Supplementary Information

ECL emission linewidth and wavelength stability analysis

Typically, ECL systems utilize first-order diffraction from a grating to provide the optical feedback and wavelength selection, as in typical Littrow and Littman–Metcalf configurations.[1, 2] Guided mode resonant filters and photonic crystal reflection filters have also been demonstrated as efficient wavelength selective mirrors for ECL systems,[3-5] including the ability to tune an ECL through electronic and optical modulation of a PC with a liquid crystal medium on its surface.

One of the most striking features of the external cavity laser is its extremely narrow linewidth, which has been extensively studied both theoretically[6-9] and experimentally.[10, 11] The linewidth of a semiconductor laser single longitudinal lasing mode is given by the modified Schawlow and Townes formula that incorporates the Henry linewidth enhancement factor α_H:[12]

$$\Delta v = \frac{h v v_g^2 (\alpha_i + \alpha_m)\alpha_m n_{sp}}{8\pi P_{out}}(1+\alpha_H^2) \quad (1)$$

where hv is the photon energy, v_g is the group velocity, n_{sp} is the population inversion factor, P_{out} is the single-facet output power, and α_m is defined as the mirror loss.

$$\alpha_m = \frac{1}{2L}\ln(\frac{1}{R_1 R_2}) \quad (2)$$

Here, L is the laser cavity length, and R_1 and R_2 are the power reflectance of the two mirrors of the laser cavity. Equation (1) describes the spectral broadening of the laser linewidth due to phase and amplitude fluctuations caused by the unavoidable addition of spontaneous emission photons to the coherent lasing mode. These so-called quantum noise fluctuations define a lower

limit on the laser linewidth, which may be masked by larger noise fluctuations caused by mechanical/acoustic vibration or thermal variation. Extending the length of the cavity will decrease α_m (see Eq. 2), which reduces the linewidth. This can be understood by viewing the quantum noise-limited linewidth as being proportional to the ratio of the number of spontaneous emission photons in the lasing mode.[13] Increasing the cavity length both reduces the number of spontaneous emission photons (by decreasing the "cold-cavity" spectral width of each longitudinal mode) and increases the total number of photons in the cavity for a fixed output power. This is why the cavity length term appears twice in the Schallow-Townes equation. In short, the elongated resonator reduces the damping rate of intracavity light and the spontaneous recombination phase fluctuation, and therefore achieves low phase noise and narrow laser emission linewidth, with values typically below 1 MHz (0.0075 pm).[10, 14, 15] Additionally, the high gain of a semiconductor laser allows for continuous wave operation, which permits simple detection, dynamic monitoring, and an inexpensive, small, robust electrical pump system.

ECL performance can be optimized if the reflectance of the laser diode's output facet is extremely low,[15] as strong external feedback requires that the mirror losses of the solitary diode cavity be much (>20 dB) greater than the combined mirror, mode selection filter, and external cavity coupling loss.[16] Alternatively, the diode can be coupled to an optical fiber as a means for extending the external cavity length up to several meters. The smallest increment of wavelength tunability in an ECL is determined by the cavity mode spacing. For stable operation, it is necessary to control the laser injection current and temperature, which can be accomplished through closed-loop thermal control with a temperature sensor mounted into the same package as the SOA. Commercially available SOA controllers stabilize injection current to better than +/- 1 mA, and temperature to within +/- 0.3 mK over time periods of several hours.[17]

Methods: Biotin-streptavidin binding protocol

First, a proprietary polymer solution was introduced to the sensor that contains a high density of amine functional groups, and allowed to incubate with the sensor for 28 hours. This type of polymer forms a 2D structure on the photonic crystal surface. After removal of the polymer solution from the biosensor microplate wells and rinsing three times with PBS buffer solution, the second step of the protocol was performed, which was to functionalize the amine groups within the deposited polymer by exposure to glutaraldehyde (GA; MW = 100 Da; Sigma-Aldrich), which served as a bi-functional linker. The GA formed a stable covalent attachment to the polymer amine groups with one of its aldehyde groups, while its second aldehyde group was available to form a covalent bond with exposed amine groups on the outer surface of SA. The sensor surface was exposed to GA solution (25% in PBS buffer solution) for 3.5 hours, followed by a PBS wash. The final step in the protocol was the exposure to SA solution (0.5 mg/mL in PBS). After 30 hours of SA incubation, the sensor chip was rinsed with PBS. Unlike the DNA hybridization detection experiment, a blocking step was not incorporated in the small molecule detection assay. This is because, even without a blocking step, the nonspecific binding observed was very low, so the step was eliminated for the sake of simplifying the assay protocol.

The ECL wavelength shift was recorded for each of the steps in the process, resulting in $\Delta\lambda = 0.48$ nm for the polymer layer, $\Delta\lambda = 0.24$ nm for the GA layer, and $\Delta\lambda = 1.89$ nm for the SA deposition (data not shown).

The expected biosensor LWS can be calculated from the following equation:

$$\Delta \mathrm{LWS}_{\mathrm{biotin}} = \frac{4 \times \mathrm{MW}_{\mathrm{biotin}}}{\mathrm{MW}_{\mathrm{SA}}} \times \Delta \mathrm{LWS}_{\mathrm{SA}}$$

Our measured LWS from the biotin is smaller than the theoretical LWS value, which is 30.5 pm, which occurs because SA molecules were not able to bind four biotin targets due to blocked binding sites.

Estradiol-estrogen receptor (ER) binding

The sensor surface was first functionalized with a high density layer of GA following the same procedure as described above. Then the sensor surface was exposed to ER solution (2 μM in PBS). After 20 hours of incubation, the sensor chip was rinsed with PBS. The measured LWS for the ER was 2.10 nm. The expected biosensor LWS resulting from the estradiol binding can be calculated from the following equation:

$$\Delta LWS_{estradiol} = \frac{MW_{estradiol}}{MW_{ER}} \times \Delta LWS_{ER}$$

When the estradiol concentration was above the K_d value (0.1 nM), a condition corresponding to a full sensor surface coverage, a ~8.67 pm resonant wavelength shift was measured due to the specific binding between the estradiol and the estrogen receptor. Our measurement results agreed well with the theoretical prediction, which is 9.07 pm, a calculated value based on the molecular weight ratio of estradiol/ER and a 1:1 binding stoichiometry. At the K_d concentration, a concentration corresponding to 50% surface coverage, a resonant wavelength shift of 4.33 pm is expected. As discussed in our Discussion Session, our study of the sensor's stability reveals a standard deviation of laser wavelength shifts of ~ 1.2 (1 σ) pm without referencing and ~ 0.39 (1 σ) pm with referencing. These correspond to wavelength shift detection resolution of 3.6 pm (3 σ, without referencing) and 1.17 pm (3 σ, with referencing), respectively. Thus, we expect that the system will be capable of detecting estradiol at the K_d

concentration. If we are aiming to detect a concentration at 0.5 K_d, which corresponds to occupancy of approximately 25% of the available estrogen binding sites, the expected laser emission wavelength shift is 2.16 pm, a value can still be resolved using a referencing method or a quartz-based device. As such, based on our experimental results and the theoretical analysis, our sensor has the potential to estimate the K_d value of a small molecule-protein interaction using a dose-response analysis.

Simulations

A commercial implementation (DiffractMOD, RSoft Design Group) of the RCWA code was used to simulate the surface mass detection limit of the ECL system. One period of the PC resonator filter was simulated, with periodic boundary conditions applied to both extents. The incident light was set to be transverse magnetic (TM) polarized plane wave incident from the bottom of the device. The materials were assumed lossless and the RI dispersion was assumed to be flat at a wavelength of 860 nm. For the simulation, only the PC reflection peak wavelength shift was calculated.

Fabrication of quartz photonic crystal

Electron-beam lithography (JEOL JBX-6000FS) was used to define a one-dimensional photonic crystal surface structure of period Λ = 550 nm and duty cycle of 50% on a fused quartz substrate with ZEP (ZEP520A-7, Zeon chemicals) as the mask layer. The pattern was exposed to a size of 1×1 mm^2 followed by development and dry-etching in a CHF_3 reactive-ion etching process. A layer of high refractive index TiO_2 (t = 90 nm) was then sputtered (Lesker PVD 75) to form the final device.

Figure of Merit Table:

Table 1. FOM comparison among a variety of optical resonator biosensor technologies

Table 1. Figure of merit (FOM) comparison with other optical label-free biosensors

Methods	*Q*-factor	S_b (nm/RIU)	*Q*-factor × S_b (FOM)
SPR[18]	~ 10	2600	2.6×10^4
1D PC[19]	850	212	1.8×10^5
Microring[20]	2×10^4	140	2×10^6
Microsphere[21-25]	2×10^6	26	5.2×10^7
OFRR[26]	10^6	50	5×10^7
DFB laser[27]	2×10^4	100	2×10^6
PC laser[28]	7×10^4	350	2.5×10^7
ECL (this work)	2.8×10^7	212	5.9×10^9

S_b, bulk refractive index sensitivity; SPR, surface plasmon resonance; 1D PC, one-dimensional photonic crystal biosensor; OFRR, optofluidic ring resonator; DFB laser, distributed-feedback laser; PC laser, photonic crystal laser, ECL, external-cavity laser

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