing technique utilizing one of the two lasing modes as reference signal. This system achieves high-*Q* resonance, high sensitivity label-free detection and eliminates common-mode sources of sensor noise.
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The external cavity laser (ECL) biosensor, which utilizes a photonic crystal (PC) reflectance filter as the wavelength-selective element and a semiconductor optical amplifier (SOA) as the gain medium to form an active cavity, has been demonstrated to operate with high *Q*-factor resonance through the process of stimulated emission and with high sensitivity label-free detection by maintaining the bulk refractive index sensitivity of the PC passive resonator. [1, 2] To improve the ability of an external cavity laser biosensor to more easily distinguish true signals caused by biomolecular binding from a variety of sources of background noise, we developed a self-referencing approach based upon a dual-mode ECL, which represents the first application of dual-mode ECL in optical biodetection. [3]

In the dual-mode ECL sensor system, the two PCs are bonded to the opposite sides of a thin chamber frame, forming a flow cell that enables a test sample to be exposed to two PC sensors simultaneously. Both PCs serve as wavelength-selective mirrors for the external cavity laser cavity, which operates at two distinct lasing wavelengths corresponding to their own peak reflection wavelengths. A schematic drawing of the dual-mode external cavity laser setup is shown in Fig. 1. Because the test sample is introduced to both sensor surfaces simultaneously, any refractive index change of the test sample will induce equivalent shifts on both lasing modes. Likewise, because both the active and reference sensor share the same thermal environment and the same optical components of the ECL system, small temperature fluctuations of the test sample or any unintentional change to the instrument will be a common-mode effect. Finally, the active and reference sensors are exposed to the test sample and are thus subjected to the same nonspecific biomolecule binding. By simply subtracting lasing wavelength shifts of the reference sensor from the lasing wavelength shift of the active sensor, the system can accurately separate the biochemical binding signal from common mode noise signals.

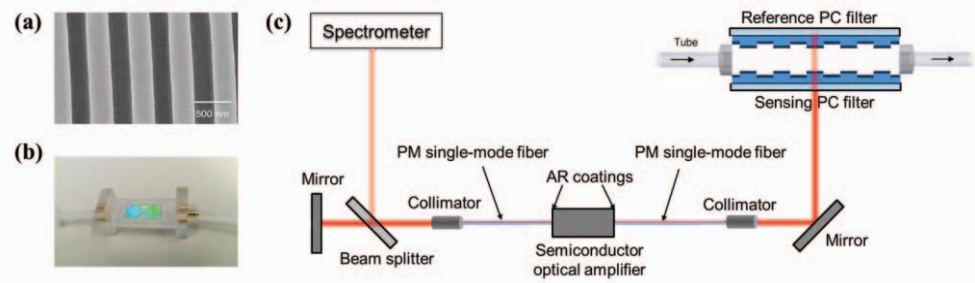

Figure1. (a) SEM image of the PC structure (b) Photograph of the flow cell where sensing and referencing photonic crystal sensors are incorporated as the upper and lower surfaces (c) Schematic drawing of the dual-mode external cavity laser biosensor system.

We demonstrated dual-mode lasing in this system. The reflection spectrum of the PC filters and the emission spectrum of the dual-mode ECL are shown together in Fig. 2. Both measurements were taken with sensing and reference PC surfaces immersed in deionized water (DI). Three flow experiments were performed to demonstrate the feasibility of the self-referencing technique. In the first test to demonstrate the compensation of environmental fluctuations, lasing wavelength values (LWV) for both modes were simultaneously monitored over time, with the measurement results represented in Fig. 3(a) and (b). Both of the signals gradually drifted downward, showing the impact of the room environment upon the sensors, for which no thermal control was applied. For a 3-minute measurement, the standard deviations for these two signals are 1.4 pm and 1.3 pm, respectively. By subtracting these two signals, a self-referenced signal was obtained exhibiting reduced drift, as shown in Fig. 3(c)

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with the standard deviation of 0.8 pm. In the second experiment to demonstrate the effect of a large common-mode shift, we induced bulk refractive index LWV shifts by the introduction of the solvent dimethyl sulfoxide (DMSO) to DI water with 1% concentration through the inlet into the flow cell.[4] LWV was initially measured with DI water in the flow cell and time sequence lasing wavelength data were measured after replacement of DI water with 1% DMSO solution. We observed a upward shift of 49 pm on both lasing modes, indicating the higher refractive index of the DMSO solution. Simple signal subtraction of the reference sensor from the sensing sensor results in no measurable shift, as desired, and had a standard deviation of 2.1 pm.

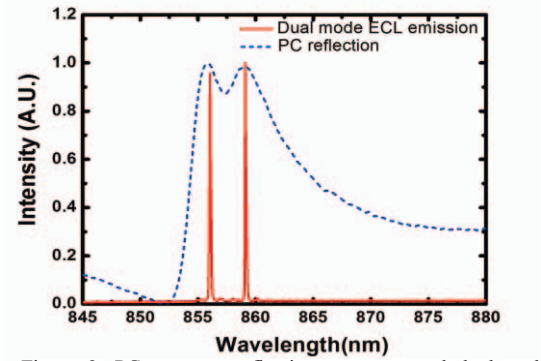

Figure 2. PC resonant reflection spectrum and dual-mode ECL laser emission spectrum when both PC sensor surfaces were immersed in DI water

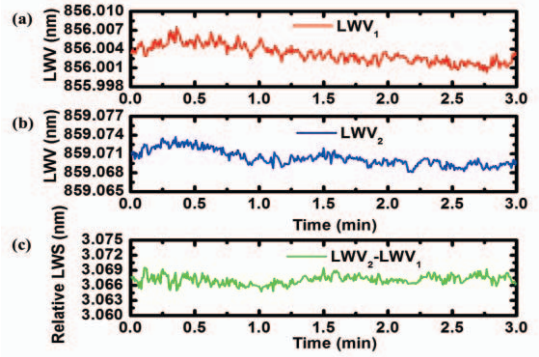

Figure 3. Response plots of laser emission wavelengths demonstrating the ability to correct for the effects of small fluctuations

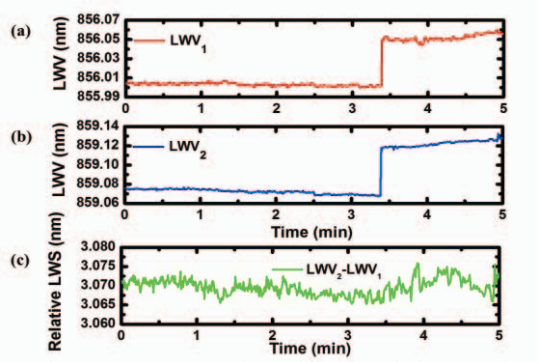

Figure 4. Response plots of laser emission wavelengths demonstrating the ability to compensate for errors induced by bulk refractive index variation of the analyte.

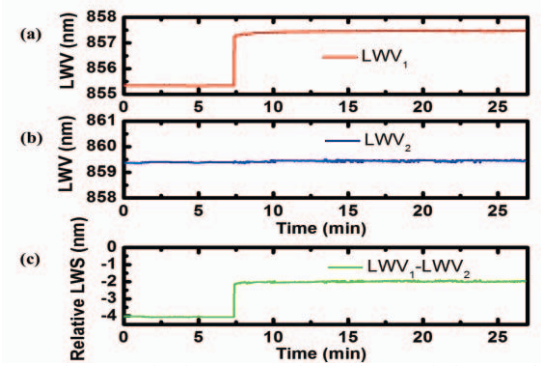

Figure 5. Kinetic response plots of laser emission wavelengths of the Protein A and pig IgG binding experiment

To demonstrate the self-referencing technique for detection of biomolecules, a simple assay for detection of pig immunoglobulin G (IgG) by immobilized Protein A was performed. The surface of the active sensor was prepared with immobilized Protein A (Pierce Biotechnology) layer, while the reference sensor PC was not functionalized with any biomolecular recognition layer. In the binding experiment, PBS solution was first injected into the flow cell to establish the baseline signals. Pig IgG (Sigma- Aldrich) diluted with 0.01 *M* PBS to a concentration of 0.5 mg/ml was introduced into the flow cell and allowed to incubate for 30 min, followed by a wash step to remove any unbounded IgG. Kinetic LWV responses of two differential binding signals were obtained for the detection and reference sensors, as shown in Fig. 5(a) and (b). The self-referenced signal was obtained by subtracting the non-binding signal from the binding signal, showing a ΔLWV=2.15 nm, which indicates only the effect of the pig IgG binding, referencing out environmental fluctuations, bulk refractive index changes, as well as non-specific binding signals.

In summary, a self-referencing ECL biosensor with dual-mode operation has been demonstrated and characterized. Future work on this self-referencing method will involve detection small molecule-protein interactions for applications in pharmaceutical development and life science research.

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