A CMOS optical detection system for point-of-use luminescent oxygen sensing

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**Abstract**

This work describes a stable and sensitive optical oxygen sensor based on a consumer CMOS image sensor array and polarization signal isolation. The consumer CMOS image sensor is inherently color discriminating, while the polarization is a wavelength-independent scheme for filtering excitation light. Combination of these two components generates a compact, multi-color detection system applicable to luminescence-based sensing. The optical system is applied to point-of-use oxygen sensing based on quenching of platinum octaethylporphine (PtOEP) luminophore. Sensitivity of the demonstrated portable oxygen sensor is comparable to that of benchtop spectroscopy-based systems. Taking advantage of the spatial resolution of the CMOS image sensor, an oxygen insensitive reference can be integrated to improve the reliability when powered by a battery. The low-cost and high sensitivity of the demonstrated optical sensing approach make it promising for point-of-use applications.

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1. Introduction

Current research in point-of-care (POC) biotechnology has focused on increased miniaturization and integration for higher throughput, decreased reagent cost, and increased sensitivity. Most of the mainstream biological analyses are based on optical methods (i.e., luminescence, absorption). These optical detection systems typically require sophisticated instrumentation, such as spectrophotometers or photomultiplier tubes, and trained personnel. The need for portable and low-cost optical sensing has led to intense focus on miniaturization of sensor components. Semiconductor and organic LEDs are gaining wide acceptance as portable light sources in such systems\cite{1}, and have been used in immunosensors\cite{2,3} and chemical sensors\cite{4,5}. Signal detection and isolation, however, remain active areas of research.

Filtering of excitation light from signal light is critical in microscale fluorescing systems. A common system design places an excitation LED orthogonal to the interrogation region and detector of the microfluidic system. This reduces system noise due to leakage of unabsorbed excitation light; however, such systems are bulky. The systems using a stacked arrangement, where the light source is in-line with the interrogation region and detector, are much more compact and preferable in portable systems. In such a system, however, the emission signal is drowned out by the leaking excitation light. A variety of techniques have been reported for filtering in these stacked micro fluorescence systems, including interference filters\cite{6}, color filters\cite{7}, and dye-based filters\cite{8}. These approaches however are wavelength-specific, making the system application-specific and far less versatile. In this work, signal isolation is achieved by cross-polarizing the excitation and emission light; an approach we introduced recently\cite{9,10}. Two perpendicularly placed polarizers, with extinction ratio of 28 dB, are able to separate the emission light from the strong excitation light (Fig. 1a). Compared with using filters, this approach is inexpensive, easy to integrate, and is wavelength independent.

In addition to filtering, a sensitive and reliable detector is critical to developing a portable sensor. Conventional photomultiplier tubes have been used for benchtop microfluidic systems\cite{11,12}. Silicon photodiodes\cite{13,14}, or avalanche photodiodes\cite{15} can be used in POC or point-of-use systems. Integration of these external components however presents alignment and assembly challenges. In the past, we\cite{9,10} and others\cite{16,17} have used organic photodiodes (OPDs) as low-cost integrated detection alternatives, being flexible and miniaturizable\cite{18}. However, OPDs suffer from low power conversion efficiency\cite{19} and short lifetime\cite{20,21}.

Recently, CMOS image arrays have attracted interest as detectors for POC/point-of-use systems because of the high-speed readout and low power consumption. El Gamal and Eltoukhy\cite{22} recently reviewed developments in CMOS image sensors and suggested applications to lab-on-a-chip as one of the future directions. CMOS image sensors, commonly used today in digital cameras, are built using the same process as ICs and consist of an array of photodetectors covered by a mosaic of microscale band-pass (Bayer) filters (Fig. 1b). Raw data taken by a CMOS image sensor are grids of...
three primary colors: red, green and blue (RGB). Each pixel contains the intensity information of one color. The intensity of each color is described within a given range from a minimum and a maximum which are dependent on the resolution of the analog to digital converter. This approach of using CMOS detector as detector has been successfully applied for bioassays [23] and fluorescence-based cell detection [24–27].

In this work we describe an optical sensing system based on a low-cost consumer CMOS image sensor and explore the feasibility of using it as a detector in a portable oxygen sensor. Oxygen detection is important in clinical diagnosis, biological research and environmental monitoring. The conventional oxygen sensors based on Clark electrodes consume oxygen and are easily poisoned by various organic compounds [28]. On the other hand, optical oxygen sensors offer quick response time, no oxygen consumption, and high sensitivity [29]. The developed oxygen sensor (illustrated in Fig. 1c) is based on oxygen sensitive metalloporphyrin luminophore platinum octaethylporphine (PtOEP) and oxygen insensitive film Rhodamine B in the portable oxygen sensor.

![Image](https://example.com/image.png)

**Fig. 1.** (a) Illustration of polarization filtering which permits isolation of optical signal (Rhodamine B emission centered at 520 nm) from high background signal due to leaking excitation light (530 nm); (b) Schematic of a photodetector array covered by Byer filters in a CMOS detector; (c) Arrangement of the light source (LED), detector (CMOS), polarizers, oxygen sensitive PtOEP film and oxygen insensitive film Rhodamine B in the portable oxygen sensor.

2. Experimental methods

A CMOS image sensor with a USB interface (OmniVision, OV9810) was used in this work. The sensor has a packaged footprint of 8.195 mm × 7.535 mm, with 6.160 mm × 4.606 mm imaging area. The active array size was 3488 × 2616 pixels (9 megapixel resolution), each approximately 1.75 μm × 1.75 μm. The sensor was placed at the bottom of the device stack as the detector and was controlled by the supplied software. Captured raw images were split to three grayscale images (each representing a separate RGB color) and quantitatively analyzed using ImageJ (ver. 1.43u). Each grayscale image yielded an average pixel intensity value.

In the spectral response test, the white LED was biased at a constant potential of 3.0 V (~16 mA forward current, based on the manufacturer provided data sheet). Illumination light in the 430–680 nm range was selected by the monochromatorin 10 nm increments. Intensities in each of the RGB channels of the CMOS image sensor were recorded. Spectral response of each channel was obtained when the recorded intensities were normalized to the white LED emission spectrum.

The sensor is based on the oxygen-sensitive PtOEP encapsulated in ethyl cellulose (EC). A green LED (Bivar R20GRN-F-0160) with a central emission wavelength of λ_\text{ex} = 520 nm was used as the excitation light source (Fig. 1c). The excitation light was linearly polarized by passing through polarizer 1 (NT45667, Edmund Optics). The red emission of the PtOEP film (λ_\text{em} = 644 nm) passed through polarizer 2. The polarized excitation light not absorbed by the PtOEP film was blocked by polarizer 2. When cross-polarized, the extinction ratio of excitation light to leakage light is 28 dB [30]. The oxygen sensitive film was prepared by completely dissolving 10 mg of PtOEP (PT0534, Frontier Scientific) in 10 mL tetrahydrofuran. The mixture was added to a polymer solution prepared by dissolving 3 g EC (48% ethoxyl) in the mixture of toluene (24 mL) and ethanol (6 mL).

For oxygen measurements, all automatic CMOS image sensor functions, such as exposure time control, gamma correction, and auto white balance (AWB), were set to manual. We used two flow meters to control the ratio of oxygen and nitrogen supplied from tanks. A mixing chamber was used to pre-mix the gases prior to introduction to the gas testing chamber. Our sensor was located in the center of the testing chamber; the chamber oxygen concentration was indicated by a commercial oxygen sensor (Nuvair, Pro O2 remote analyzer). During measurements, oxygen concentration in the chamber was changed by adjusting input gas flow rates. Signals and background were obtained by taking images of the PtOEP film and a bare glass wafer. The values in the red channel were used for oxygen measurement. By subtracting the background from the signal, the emission intensity of PtOEP at different oxygen concentration was obtained. To test stability of the sensor performance under an unstable light source, the forward voltage of the LED was manually varied by ±0.02 V during oxygen measurements. The emission intensities of the RhB reference were standardized and the emission intensities of the PtOEP were calibrated accordingly.

3. Results and discussion

We first tested the spectral response of the CMOS chip RGB channels to examine the color discriminating capability. We used a monochrometer to select specific wavelengths of illumination light and recorded the spectral response in the 430–680 nm range in 10 nm step increments. As illustrated in Fig. 2a, each channel of the CMOS detector exhibits a strong response to the corresponding wavelength range, as expected. The blue pixels exhibit a peak response at 460 ± 30 nm, while the green and red pixels peak at 540 ± 40 nm and 600 ± 50 nm respectively. This test confirms that the CMOS detector we used is inherently discriminating to the RGB colors and demonstrates the spectral response range of each color pixel type. These results are helpful in the development of sensors that take advantage of the CMOS detector’s color discriminating capability.

While Bayer filters permit color discrimination in the CMOS detector, response of each color pixel type does overlap. In a consumer camera this is desirable as the overlap permits capture of colors that fall between the standard RGB bins, such as yellow at 580 nm. In sensor applications, however, when individual color channels in the CMOS detector are used to distinguish between the excitation and emission of analyte, the spectral overlap leads to noise. For example, a sensor based on detection of fluorescein isothiocyanate (FITC) – a fluorescent dye commonly used in bioapplications – will detect emission signal in the green channel (521 nm) and unabsorbed excitation light (488 nm) in the blue channel. The overlap between the two channels (Fig. 2a) will lead to high background noise as a fraction of the excitation light will be picked-up by the green channel.
Fig. 2. (a) Responsivity of the CMOS detector in each of its color channels. Inset images taken by the CMOS detector illustrate response at center wavelengths 460 nm, 540 nm and 620 nm. (b) Response of the CMOS detector to 620 nm light as compared to a current measured by a silicon photodiode. The gamma correction function of the CMOS detector distorts the linear response.

Fig. 3. The PtOEP absorption and emission spectra overlaid with the green LED emission and the red channel response of the CMOS detector.

Optical signal isolation based on cross-polarization is a simple, wavelength-independent approach that ideally complements the CMOS detector. Cross-polarization offers a high extinction ratio, on the order of 28 dB, and is able to separate weak emission light from a strong background excitation in a stacked configuration where the excitation light source and detector are positioned in-line (Fig. 1c). The approach is wavelength independent, as the high-efficiency linear polarizer films exhibit high-transmittance of ~40% at wavelengths above 400 nm. We recently used cross-polarization to demonstrate quantitative measurements down to 10 nM concentration of Rhodemine 6G fluorophore in a microfluidic lab-on-a-chip, a hundred-fold improvement from previously published results [16,17]. Herein, the use of cross-polarization will permit us to sensitively detect small changes in emission of PtOEP due to quenching by oxygen in presence of the leaking excitation light. However, before proceeding with discussion of the oxygen sensor, we first describe further CMOS detector characterization focused on integrated detector functions capable of influencing sensor performance.

While CCDs offer better low-light performance, a CMOS image sensor was selected for this work based on the overall sensor needs. The two image sensor types were recently compared by Gamal and Eltoukhy [22], who suggested that CMOS sensors are better suited for low cost portable sensors. Most important to our application, since most camera control functions of a CCD are integrated off-chip, the CCD-based detection systems tend to increase in size and cost very rapidly. A CMOS array integrates sensing with A/D converter and gain amplifier at the pixel level, making it highly customizable [22]. Although these features make CMOS sensors popular with the consumer market, their impact must be fully understood for application to the light intensity-modulated optical sensing. Thus, in the next set of experiments we manually controlled these functions (by writing the registers through the standard serial camera control bus interface) to investigate capabilities of a consumer CMOS detector.

In photography, gamma correction is used to compensate non-linear decoding process of displays by encoding the signal inversely. The gamma correction is given by:

\[ I_{\text{out}} = I_{\text{in}} \left( \frac{I_{\text{in}}}{I_{\text{max}}} \right)^\gamma \]  

(1)

Fig. 4. (a) Emission intensity of PtOEP as a function of oxygen concentration. (b) Stern–Volmer plot of the oxygen sensor. Inset images illustrate emission of PtOEP at low and high oxygen concentrations.
where $I_{\text{out}}$ is the displayed intensity, $I_{\text{in}}$ is the detected intensity, and the exponent $\gamma$ is the gamma correction index. The $I_{\text{max}}$ is the upper limit which depends on the color depth of the image sensor. The OV9810 is a 24-bit color image sensor so its $I_{\text{max}}$ is 255. The default value of $\gamma$ is 1/2.2 which makes the response of the CMOS detector non-linear to the light intensity [31]. Turning off the gamma correction by setting $\gamma = 1$ leads to the CMOS detector exhibiting perfect linearity satisfying a fundamental requirement as a detector. Fig. 2b illustrates the red channel response to monochromatic 620 nm light generated by biasing LED from 2.6 V to 2.88 V to yield different intensities directly measured by a silicon photodiode. The blue and green channels were tested in the same way and they demonstrated good linearity as well. The effect of gamma correction is clearly evident. As these results indicate, gamma correction must be turned off for the detector to yield a linear response over the entire sensor range. However, gamma could provide additional signal amplification for low-light conditions if sensor range is limited. The auto white balance (AWB) and auto exposure were tested similarly. They were also manually controlled to keep the consistency during the following experiments.

Having examined the CMOS detector features, in the next set of experiments we explored application to luminescent oxygen sensing using PtOEP, which is known to be sensitive to oxygen. The absorption and emission of PtOEP are centered at 535 nm and 620 nm, respectively, and are manually controlled to keep the consistency. The auto white balance (AWB) and auto exposure were tested similarly. They were also manually controlled to keep the consistency during the following experiments.

This oxygen sensor based on CMOS detector exhibited high sensitivity as well as good linearity in the Stern–Volmer analysis. Fig. 4a illustrates the steady state emission intensities of PtOEP sensor subject to different oxygen levels. The red pixel values decrease significantly with the increasing oxygen concentration as the quenching effect becomes stronger. The intensities in green and blue channels are much more stable because these two channels are inert to the red emission of PtOEP which varies with the oxygen concentration. The standard approach to characterizing luminescence quenching based oxygen sensors is using Stern–Volmer analysis in which,

$$\frac{l_0}{I} = 1 + K_{SV}[O_2]$$

where $l_0$ and $I$ are the emission intensities in the absence and presence of oxygen at concentration of [O$_2$], respectively, and $K_{SV}$ is Stern–Volmer constant. Fig. 4b illustrates a typical Stern–Volmer plot of the developed oxygen sensor. The high correlation coefficient (0.9982) indicates a linear relationship between the oxygen concentration and the ratio of emission intensities in the absence ($l_0$) and presence ($I_{100}$) of oxygen. The ratio $l_0/I_{100}$ as sensitivity of the oxygen sensor is estimated to be $\sim$41. This result is comparable to the $\sim$50 values reported by others using an external spectrophotometer [33,34] and is much higher than that of an integrated lab-on-a-chip sensor ($\sim$1.4) reported recently [35]. The insets in Fig. 4b illustrate images taken at low and high oxygen concentrations. As emission of PtOEP is strongly quenched by oxygen, little emission light is observed and the corresponding image was dark (right inset). In low oxygen conditions, however, the emission of PtOEP film increases and the image becomes red.

The oxygen sensor exhibited reversible quenching and good repeatability when exposed to an alternating atmosphere of oxygen and nitrogen. A typical dynamic response of the sensor is illustrated in Fig. 5. The response time of an optical oxygen sensor is considered as the time of 90% total intensity change when switching between oxygen and nitrogen conditions. The response time of the oxygen sensor in this work was $\sim$4 s upon switching from nitrogen to oxygen and the reverse switch time, from oxygen to nitrogen, was $\sim$80 s. The asymmetric response curve with a slow O$_2$ to N$_2$ response compared to N$_2$ to O$_2$ response is typical of the luminescent thin film oxygen sensors [36]. The response is comparable to the reported sensor using polystyrene as luminophore-encapsulating matrix (18 s/60 s) [33]. We believe the response time is limited by the mass-transport in our gas test chamber ($\sim$3 L volume), which can take 22–60 s to fill depending on input gas flow rate (up to 8 l per min). Reducing the test chamber volume will improve sensor response time in the gas cycling test. A number of methods may also be used to improve the film response time, such as using Pt porphyrins bearing side groups designed to increase solubility at higher concentration in a thinner layer [30] or use a material with high diffusivity to oxygen [34].

The oxygen sensor showed excellent reliability after 30 h exposure to a green LED in ambient air (21% oxygen condition). Fig. 6 illustrates intensities of the RGB channels during the test. At 21% O$_2$, emission of PtOEP was not strong but very stable during the

![Fig. 5. Response of the oxygen sensor exposed to an alternating streams of oxygen and nitrogen gases. The arrows indicate the time when the gases were switched (filled arrows indicate nitrogen; hollow arrows indicate oxygen).](image)

![Fig. 6. Stability of the RGB channels in the CMOS detector of the oxygen sensor in ambient air.](image)
Fig. 7. (a) Emission of PtOEP is quenched by oxygen while emission of the reference Rhodamine B remains unchanged. Light emission in the red channel of CMOS detector at (b) low oxygen and (c) high oxygen conditions. Dashed lines indicate approximate boundaries of each film.

1800-min test. The signals fluctuated slightly initially but stabilized during the first ten minutes. The fluctuation may be caused by the LED or the power supply. This result suggests that a ten-minute warm-up time is necessary for the system to reach a stable condition for accurate measurement. After the warm-up, the mean value of the intensities in red channels is 16.5 with a standard deviation of 0.33, resulting in a coefficient of variation of only 0.02. Based on these results, the oxygen sensor is reliable and can be used for continuous oxygen sensing applications.

The oxygen sensor exhibited high sensitivity when the light source was powered by the stable external power supply. However, such a power supply is not always available outside the laboratory and batteries are used in most portable devices. While batteries are much smaller in size and lighter in weight, their output decreases over time with use or even due to storage. The lower driving voltage causes lower light source emission; as a result, the PtOEP emission is a function of the oxygen concentration and the light source intensity. Thus, an oxygen insensitive reference should be integrated to monitor the light source intensity. In the next experiment, RhB was integrated as a reference.

The RhB emission does not change with oxygen concentration while the PtOEP emission is sensitive to oxygen. To enhance RhB stability it was sealed in a glass capillary. Emission of both luminophores was recorded by taking advantage of the CMOS sensor’s spatial capabilities. The intensity profiles of two images taken at high and low oxygen conditions are illustrated in Fig. 7. The PtOEP emission decreases significantly from 190 to 20 a.u. as expected when the oxygen concentration increases. On the other hand, the emission of RhB remains constant. The sensor images clearly illustrate the quenching effect of oxygen on PtOEP. The black tape between the PtOEP and RhB blocks the bleeding light from PtOEP at low oxygen concentrations and thus enhances the stability of the RhB reference signal.

The integrated reference makes the oxygen sensor stable and accurate because any fluctuation of the power supply can be observed from the emission intensity of RhB and then negated. To demonstrate this, the LED bias was intentionally varied during oxygen measurement by ±0.02 V, which corresponds to less than 1% change. The oxygen sensor was first characterized with LED biased at 3.0 V using a Keithley 6487 voltage source. Fig. 8a illustrates the resulting RhB and PtOEP emission intensities that deviate from the calibration curve (dotted lines). After the RhB intensities were used to compensate fluctuations in the PtOEP intensities, the sensor exhibited good linearity in response (Fig. 8b). The inset images in Fig. 8 illustrate the Stern–Volmer plots based on the calibrated PtOEP intensities. The coefficient of determination ($R^2$) of 0.88 demonstrates the good linearity of the sensor. Without the RhB reference, the $R^2$ decreases to 0.54 under less than 1% of power supply output fluctuation.

4. Conclusions

In summary, a portable optical oxygen sensor using a low-cost CMOS image sensor as a detector and a cross-polarization scheme as signal isolation was demonstrated. The sensor exhibited sensitivities comparable to that of macroscale benchtop sensor systems.
The integrated oxygen insensitive reference was monitored simultaneously by the CMOS sensor, permitting compensation when the light source is not stable. We are currently in process of applying the system to multi-analyte sensing by taking advantage of the detector’s inherent color discriminating capabilities. Overall, the described approach of using the wavelength-independent polarizer scheme and color-recognizable CMOS image sensor clearly demonstrate suitability for reliable, on-site sensor applications.

Acknowledgements

This work was supported by the NSF awards ECCS-0725812 and ECCS-0930305.

References


Biographies

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