Compact Label-Free Biosensor Using VCSEL-Based Measurement System

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***Abstract*—We report an ultracompact label-free biosensor that uses a vertical-cavity surface-emitting laser (VCSEL)-based measurement system for the characterization of biomolecular interactions. It consists of a VCSEL, a plastic guided-mode resonant filter, and two p-i-n detectors. The system has demonstrated very high sensitivity to molecules on top of the sensor.**

***Index Terms*—Biomedical transducer, drugs, dynamic response, label-free, nonintrusive, protein.**

I. Introduction

BIOASSAYS are important tools used to detect interactions of various biomolecular complexes for pharmaceutical and biomedical applications. In addition, such analysis methods can provide a deep understanding on how proteins, encoded by DNA, interact with enzymes, inhibitors, or other proteins.

In general, bioassay techniques can be put into two categories: Labeling with compounds (such as fluorescent, radioactive, or colorimetric) and direct molecule identification. For the majority of bioassays currently performed for life science research and pharmaceutical screening, fluorescent or colorimetric chemical labels are commonly attached to the molecules under study [1]. Labels can be readily visualized and the measurement technique is simple. However, the attachment of labels may substantially increase the assay complexity. It may also alter the functionality of molecules through conformational modification or epitope blocking, which ultimately leads to errors in the data interpretation.

A label-free sensor is a bioassay tool that enables direct molecule detection, and it is generally desirable due to its nonintrusive nature of detection. The sensor typically consists of two parts. The first part is the binding surface, which is activated (coated) with a known receptor molecule that has a high affinity to the molecules to be detected. The second part is the detection mechanism that converts a recognizable molecular binding event into a quantifiable signal. The activation step is always done *a priori*. Assays using this method are much faster than the compound labeled ones, since no additional incubation and activation steps for the attachment of labels are required. Thus, the reduction in assay complexity results in faster screening or developing time.

Optical biosensors are well suited for label free sensing and utilize light as their detection mechanism. They have several advantages, such as *in situ* real-time process monitoring and high sensitivity to surface modifications, where most of the bioprocesses take place [2]. Optical methods can be measured by different quantities, such as angle, polarization, phase, amplitude, and frequency. The versatility of optical methods is noticeable by the success of several devices such as surface plasmon resonance, output grating couplers, ellipsometry, evanescent wave devices, and reflectance interference spectroscopy [1], [2]. Although bioassays using those methods are fairly sensitive, they are still quite slow, bulky, and expensive.

In this letter, we present an ultracompact label-free biosensor that uses a vertical-cavity surface-emitting laser (VCSEL)-based measurement system. It has high sensitivity, high resolution, low power consumption, low cost, and the potential advantage of fabrication in two-dimensional (2-D) arrays.

II. Biosensor System

The biosensor system contains a guided-mode resonant (GMR) filter as the activated surface [3]. Light incident onto the GMR in the normal direction can yield a narrow reflectance peak with reflectivity close to unity. The resonant wavelength $(\lambda_{\mathrm{peak}})$ is a strong function of the optical thickness of the layer immediately above the grating. Hence, the wavelength shift can be used to detect minute changes in thickness (t_{bio}) and refractive index (n_{bio}) of the biomolecules that are attached to the grating surface.

To date, the GMR method has demonstrated very high sensitivity and suitability for both dry and wet samples. Moreover, the GMR can be fabricated from plastic, thus making it very low cost, disposable, and environment-friendly [4]. The previously reported detection system consists of a white light source that illuminates the GMR sensor through an optical fiber that also collects the reflected light to couple it to a spectrometer. The spectrometer signal is then monitored to detect wavelength shifts [4]. Despite its high sensitivity, resolution will always be limited by the spectrometer pixel-wise nature and the tradeoff between resolution and signal strength can limit further improvements. Major challenges remain on how to make a low-cost, compact, and portable system that has high resolution and throughput.

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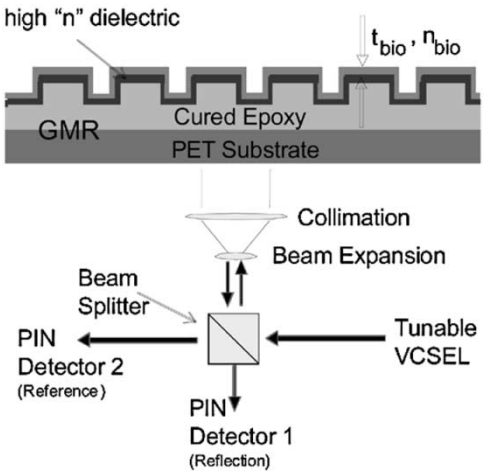

Fig. 1. Biosensor with VCSEL-based measurement system. A tunable VCSEL and two p-i-n detectors work as a readout system for a plastic GMR filter that is the binding surface. Peak reflectivity from the GMR is detected by correlating maximum normalized detector current with laser bias current.

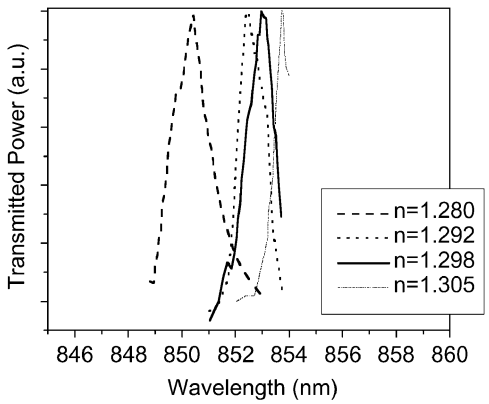

Fig. 2. Spectral shift of the resonance due to changes in the refractive index. Changes smaller than 0.005 can be detected by this system.

Here, the detection system for the VCSEL-based biosensor utilizes a tunable VCSEL and two p-i-n detectors (Fig. 1). In order to scan the position $\lambda_{\rm peak}$, we can tune the VCSEL wavelength by varying its bias current, causing a rapid thermal effect that shifts the lasing wavelength. Detector 1 is positioned to measure the reflected light from the GMR substrate, while Detector 2 enables the normalization of the incident power, since the VCSEL output power also varies with the bias current. Thus, the ratio of the two detector signals is used to monitor the reflection peak. Varying the laser bias current provides a fast but narrow range (2–3 nm) tuning of the VCSEL wavelength. Current was kept below 9 mA, assuring single-mode operation for the ion-implanted VCSEL with a 5°–7° beam divergence [5]. Temperature tuning is also used to augment the tuning range, extending it to 8–9 nm. Typical wavelength dependence for temperature and current tuning for a VCSEL is 0.08 nm/°C and 0.4 nm/mA, respectively. Sensitivity is greatly enhanced by using a laser rather than a white light source. Since the reflection peak is due to a resonance and very sensitive to incident angle [3], a coherent light source with smaller diffraction and a higher signal-to-noise ratio can improve the sensitivity of the system.

Furthermore, the analysis of the surface reaction is done in the electrical domain, simplifying data processing. Using the one-to-one correspondence between the laser bias current and lasing wavelength, at a given initial temperature, we can directly map the normalized detected current versus the injected current. The detected maximum current corresponds to a given bias, and hence, the peak resonant wavelength can be deduced. The measurement can be completed in microseconds, and the conversion to wavelength units is convenient to compare data taken at different initial temperatures. Moreover, electrical circuitry, for signal processing, driving laser and detector, and optical assembly can be made very compact.

III. Experimental Results

Two different experiments were performed in order to characterize the VCSEL readout system apart from the context of a particular assay. The first one was to investigate shifts due to changes in $n_{\rm bio}$ and the second one changes in $t_{\rm bio}$. A 3×5 in^2 GMR plastic sheet was cut and bonded to the bottom of a bottomless 96-wells microplate [4]. Thus, liquid solutions can be easily deposited on top of the GMR sheet.

The first experiment measured the refractive index variation of the specimen deposited on top of the grating. We deposited fluids with various refractive indexes into the wells and the corresponding spectrum was measured. The liquids used were standard matching fluids from Cargille Laboratories with the precision of ± 0.0002. A very low index of 1.280 (Galden fluid), from Solvay Solexis, was also used.

Fig. 2 shows the spectral shift of the resonance for four different index matching liquids. The readout system shows a very high sensitivity to small changes in the refractive index. Analysis of Fig. 2 illustrates that an index change <0.005 can be easily resolved.

The second experiment measured thickness variations of the specimen deposited on top of the grating. The experiment consisted of the deposition of successive layers of polyelectrolyte polymers and the measurement of the GMR spectral response for each layer. Polyelectrolytes are long molecules that can be either positively (polycations) or negatively (polyanions) charged and bind through electrostatic attraction. In principle, the adsorption of molecules carrying equal charges leads to the charge reversal on the surface. This has two important consequences: 1) the repulsion of equally charged molecules and thus self-regulates the adsorption to a single layer, and 2) the ability for an oppositely charged molecule to be adsorbed in the second step, on top of the first one. Cyclic repetition of the adsorption steps leads to the formation of multilayer structures. The linear increase of film thickness is thus a polyion property, independent of the surface.

The polyelectrolytes used in this experiement were cationic polyethyleneimine (PEI), anionic polysodium styrenesulfonate (PSS), and cationic polyallylamine hydrochloride (PAH), from Aldrich. The polyions were dissolved in a buffer solution of 0.9 M of NaCl, at the concentration of 5 mg/ml. The same buffer solution was also used to rinse the surface after the adsorption of each monolayer.

An initial base layer of cationic PEI was deposited on the GMR surface by pipeting 100 μ1 of PEI solution into the well.

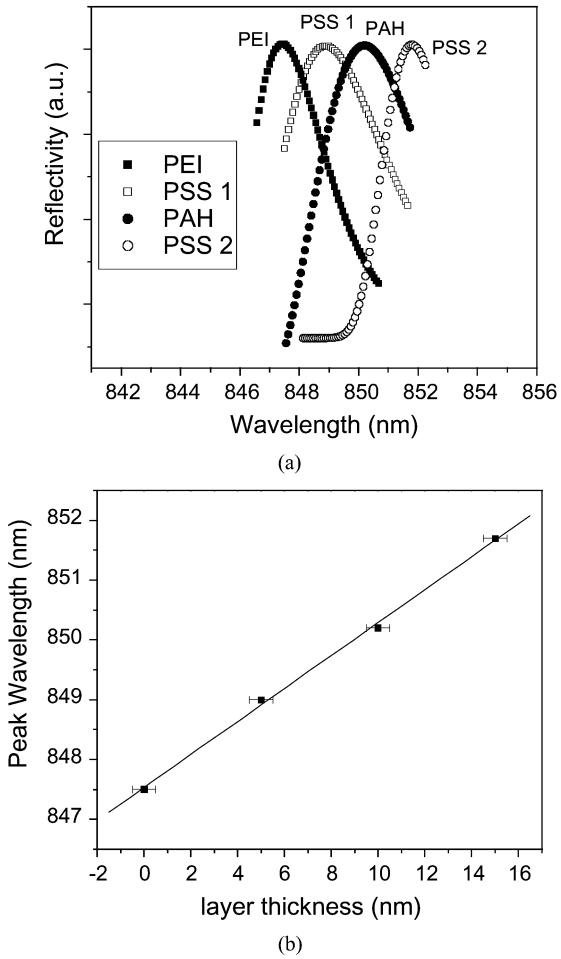

Fig. 3. (a) Spectral shift of the resonance due to the addition of successive layers of polyelectrolytes. (b) Peak wavelength as function of layer thickness on top of the GMR. Error bars are due to molecular nonuniformity [6].

The spectral response was measured by scanning the VCSEL wavelength. Then the well was rinsed with the buffer solution in order to remove the remaining PEI. The same deposition–wash procedure was repeated for cycles of PSS and PAH. Since the growth of the layers is self-limiting, the thickness of each layer is found to be $\sim$50 Å (10% error expected due to molecular nonuniformity) [6], [7].

Fig. 3(a) shows the spectral shift of the resonance detected as layers of polyelectrolyte polymers are deposited, where one-to-one correspondence between the bias current and wavelength is used. Fig. 3(b) shows the resulting linear relationship between the peak reflection position and deposited polymer thickness. Thus, the system has the sensitivity to resolve much less than 10 Å of thickness variation on top of the GMR surface, as can be seem from the linearity of the obtained curve in Fig. 3(b).

It is worthwhile to mention that two different batches of GMRs were used here and, hence, Figs. 2 and 3 should not be directly compared against each other. In addition, our data are direct measurement and, hence, resolution is limited by physical resolution. With significant numerical processing, this can be improved further as method used in [4]. In the GMR used, also described in [4], the epoxy has index of reflection matched to glass (1.5). Silicon Nitride layer has its index strongly dependent on Hydrogen concentration in the film. The deposited material is actually SiN_xH_y with index in the range 1.8–2.2. Even though our current design of the VCSEL-based biosensor may be limited to interactions that cause small wavelength shifts, a larger dynamic range is also being pursued by means of two different approaches: tunable VCSEL [8] and tunable detector [9]. Both approaches can easily enable about 40 nm of peak wavelength scanning.

IV. Conclusion

An ultracompact label-free biosensor that uses a VCSEL-based measurement system is presented. The configuration is very simple, consisting of a VCSEL that is tuned by bias current and temperature, a plastic GMR surface, and two p-i-n detectors. The system has several advantages such as extreme compactness, high sensitivity, high throughput, low power consumption, low cost, and the potential to become portable. Experimentally, the device has shown to be highly sensitive to surface modifications, with the ability to detect the thickness variations <10 Å and <0.005 of change in the index of refraction. Finally, as all technologies being used are naturally 2-D, the system can be extended to an array format and hence further increases its throughput.

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