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Integrated bio-fluorescence sensor

Evan Thrush^{a,*}, Ofer Levi^a, Wonill Ha^a, Ke Wang^a, Stephen J. Smith^b,
James S. Harris Jr.^a

^a*Solid State and Photonics Laboratory, Stanford University, Stanford, CA 94305, USA*

^b*Department of Molecular and Cellular Physiology, Stanford University, Stanford, CA, USA*

Abstract

Due to the recent explosion in optoelectronics for telecommunication applications, novel optoelectronic sensing structures can now be realized. In this work, we explore the integration of optoelectronic components towards miniature and portable fluorescence sensors. The integration of these micro-fabricated sensors with microfluidics and capillary networks may reduce the cost and complexity of current research instruments and open up a world of new applications in portable biological analysis systems. A novel optoelectronic design that capitalizes on current vertical-cavity surface-emitting laser (VCSEL) technology is explored. Specifically, VCSELs, optical emission filters and PIN photodetectors are fabricated as part of a monolithically integrated near-infrared fluorescence detection system. High-performance lasers and photodetectors have been characterized and integrated to form a complete sensor. Experimental results show that sensor sensitivity is limited by laser background. The laser background is caused by spontaneous emission emitted from the side of the VCSEL excitation source. Laser background will limit sensitivity in most integrated sensing designs due to locating excitation sources and photodetectors in such close proximity, and methods are proposed to reduce the laser background in such designs so that practical fluorescent detection limits can be achieved.

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1. Introduction

There has been and continues to be considerable interest in area of integrated sensors for biological applications [1,2]. A variety of molecular sensing schemes have been developed for biochip applications, such as electrochemical, refractive index, UV absorption and fluorescence [3–7]. Fluorescence detection offers several advantages over other methods such as state of the art sensitivity and specificity. The integration of fluorescence detection systems has

received particular attention due to the large expense and size of current bio-fluorescence detection systems. The potential applications of integrated bio-fluorescence sensors are numerous including high throughput experimentation, portable diagnostic devices and in-vivo sensing.

As a result of the recent explosion in optoelectronics for telecommunication applications, a variety of interesting and useful integrated optical sensing architectures can now be realized [8–14]. Unfortunately, optoelectronic technologies for visible wavelength applications are relatively immature compared with their near-IR alternative because the telecommunication industry has focused on the in-

*Corresponding author.

E-mail address: ethrush@stanford.edu (E. Thrush).

frared spectral regime, where optical fiber is less absorbing. As a result, the level of integration and complexity that can be achieved towards sensing systems in the spectral range 0.65–1.0 μm is unparalleled by the current material and fabrication technologies available for visible wavelength applications. Fortunately, fluorescence sensing in the deep red to near-IR spectral regions has been proven to be an advantageous technique. The lower background fluorescence and lower scattering at longer wavelengths offers key advantages over working with visible fluorescence, and researchers have turned to deep red and near-infrared dyes to solve important engineering problems [15–24].

The possible applications of near-infrared fluorescence sensors are numerous. Companies have demonstrated a commitment to developing deep red and near infrared dye technology for research instrumentation. For example, in DNA sequencing, single-color red experiments are found to be preferable over standard four-color methods due to simplicity, reduced cost of instrumentation and low background fluorescence at the longer wavelengths [16]. In medical diagnostics and field-based applications, fluorescent detection sensitivity can be greatly enhanced with long wavelength dyes due to the lower background. For example, it was shown in point-of-care rapid diagnostic assays (Biosite, San Diego, CA, USA) that near-IR dyes (excitation at 670 nm) can be used as fluorescent markers attached to antibodies to increase sensitivity and overcome background fluorescence problems involved with using visible dyes in blood samples [15]. Commercially available near-infrared dyes have been shown to be useful for a variety of analytical techniques, such as DNA sequencing [20].

Integrated fluorescence sensing systems have been realized in the literature [11–14]. Our approach is to integrate vertical-cavity surface-emitting lasers (VCSELs), PIN photodetectors and emission filters monolithically on the same substrate. The benefits of integration include reduced cost and size and increased parallelism. For example, sensor channel spacing or sensor separation can be reduced to less than 100 μm using a monolithic approach. These dimensions cannot be reached in a discrete component system. Also, many sensor units can be fabricated in parallel using conventional semicon-

ductor fabrication techniques and used as building blocks for highly parallel detection systems, such as flow channel based detection systems.

We believe that this work marks the first attempt to integrate these optical components at such a small scale to achieve fluorescent detection. By bringing the excitation source and photodetector in such close proximity, an increase in laser background is observed and limits the sensor sensitivity. The aim of this study is to examine the causes and magnitude of laser background and propose ways to reduce laser background and increase sensitivity. The design concepts learned and invented will be applicable to other sensor designs, spectral ranges, technologies, etc.

2. Experimental

2.1. Optical system design

In regards to the optical system design, current work is being directed to filter through the many possible design alternatives to find the most optimal and practical solutions. Fig. 1 illustrates some general sensor architectures that are possible with vertical oriented optical devices, such as VCSELs, PIN photodiodes and emission filters. The imaging architecture (Fig. 1a) utilizes micro-optics, refractive or diffractive, for focusing the laser beam and

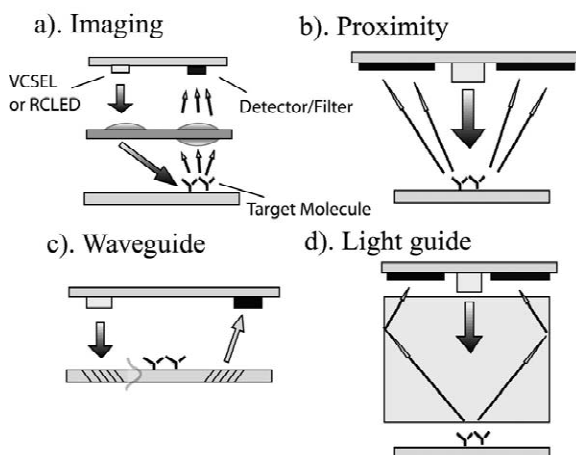


Fig. 1. Schematic of system architectures: (a) imaging, (b) proximity, (c) waveguide and (d) light guide.

collecting the fluorescence. The proximity (Fig. 1b) sensor allows the laser beam to propagate freely to the biological sample, and a large area detector, which surrounds the VCSEL, collects fluorescence. The waveguide architecture (Fig. 1c) utilizes gratings to couple the laser beam into the waveguide, where the evanescent tail of the waveguide mode excites the biological sample. Then, the fluorescence is collected by the waveguide and coupled out onto the detector. Fig. 1d shows a light guide architecture where a glass or plastic light guiding block is located between a sensor and the biochip.

The system architectures discussed above offer several advantages. In contrast to a “starring” or transmission architecture, where the excitation source is emitting directly into the detector, it is advantageous to have the laser and detector located on the same substrate for several reasons. One-sided architectures offer the possibility for significant reduction in scattered background from the laser. By not shining the laser directly into the detector, significant spatial filtration is achieved, which is extremely important for high sensitivity applications [7,8]. Also, the alignment between the photodetector, filter and laser is achieved through micro-fabrication procedures, simplifying device packaging and improving robustness. One could imagine simply bonding these sensors to a capillary and designing the sensor (varying such parameters as size and location of photodetector) such that the scattered laser light does not illuminate the photodetector.

2.2. Optoelectronic design

There are many possible optoelectronic designs that could be utilized in the configurations shown in Fig. 1. However, achieving lasing, photo-detection and filtration by a monolithically integrated device fabricated using one GaAs substrate in a practical and inexpensive way is a design challenge. We designed and fabricated a device that capitalizes on current VCSEL technology. Fig. 2a shows a schematic of the optoelectronic design. The VCSEL on the left includes two mirrors or distributed Bragg reflectors (DBRs) and a laser gain region. Adjacent to the VCSEL, a simple PIN photodetector is realized by adding an intrinsic GaAs region underneath the

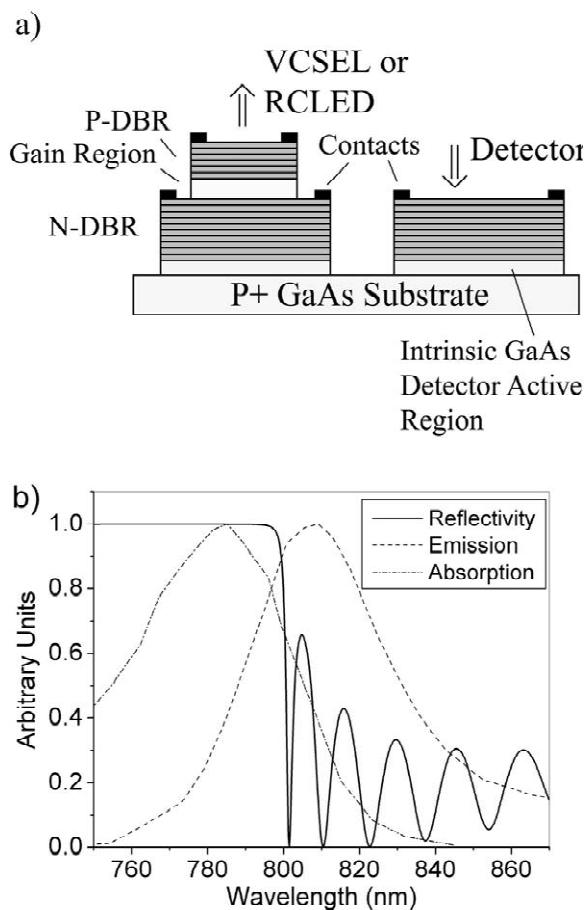


Fig. 2. Optoelectronic device design. (a) VCSEL, PIN photodetector and emission filter are monolithically integrated. (b) Theoretical filtering behavior of the DBR is illustrated, together with Li-Cor dye IR-800 excitation and emission spectra.

standard VCSEL epitaxial structure. The PIN photodetector utilizes the N-doped DBR (N-DBR) as both an emission filter and electrical contact. The filtering behavior of the DBR is illustrated in Fig. 2b. The DBR is highly reflecting at the lasing wavelength of 770 nm ($R > 99.99\%$) and relatively transparent at the Stokes shifted dye emission. The mirror design can be easily modified to give more ideal filter behavior without comprising mirror reflectivity. It is important to note that this design results in a high quality photodetector and emission filter by one simple modification to a typical VCSEL design. This will result in reduced costs and higher yield when compared to other integration schemes. Also, this

technology can be scaled to other wavelength regions and be compatible with fluorescent dyes spanning the spectral range 0.6–1.0 μm .

The above filter design offers another significant advantage other than simplicity of fabrication. The high index AlGaAs material used to make the filter reduces the angular sensitivity of the filter. Simulations (Fig. 3) show that filtration of 10^4 is possible for light rays incident at highly off normal angles ($\theta=60^\circ$). In an integrated system, where scattered laser light may be incident over a wide range of angles, maintaining filtration over a large range of angles is clearly an advantage.

Fig. 4 shows scanning electron microscopy (SEM) images of processed sensors in proximity architecture (Fig. 1b). Fabrication details can be found elsewhere in the literature [25]. The laser is located in the center of the annular shaped photodetector, Fig. 4a. Two interconnect lines can be seen making contact to the VCSEL structure in the center of the photodetector and another can be seen making contact to the photodetector. Fig. 4b illustrates that one- or two-dimensional arrays of these sensors are possible.

2.3. VCSEL characterization

The VCSEL modules of the sensor have been

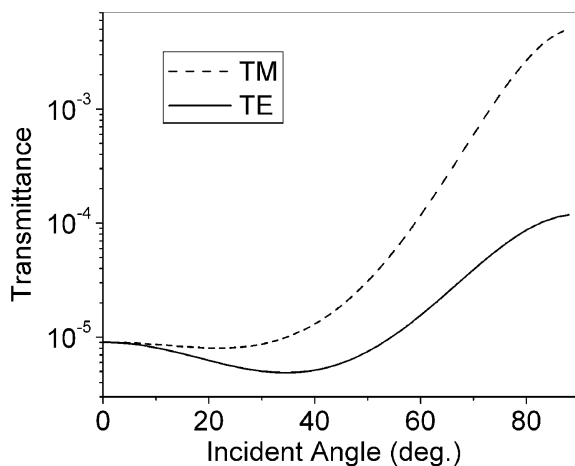


Fig. 3. Angular dependence of emission filter. High optical index of AlGaAs material results in good filtration over a large numerical aperture.

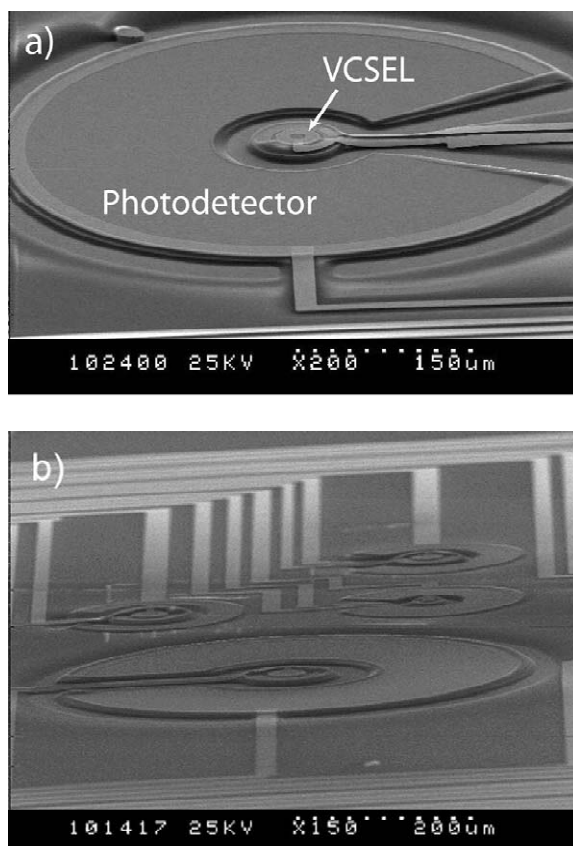


Fig. 4. SEM images of proximity sensor. (a) VCSEL located in center of photodetector. (b) Sensor array with different size sensors.

characterized. Continuous wave (CW) operation at 20 °C for VCSELs with wavelengths of 773 nm is achieved. The threshold current increases with aperture diameter from 0.5 to 2.1 mA for aperture diameters of 4 and 20 μm , respectively. The maximum output power also scales with aperture diameter from 0.6 to 4.0 mW for aperture diameters of 4 and 20 μm , respectively. These power levels should provide sufficient optical power for most quantitative fluorescence experiments. The VCSELs demonstrate multimode operation for aperture diameters greater than 7 μm . For the purpose of fluorescence sensing, single mode operation does not offer significant advantages over multimode operation, since the laser light is absorbed in the dye as long as the laser light propagation is controlled and not highly divergent.

2.4. Photodetector characterization

High-quality heterostructure PIN photodetectors are fabricated and fully characterized [25,26]. The processed photodetectors show a linear response over a wide dynamic range of nine orders of magnitude. High quantum efficiencies of 85% are observed over the spectral range of interest (800–850 nm). Despite the N-DBR on top, the series resistance of the photodetectors is negligible for detector diameters greater than 100 μm . In addition, the photodetector dark current is extremely small (500 fA/mm detector diameter). Low dark current is important for high-sensitivity and high-speed fluorescence sensing applications. We found that the photodetector response is independent of detector bias, which will allow low speed operation at 0 V applied bias [25]. At 0 V applied bias, the photodetector dark current is negligible.

2.5. Laser background results and discussion

The sensor demonstrated a large amount of laser background (i.e., the laser light detected by the photodetector), which drastically limits the sensor sensitivity. If one neglects noise from laser background, previous simulations have shown that detection of less than 5000 molecules in an area of $10^4 \mu\text{m}^2$ should be possible with the proximity architecture [27]. These detection sensitivities are not possible with the current level of laser background in the system.

The large amount of optical background is caused by spontaneous emission emitted from the VCSEL side and subsequent detection by the photodetector [28]. All excitation sources are sources of spontaneous emission [29]. Before threshold is reached, a laser essentially behaves like a light emitting diode (LED), producing spontaneous emission. Once threshold is reached, the additional input power is coupled into the coherent lasing radiation mode and is superimposed on the background LED spontaneous emission. Spontaneous emission can be disastrous for miniature fluorescence systems because this radiation is extremely hard to filter and, as a result, can cause a large amount of laser background. Spontaneous emission is difficult to filter because it is emitted in a broad range of angles (i.e., like a

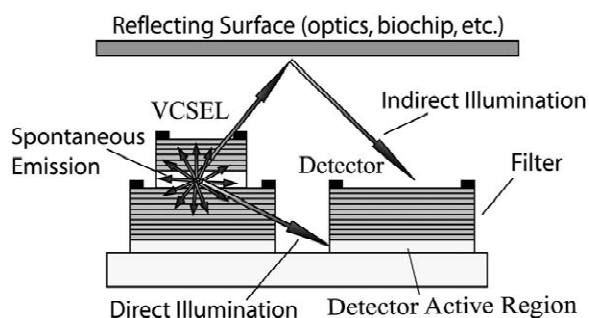


Fig. 5. Spontaneous emission causes laser background through direct illumination and indirect illumination.

point source) and is spectrally broad. In our particular sensor design, spontaneous emission can directly illuminate the detector side (Fig. 5), avoiding the interference filter and causing a large background. Another source of laser background is indirect illumination (Fig. 5), where radiation bounces off an optical interface and impinges onto the detector at a highly off normal angle. The indirect illumination is a problem because the filter's angular performance degrades as one moves off normal. These direct and indirect illumination problems are not unique to our particular design and one must take considerable efforts to avoid these parasitics when designing integrated fluorescence sensing systems.

An optical image of the VCSEL structure during lasing is shown in Fig. 6. The parasitic spontaneous emission rings, at the VCSEL mesa edge, can be seen circling the central coherent laser beam. For the proximity sensing architecture, Fig. 7 shows the measured detector current as function of laser input current. The measurement is conducted with no optical interface above the sensor so that we could specifically analyze the direct illumination cause of laser background (Fig. 5). At an input drive current of about 6 mA (1 mW operation), the detector current is 5 μA . This large laser background will reduce the sensitivity of the sensor below practical levels. It is also interesting to note the kink in the curve of Fig. 7. In theory, above laser threshold current the spontaneous emission does not increase; however, in practice, the spontaneous emission does increase, but at a smaller rate when compared to below threshold operation. A kink is observed at the

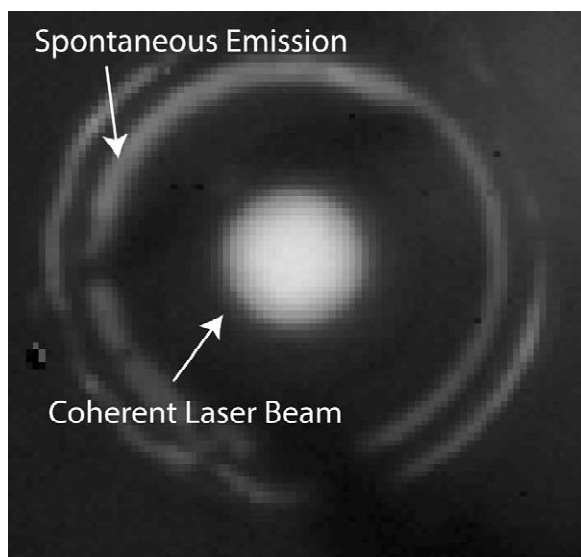


Fig. 6. Optical image of VCSEL lasing. Spontaneous emission rings can be seen due to scattering off the VCSEL mesa.

threshold point of the laser, supporting the theory that the laser background is caused by spontaneous emission emitted from the VCSEL side.

Laser background levels are also measured for imaging architectures with no optical interface located above the sensor. Fig. 8 shows the laser background as a function of the separation distance between the laser and photodetector. The laser background is as low as 1 nA for a separation

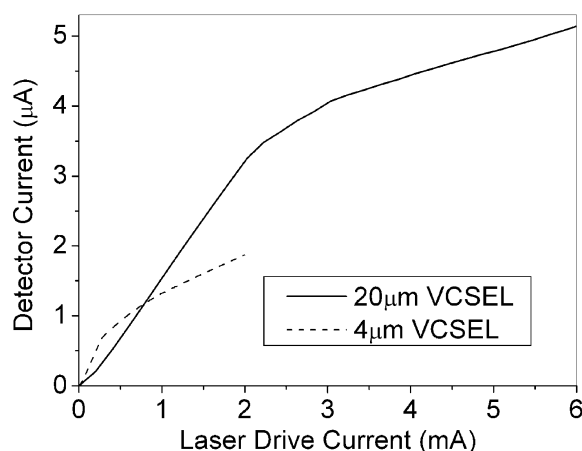


Fig. 7. Laser background in a proximity architecture. The laser background increases as the output power of the laser increases.

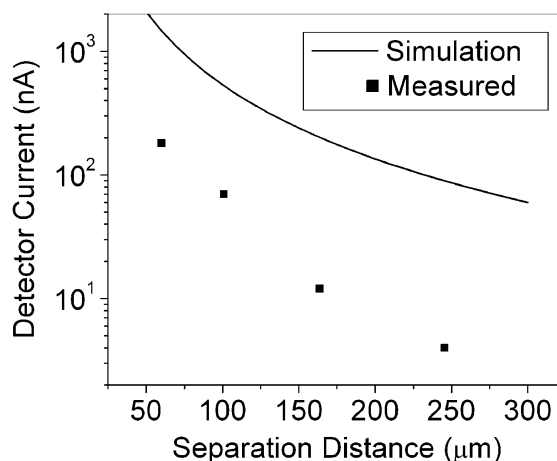


Fig. 8. Laser background in imaging configuration. Spacing is varied between the laser and photodetector.

distance of 250 μm . It is interesting note that this value is much smaller than the proximity sensor laser background. The imaging architectures show much less laser background because of the ability to use a small area photodetector and have larger separation between the excitation source and photodetector. The measured data follows a $1/r^2$ dependence, which is expected for a point source producing spontaneous emission. This supports the theory that spontaneous emission causes the laser background.

Fabrication work has been conducted to realize optical blocking layers that will isolate the detector from spontaneous emission emitted from the side of the light source [25]. Fig. 9 illustrates how optical blocks can be fabricated to reduce the laser background. Preliminary results are promising and we believe that a significant reduction in the laser background is possible with metal optical blocks. In

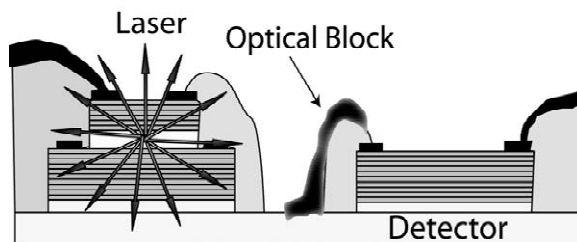


Fig. 9. Metal optical blocks will reduce laser background and increase sensor sensitivity.

addition, the high index AlGaAs material used to fabricate the emission filter will help reduce laser background, as discussed above. Also, optical simulations indicate that the system can be designed such that the laser background contribution from direct specular reflection is negligible, i.e., significant spatial filtration is possible [26].

3. Conclusions

A novel optoelectronic design is presented. The design offers several key advantages. First, this design capitalizes on current VCSEL technology, which has been highly developed for telecommunication applications and continues to be an area of intense focus. Secondly, a high precision and high index emission filter can be achieved, which is important in reducing the angular sensitivity of the filter. These sensors can be arrayed in sensor spacing of less than 100 μm . Also, the sensors can be permanently bonded to capillaries or microfluidics, which may allow the designer to significantly reduce experimental variation and noise caused by laser background.

This technology holds much promise towards an inexpensive, highly sensitive, robust, and massively parallel fluorescence sensing solution. Sensitivity is currently limited by laser background caused by spontaneous emission. We feel that through novel optical isolation methods, such as implementing metal optical blocking layers, laser background can be significantly reduced, allowing for highly sensitive detection.

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