Label free biosensor incorporating a replica-molded, vertically emitting distributed feedback laser

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A label free biosensor based upon a vertically emitting distributed feedback (DFB) laser has been demonstrated. The DFB laser comprises a replica-molded, one-dimensional dielectric grating coated with laser dye-doped polymer as the gain medium. Adsorption of biomolecules onto the laser surface alters the DFB laser emission wavelength, thereby permitting the kinetic adsorption of a protein polymer monolayer or the specific binding of small molecules to be quantified. A bulk sensitivity of 16.6 nm per refractive index unit and the detection of a monolayer of the protein polymer poly(Lys, Phe) have been observed with this biosensor. The sensor represents a departure from conventional passive resonant optical sensors from the standpoint that the device actively generates its own narrowband high intensity output without stringent requirements on the coupling alignments, resulting in a simple, robust illumination and detection configuration. © *2008 American Institute of Physics*. [DOI: 10.1063/1.2913007]

Label free biosensors based upon detecting shifts in emission wavelength, coupling angle, or the magnitude of optical resonances have become an effective and commercially viable means for characterizing biomolecular interactions.[1–3] Desirable properties for such sensors include ease of fabrication over large surface areas, robust noncontact illumination/detection optics, the ability to perform independent assays in parallel, and the option of incorporating the sensor into common biochemical assay formats such as microplates or microfluidic channels.[4,5] The ability to resolve changes in the adsorbed mass density below 1 ng/mm^2 is essential for assays requiring the detection of samples at low concentration, or of biomolecules with low molecular weight, such as drug compounds. To address this challenge, researchers have designed label free biosensor structures with passive optical resonators having Q factors as large as 10^7 (Ref. 6–8) thereby reducing dramatically the shift in wavelength of the sensor that can be detected reliably.[9] However, the drawbacks of such evanescent coupled, passive resonators generally include the necessity for precise optical alignment with the illumination source which limits their application to biomolecule screening. In contrast, active sensors[10] facilitate the coupling of the excitation source with the collection optics since the optical source is integrated with the sensor.

This letter reports the fabrication and characterization of a distributed feedback (DFB) laser biosensor. Fabricated as a one-dimensional, dielectric subwavelength grating incorporating a dye-doped polymer gain region, the DFB laser source features a second-order Bragg grating that supports a vertical emission mode by first-order diffraction.[11–13] In addition to simplifying the excitation of the laser and extraction of the output radiation, this monolithic structure is stable and sensitive. Experiments demonstrate that the bulk sensitivity of this detector, operating in the blue-green ($\lambda \sim 496$ nm), is 16.6 nm/refractive index unit (RIU), and a single monolayer of the protein polymer poly(Lys, Phe) can readily be observed. Since DFB laser structures can be fabricated uniformly and inexpensively over surface areas of at least 1 m^2 by employing recently developed nanoreplica molding techniques[14–16] to produce the periodic grating structure, this biosensor is potentially inexpensive and amenable to mass production.

A cross-sectional diagram (not to scale) of the DFB laser structure adopted for the present experiments is shown in Fig. 1. After spinning a 1 μm thick film of nanoporous glass (n_d=1.17) onto the surface of a glass substrate having a refractive index (n_g) of 1.5 and a surface area of 10.8 $\times 7.2$ cm^2, a grating was produced in the nanoporous glass film with a polydimethylsiloxane (PDMS) mold bearing a negative volume image of the desired grating pattern.[16] The flexible PDMS mold used for imprinting the device grating pattern was itself molded from a "master" mold which was fabricated on a 200 mm diameter silicon wafer by conventional photolithographic and dry etching processes. Atomic force microscopy verified that the replicated gratings on the finished device have a periodicity and depth of 360 and 78 nm, respectively. The waveguide/gain medium region

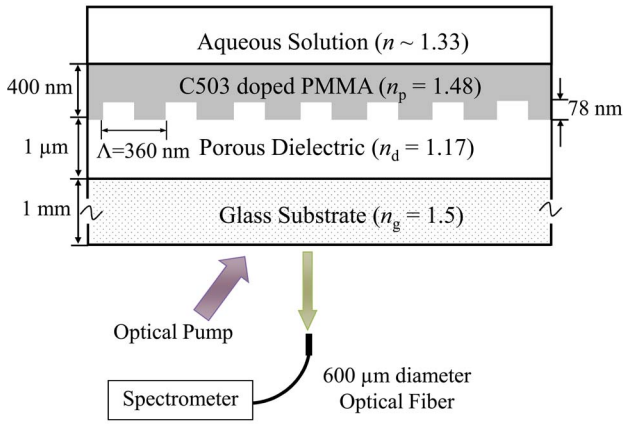

FIG. 1. (Color online) Cross-sectional diagram (not to scale) of the DFB laser-based biosensor structure and partial schematic of the instrumental arrangement. The period and depth of the grating structure are 360 and 78 nm, respectively.

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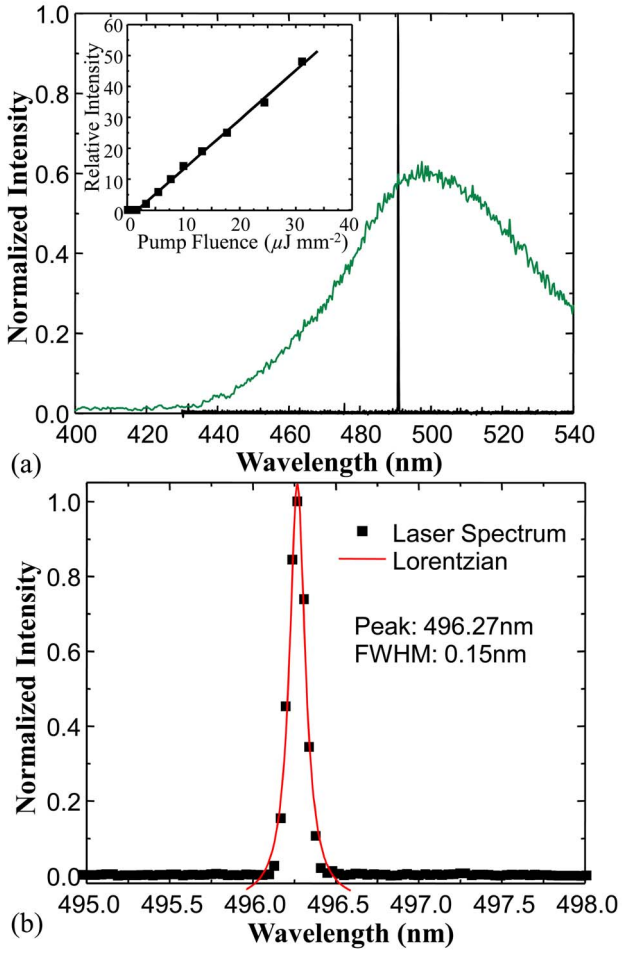

FIG. 2. (Color online) (a) Spontaneous emission and laser spectra for the DFB laser, recorded for pump fluences above and below threshold (1.0 and 8.5 μJ mm^{-2}, respectively). The inset displays the dependence of the relative laser output power on the pump fluence. From these data, the threshold pump fluence is calculated to be 1.8 μJ mm^{-2}. (b) Observed laser spectrum when the sensor surface is immersed in DI water.

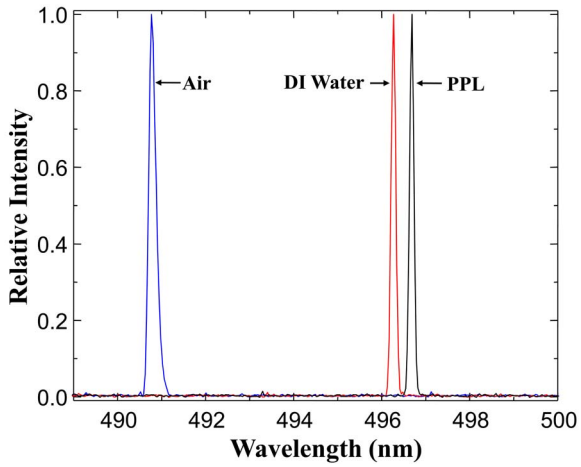

FIG. 3. (Color online) Normalized laser emission spectra for the sensor surface in contact with air, DI water, and PPL solution. The sensor was pumped at a fluence of 10.2 μJ mm^{-2} for all three measurements.

was fabricated by preparing a 10 mg/ml solution of Coumarin 503 dye (Exciton) and CH_2Cl_2 mixed with polymethyl methacrylate (950 PMMA C4 resist, Microchem) to a volume percentage of 10%. This material was sonicated for improved homogenization and subsequently spin coated onto the dielectric grating/substrate assembly at 4000 rpm for 30 s. The device was baked on a 110 °C hotplate for 2 min to remove the solvent from the dye-doped PMMA layer and harden the film. Finally, the PMMA surface was exposed to an O_2 plasma for ~30 s for final surface cleaning, leaving the surface in a hydrophilic state. The completed DFB active layer has an overall thickness of ~400 nm and a refractive index of $n_p=1.48$ as measured by ellipsometry.

Device characterization experiments entailed photoexciting the dye-doped polymer with ~10 ns pulses from a frequency-tripled, *Q*-switched Nd:YAG (yttrium aluminum garnet) laser ($\lambda=355$ nm) at a repetition rate of 10 Hz. V_{iii} as laser emission emanating from the DFB grating was coupled through an optical fiber to a spectrometer having a resolution (in first order) of 0.05 nm full width at half maximum (FWHM). With the DFB laser surface exposed to air, spectra representative of those recorded for pump fluences of 1.0 and 8.5 μJ mm^{-2} are presented in Fig. 2(a). For a fluence below threshold (0.8 μJ mm^{-2}), the fluorescence from the dye/polymer matrix is broad ($\Delta\lambda\sim70$ nm FWHM). At higher intensities, laser oscillation occurs at $\lambda=490.77$ nm. As illustrated by the inset of Fig. 2(a), the dependence of the relative laser pulse energy on the pump fluence (measured by a pyroelectric detector) exhibits a clear threshold fluence of ~1.8 μJ mm^{-2}. Above this value, the output pulse energy is observed to rise linearly with increasing fluence. Panel (b) of Fig. 2 presents the laser spectrum observed when the sensor surface is immersed in de-ionized (DI) water ($n_{DI}=1.33$) and the pump fluence is maintained at 10.2 μJ mm^{-2}. Note that the laser emission wavelength has shifted to $\lambda=496.27$ nm and the measured linewidth is 0.15 nm FWHM. It should be mentioned that, in an effort to maximize the resolution with which the peak laser wavelength was measured, each laser spectrum was fitted with a Lorentzian profile, as illustrated in Fig. 2(b). From the best fit to the spectrum, the peak wavelength was determined to within an estimated uncertainty of 3.3 pm.

Sensitivity to surface mass adsorption was characterized by introducing to the laser surface a solution of the protein polymer poly(Lys, Phe) (denoted PPL, Sigma-Aldrich) which has been shown to deposit a self-limited, single monolayer having a refractive index, thickness, and mass density of ~1.45, ~15 nm, and ~2.5 ng mm^{-2}, respectively. Figure 3 compares the laser spectra recorded when the device surface was exposed to air, DI water, and the PPL solution, from which the lasing wavelengths were determined to be 490.77, 496.27, and 496.69 nm, respectively. Throughout the experiment, the spectral linewidths remain narrow (<0.18 nm) which indicates that the breadth of the laser profile is insensitive to changes in the surface refractive index. On the basis of Fig. 3, the bulk refractive index sensitivity $S_b\equiv\Delta\lambda/\Delta n$ can be determined. From the air/water transition, for example, S_b for this DFB laser sensor is found to be 16.6 nm/RIU.

By monitoring the spectral output of the sensor over time, the kinetic characteristics of mass adsorption can be recorded. Figure 4 illustrates the dynamic detection of the growth of a single PPL layer. These data were obtained by initially establishing a baseline emission wavelength when the sensor surface was soaked in a phosphate-buffered saline (PBS) solution with *p*H=7.4. After 3–4 min, the PBS solution was replaced with PPL solution and stabilized for

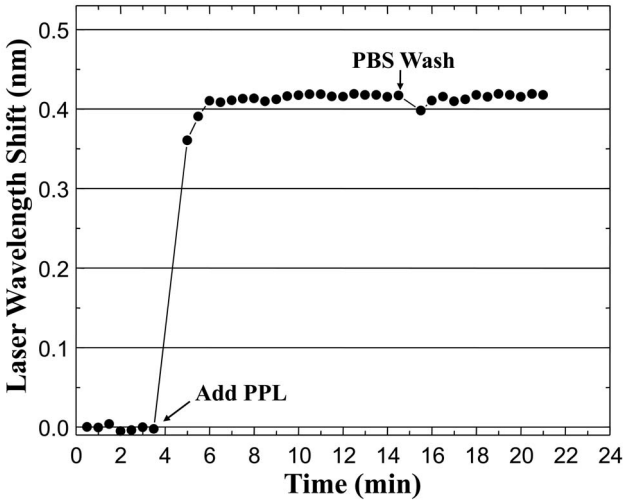

FIG. 4. Temporal variation of the shift in the peak laser wavelength when a PPL monolayer is deposited onto the DFB laser sensor surface. During the experiments, the pump fluence was fixed at 12.5 μJ mm^{-2}.

10 min. Then, the sensor surface was rinsed with PBS solution to remove any PPL that was not firmly attached to the sensor. In this manner, the sensor was observed to exhibit an emission wavelength shift of ~0.42 nm for PPL monolayer adsorption, and no drift of the lasing wavelength was detectable over time periods up to 1 h. The long term stability and spectral resolution of the sensor were explored further by exposing the sensor surface to DI water and capturing spectra every 30 s over a total time period of 15 min. Throughout this period, the pump fluence was maintained at 10.2 μJ mm^{-2} ($\pm 10\%$) and the fluctuation in the peak laser wavelength was characterized by a standard deviation (σ) of 3.3 pm. Therefore, the apparent system resolution (3σ) is 9.8. All measurements were made with the sensor at ambient temperature with no effort given to stabilizing temperature. Future implementation of a reference device or temperature control will undoubtedly improve the system resolution.[17]

The results presented here represent the first reported use of a DFB laser for label free optical biodetection. While the device in this work is fabricated upon a glass substrate with a dielectric DFB grating, alternative structures are anticipated that are fabricated upon flexible plastic substrates and dye-doped polymer DFB gratings having the potential for lower cost and larger surface areas. The high intensity, narrow bandwidth output of the DFB laser affords the capability for resolving extremely small wavelength shifts, and alternate wavelength measurement instruments (such as interferometers) will increase the sensitivity of this sensor by detecting yet smaller shifts in the laser wavelength.

In summary, a DFB laser biosensor has been demonstrated and characterized. The laser exhibits a single spectral peak having a linewidth of nominally 0.15 nm. The bulk sensitivity of 16.6 nm/RIU and the surface sensitivity of the biosensor result in a shift of the laser wavelength of 0.42 nm when a monolayer of PPL is adsorbed onto the sensor surface. Wavelength shifts as small as 9.8 pm can currently be resolved with an accuracy exceeding 99%. To achieve a portable biosensor system, alternative microchip pumping light sources are[18] under consideration. Alternative DFB laser sensor structures that can be fabricated upon plastic substrates in an inexpensive roll-to-roll process render this sensor of value for applications in life science research, diagnostic testing, and environmental detection.

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[1] B. Cunningham, B. Lin, J. Qiu, P. Li, J. Pepper, and B. Hugh, Sens. Actuators B **85**, 219 (2002).
[2] J. Homola, S. S. Yee, and G. Gauglitz, Sens. Actuators B **54**, 3 (1999).
[3] R. Narayanaswamy and O. S. Wolfbeis, *Optical Sensors: Industrial, Environmental, and Diagnostic Applications* (Springer, Berlin, 2004).
[4] C. J. Choi and B. T. Cunningham, Lab Chip **7**, 550 (2007).
[5] B. T. Cunningham and L. Laing, Expert Rev. Proteomics **3**, 271 (2006).
[6] F. Vollmer, D. Braun, A. Libchaber, M. Khoshsima, I. Teraoka, and S. Arnold, Appl. Phys. Lett. **80**, 4057 (2002).
[7] C. Y. Chao, W. Fung, and L. J. Guo, IEEE J. Sel. Top. Quantum Electron. **12**, 134 (2006).
[8] I. M. White, H. Oveys, and X. D. Fan, Opt. Lett. **31**, 1319 (2006).
[9] I. M. White and X. D. Fan, Opt. Express **16**, 1020 (2008).
[10] W. Fang, D. B. Buchholz, R. C. Bailey, J. T. Hupp, R. P. H. Chang, and H. Cao, Appl. Phys. Lett. **85**, 3666 (2004).
[11] H. Temkin, G. J. Dolan, R. A. Logan, R. F. Kazarinov, N. A. Olsson, and C. H. Henry, Appl. Phys. Lett. **46**, 105 (1985).
[12] S. Balslev, T. Rasmussen, P. Shi, and A. Kristensen, J. Micromech. Microeng. **15**, 2456 (2005).
[13] R. F. Kazarinov and C. H. Henry, IEEE J. Quantum Electron. **21**, 144 (1985).
[14] J. R. Lawrence, G. A. Turnbull, and I. D. W. Samuel, Appl. Phys. Lett. **82**, 4023 (2003).
[15] J. A. Rogers, M. Meier, A. Dodabalapur, E. J. Laskowski, and M. A. Cappuzzo, Appl. Phys. Lett. **74**, 3257 (1999).
[16] I. D. Block, L. L. Chan, and B. T. Cunningham, Sens. Actuators B **120**, 187 (2006).
[17] J. D. Suter, I. M. White, H. Y. Zhu, and X. D. Fan, Appl. Opt. **46**, 389 (2007).
[18] G. A. Turnbull, P. Andrew, W. L. Barnes, and I. D. W. Samuel, Appl. Phys. Lett. **82**, 313 (2003).