A Versatile Multichannel Instrument for Measurement of Ratiometric Fluorescence Intensity and Phosphorescence Lifetime

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ABSTRACT Optical biosensing is being actively investigated for minimally-invasive monitoring of key biomarkers both in vitro and in vivo. However, typical benchtop instruments are not portable and are not well suited to high-throughput, real-time analysis. This paper presents a versatile multichannel instrument for measurement of emission intensity and lifetime values arising from luminescent biosensor materials. A detailed design description of the opto-electronic hardware as well as the control software is provided, elaborating a flexible, user-configurable system that may be customized or duplicated for a wide range of applications. This article presents experimental measurements that prove the in vitro and in vivo functionality of the system. Such tools may be adopted for many research and development purposes, including evaluation of new biosensor materials, and may also serve as prototypes for future miniaturized handheld or wearable devices.

INDEX TERMS Implantable sensors, bio-instrumentation, phosphorescent, fluorescent, lifetime measurement, continuous glucose monitoring, diabetes.

I. INTRODUCTION
Sensing key biomarkers over time provides direct insight into disease progression, enabling management of chronic conditions and tracking response to treatments. Enhanced biosensing of metabolic signatures offers promise to improve the prognosis of various types of diseases, including cancer and diabetes [1], [2]. A hallmark of cancer, as an example, is the Warburg effect—a result of the hyperactivity of cancer cells—that substantially alters the local tissue environment surrounding a tumor. Hypoxia, depleted glucose levels, and elevated lactate levels arise due to the increased tumor metabolism relative to the surrounding healthy tissue [3]. Similarly, type I and type II diabetes are marked by increased glucose levels in the blood due to the body’s inability to produce insulin or developed resistance to insulin. In contrast, insulin administration intended to counteract elevated blood glucose can drive glucose levels dangerously low, placing individuals at risk for serious acute effects, even death. In these examples—and in any chronic condition—once key metabolic biomarkers associated with the native condition or its treatment are identified, tools for tracking these rapidly-changing molecules - on a scale of seconds to minutes - are needed. These tools are intended to support studies of distribution and metabolism that may then be linked to management through lifestyle, pharmaceuticals, etc. [4]–[6]. Beyond clinical use, real-time biosensing is needed for benchtop in vitro applications supporting development of effective treatments and efficient manufacturing of cell and tissue products [7], [8].

Optical methods are being actively investigated for their application in biosensing, as they offer a number of advantages over other sensing mechanisms such as those based on electrochemical sensing. While noninvasive optical approaches are limited in specificity, optical methods that employ chemical-optical transducers may be even more...
specific, sensitive, reversible, and also generally exhibit a higher dynamic range than other methods [9]. Most importantly, such optical sensors can still be minimally invasive, as long as the probe used to transduce or amplify the signals [10]–[20] has small dimensions and is subcutaneously implanted into the patient’s body [21]. Optical sensing modalities include, but are not limited to, colorimetry [22], absorbance [23], surface-enhanced Raman scattering (SERS) [24], and luminescence [25]. Each of these options has advantages and disadvantages, with weak signals and the turbidity of tissue being key confounding factors for biomedical applications.

Luminescence methods (fluorescence, phosphorescence) are attractive for achieving higher signal-to-noise ratios [26], but their use in biological systems is typically challenged by the presence of endogenous fluorophores such as melanin, collagen, and elastin that exhibit fluorescence emission that overlaps with that of target biomarkers or the exogenous agents used as chemo-optical transducers (“reporters”) [27]. It is noteworthy, however, that native autofluorescence has a maximum emission lifetime of about 10 ns. Further, these endogenous autofluorophores often exhibit an emission spectrum peaking at shorter spectral wavelengths [28], [29]. Thus, specialized phosphorescent probes exhibiting emission lifetimes of hundreds of nanoseconds to many minutes or even hours can be used to temporally distinguish between signals from endogenous fluorophores and the sensor emission. Alternatively, the specialized phosphorescent probes can be designed with long-wavelength excitation and emission spectra peaking at longer wavelengths, making them readily distinguishable from signals arising from autofluorescence.

These phosphorescence emission optical signals from chemical biosensors can be analyzed for their lifetime, phase shift, or intensity to relate to an analyte concentration value. As an example, palladium and platinum porphyrins are a major class of oxygen-sensitive phosphorescent probes that have been reported in the literature [30], [31]. Combining these oxygen-sensing porphyrins with enzymes that consume a biomarker of interest enables new biosensors that exhibit emission properties sensitive to the concentration of those biomarkers within biological test beds, including live animals and human subjects [32]–[35].

Figure 1 shows a conceptual illustration of an optical biosensor, wherein luminescent materials (e.g. microparticles) are embedded in a biological testbed. These biosensors are excited via a light source; the emitted light is then received by a photodetector and processed to obtain information from the ambient condition. The biosensors are usable at a depth ranging from several millimeters to a few centimeters - depending upon the wavelengths and measurement approaches involved [36]–[38] - allowing for rapid, facile monitoring of analyte levels in vivo.

A large body of literature describes the ongoing efforts to develop various luminescent optical biosensors for relevant biomarkers; these are reviewed elsewhere and are not discussed further herein [39]–[43]. However, as these phosphorescent biosensing materials are continuously developed to effectively transduce chemical information to measurable optical quantities, appropriate instrumentation is needed to “read” these optical signals [44]–[46]. Towards that goal, this paper introduces a versatile instrumentation system that is capable of measuring properties of phosphorescent and fluorescent materials. The presented system is unique in its versatility, throughput, and user customizability, making it suitable for a wide range of applications. This paper reports on the design and testing of this system with model oxygen-responsive biosensing luminescent materials, providing a description of how the optical signals are formed, the design requirements of the instrumentation, and the choices for the current implementation. Such detailed representation helps those in this field of science to replicate and develop similar optoelectronic readout systems.

II. BACKGROUND
A. FLUORESCENCE AND PHOSPHORESCENCE
Luminescence - which includes fluorescence and phosphorescence - is a phenomenon of energy-shifted photon emission that stems from the excitation of atomic particles to specific levels of energy [47]. Luminescence-based biosensors are designed such that the emission properties (intensity emission rate and/or “lifetime”) are sensitive to the concentration of the analyte of interest. The vibrational loss of energy is not instantaneous and on average takes between $10^{-9}$ to $10^{-7}$ seconds [26], [48], [49], which in the simplest form follows a single exponential relationship written as

$$I = I_0 e^{-t/\tau},$$

where $I_0$ represents the maximum emission intensity and the decay constant $\tau$ represents the lifetime. Materials with short decay lifetimes are associated with rapid emission after a fast internal conversion process, a process referred to as “fluorescence”. 

![FIGURE 1. A conceptual illustration of optical biosensing. Biosensing elements (e.g. microparticles localized within biocompatible hydrogel matrix) serving as chemo-optical transducers are embedded in a biological test bed (e.g. living tissue, cells in Petri dish, etc). Incident light excites the biosensors by transmitting directly through the surrounding medium without requiring physical contact. The properties of the emitted light, collected by an emission photodetector, provide indication of the biomarker levels in the vicinity of the biosensors.](image-url)
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FIGURE 2. Graphical depiction of intensity and lifetime measurements from an example (oxygen-sensitive) fluorescent or phosphorescent biosensor. (Top) Excitation and emission spectrum at high and low oxygen concentrations. (Middle) Example of time-domain pattern for light source intensity cycling for excitation. (Bottom) Example of time-domain emission intensity at high and low concentrations of oxygen.

In another scenario, the excited singlet electron in the conductive band undergoes intersystem crossing (ISC) [50] where its spin changes and matches that of its pair in the ground state. The electron with a new spin enters a new triplet energy level, from which it is unfavorable to return to the ground energy level. As such, the emission rate is much slower and hence the intensity decays more slowly—as long as hours [48]—and the process is termed “phosphorescence”. When a phosphor is in the excited triplet state, oxygen molecules present in the environment may physically interact with and accept energy from the phosphor, quenching the excited electron more rapidly and thus attenuating phosphorescence emission intensity and reducing emission lifetime [51]. This phenomenon has been leveraged for biosensing by measuring the modulation of emission intensity and lifetime by oxygen to determine the concentration of oxygen in the vicinity of a phosphorescent biosensor.

B. INTENSITY AND LIFETIME MEASUREMENTS

The quenching effect of an oxygen molecule adjacent to a biosensor presents itself both in terms of the phosphorescent lifetime and intensity modulation. In one oxygen measurement protocol, the optical excitation of a phosphorescent biosensor results in an emission spectrum that includes one or multiple peaks at wavelengths longer than that of the excitation spectrum as depicted in Fig. 2 (top). While one of these emission peaks is insensitive to the oxygen levels surrounding the sensor, other peaks exhibit intensity that varies with the oxygen concentrations. This mechanism warrants a ratiometric measurement protocol where the heights of the oxygen sensitive and oxygen insensitive peaks are measured at different oxygen concentrations. The ratio of the peak heights are then calibrated and assigned to distinct oxygen concentrations in the vicinity of the biosensor. This measurement arrangement is referred to as “ratiometric sensing” in the literature [52], [53].

In another measurement protocol, the key temporal characteristics of the emission signals (e.g. \( \tau \)) are analyzed in either the time or frequency domain [49]. Here, we focus only on time-domain analysis because of the necessary advantage of rejecting (via time gating) the short-lifetime background autofluorescence that otherwise plagues frequency-domain measurements applied to biological tissue. Thus, the phosphorescent biosensor is excited with a square-wave modulated light pattern that turns off at a time instance denoted by “\( t_{\text{Off}} \)” in Fig. 2 (middle). The phosphorescence emission begins to decay after the excitation is removed; at higher concentrations of oxygen, a more rapid decay (shorter lifetime) is observed due to quenching of the phosphorescence as depicted in Fig. 2 (bottom). Such modulation of the emission lifetime values may be calibrated and assigned to distinct oxygen concentrations around the biosensor.

III. SYSTEM DESIGN REQUIREMENTS AND OVERVIEW

The instrumentation development aimed to meet the following requirements for a versatile system that would support monitoring of implantable luminescent biosensors: multiple small (handheld) “reader head” units to interface with and collect data from multiple luminescent samples in parallel; each unit individually configurable to measure intensity and/or lifetime for two independent emission wavelengths; plug-and-play optics to change excitation and emission wavelengths as needed; user view of raw signals and real-time calculation of lifetime and ratiometric intensity; portability, enabling use in field; and electrical power isolation for user and patient safety.

The top level system representation of the design - independent reader heads connected to a computer through an “aggregation unit” that provides power and performs data acquisition - are depicted in Fig. 3 and are described in detail within the following sections. In brief, the User Interface software (LabVIEW, National Instruments) is installed on a computer with a high-speed USB port that is connected to
then collecting and measuring the photons collected through the luminescent biosensors by providing excitation light and Reader heads are the compact, versatile units that interrogate instrumentation are described. Also explained. Finally, the model oxygen-sensitive phosphorescence signals are detected. The fundamental attributes of the software used to control the aggregation unit and analyze the phosphorescence signals are described. The reader heads enclose a printed circuit board (PCB) on which the excitation LED and the photodetector are mounted. The detailed electronic design external view of the casing; (b) Diagram of the reader head bottom, indicating the location of excitation and emission apertures as well as the thermistor port; (c) Cross-sectional internal view of the light path and optical components used in the excitation and emission channels.

FIGURE 4. Illustration of the reader head design. (a) Computer-aided design external view of the casing; (b) Diagram of the reader head bottom, indicating the location of excitation and emission apertures as well as the thermistor port; (c) Cross-sectional internal view of the light path and optical components used in the excitation and emission channels.

the aggregation unit. The aggregation unit encloses a multilayered electronic design (Fig. 7) that, first, produces the driving signals to the reader heads (inside circuitry depicted in Fig. 5) then collects the emission data, with all signals passed through HDMI cables. These HDMI cables transmit and receive the signals to and from the excitation LED, emission photodetectors, temperature sensors, and the actuator for the dual optical filter flipper (Fig. 4). A medical grade transformer is used to provide the aggregation unit, which in turn provides DC voltages to the computer and all reader heads.

IV. MATERIALS AND METHODS

This section covers the details of hardware elements used within the optoelectronic reader heads as well as the aggregation unit that can sense the phosphorescence lifetime and intensity of luminescent biosensors under interrogation. The electronic hardware of the aggregation unit and its function in driving and compiling the electrical signals to and from multiple different reader heads in rapid succession is described. The fundamental attributes of the software used to control the aggregation unit and analyze the phosphorescence signals are also explained. Finally, the model oxygen-sensitive phosphorescent biosensors and the experiments used to evaluate the instrumentation are described.

A. HARDWARE

1) READER HEADS

Reader heads are the compact, versatile units that interrogate the luminescent biosensors by providing excitation light and then collecting and measuring the photons collected through the emission aperture. As Fig. 4 (a) illustrates, the designed reader heads are cylindrical in shape with a 38 mm diameter base. The total height of the cylinder is 31.5 mm, including the height of the additional cylindrical protuberance (Fig. 4, a) that encloses the actuator for the two-position optical filter holder. The excitation and emission apertures are each 1 mm in diameter and are spaced 6 mm (edge-to-edge) (Fig. 4, b). These dimensions are set primarily by the physical requirements for optical, mechanical, and electrical components within the reader.

- **Optics:** The reader head optical components can be broken down to two different sets: an excitation channel for delivering photons from the source excitation LED to the luminescent test article, and an emission channel that collects and detects emitted photons (Fig. 4, c).
  - **Excitation Channel:** The excitation LED in each reader head produces photons that exit the reader heads through the excitation aperture. The excitation channel consists of an excitation LED, typically red or green (LUXEON Rebel Color Line, Red: Lumileds Mfr.#LXML-PM01-0050, Green: Lumileds Mfr.#LXML-PM01-0100, Size: 4.4 x 3.2 mm2), an excitation optical filter (e.g. Semrock, Mfr.# FF01-631/36 for the red LED), and a ball lens (Edmund Optics, Mfr.# 43-644) that focuses the LED excitation rays onto the excitation aperture (Fig. 4, c).
  - **Emission Channel:** As the excitation photons interact with the luminescent material, emission occurs isotropically and some of those photons are directed towards the reader head. Photons that impinge on the emission aperture of the reader heads are collected by the collimating lens (Thorlabs, Mfr.#354330), passed through an emission filter and trigger a silicon photomultiplier (SensL, Mfr.# FF02-809/81, shape: circular). In the studies here, where lifetime analysis was performed using only single emission bands, the filters used in the readers with red and green exciting LEDs, respectively, were from Semrock: (Mfr.# FF02-809/81, shape: circular) and (Mfr.# FF01-711/25, shape: circular). The emission and excitation wavelengths of the aforementioned readers are listed in Table 1.

- **Electronics:** The reader heads enclose a printed circuit board (PCB) on which the excitation LED and the photodetector are mounted. The detailed electronic design is external view of the casing; (b) Diagram of the reader head bottom, indicating the location of excitation and emission apertures as well as the thermistor port; (c) Cross-sectional internal view of the light path and optical components used in the excitation and emission channels.

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circuit design that is used within the readers is depicted in Fig. 5. The PCB receives electrical signal from and sends electrical signal back to aggregation unit - described in later sections of this manuscript- to drive the excitation LED and read the photodetector emission signal, respectively. Due to the large number and varying types of communicating signals, a High-Definition Multimedia Interface (HDMI) cable, with 19 connecting buses, is used to link the reader heads to the aggregation unit. Other cables such as universal serial bus (USB) with fewer connection lines, are simply insufficient to transfer all necessary signals between the reader heads and the aggregation units.

At the core of the PCB is a transimpedance amplifier (Fig. 5), which is built around an OpAmp (Texas Instruments, Mfr.#OPA656NB/250). The photodetector has to be biased in reverse, so the anode is connected to the virtual ground of the OpAmp and the cathode to a constant positive DC voltage $V_{APD}$. This voltage is produced by feeding a 2nd-order lowpass filter (LPF) that is produced by the aggregation unit. The OpAmp is powered by two supply voltages $V_{Supply+}$ and $V_{Supply−}$ (+5 V and −5 V, respectively) that are stabilized after passing through two ferrite beads ($FB_1$ and $FB_2$) and filtering capacitors ($CSupply$). The excitation LED is driven by two signals LED_A and LED_C that are set via the aggregation unit under software control. A diode (1N4148W-7-F) is placed in parallel with the excitation LED to protect it from unexpected reverse voltages. A 1 nF capacitor ($C_{Exc}$) filters high frequency components from the LED driving signal (Fig. 5). To assist the user in determining the quality of signals without requiring observation of raw signals, a dual-color (Red/Green) LED is used to provide visual feedback. The LED is driven with the two signals (RED_LEDA and RED_LEDC) that turn the LED red or green depending on their voltage polarity: the LED turns green if the signal-to-noise ratio (SNR) of the phosphorescence emission long lifetime signal is decay is greater than 10. This provides simple visual feedback to the user that the detector is aligned with the luminescent test articles.

Motors: A bistable rotary solenoid actuator (GEEPLUS, Mfr.#BRS0710-9.5) is driven by two signals $CoilN$ and $CoilP$ (Fig. 5) produced by the aggregation unit. The polarity of these two signals determines the binary location of the actuator, which places one of the two emission filters into the emission path (Fig. 4, c). This mode is used in ratiometric analysis of light intensity at two different emission wavelengths, where data may be collected for either or both emission bands passed by the filters by alternating the flipper position. It should be noted that the cylindrical protuberance hosts the bistable rotary solenoid actuator; in alternative versions of the reader heads where only lifetime measurements are desired, the actuator as well as the protuberance may be omitted from the mechanical design and replaced with a fixed single filter. In that case, the reader head height profile is reduced to 17 mm. An example of such reader head is depicted in Fig. 6 (bottom right).

Temperature sensors: A thermistor (Littelfuse, Mfr.#KS103J2) is used to continuously monitor the temperature on the surface of each reader head. The LabVIEW software uses this signal to adjust the value of $V_{BIAS}$ and $V_{APD}$, which tunes the electrical current that the photodetector introduces subject to a steady amount of incident light.

2) AGGREGATION UNIT/DAQ
The aggregation unit encloses a multilayered electrical design that drives the reader heads and collects the emission intensity information as well as the ambient temperature value. This aggregation unit is controlled by the LabVIEW software within which the emission signals are also captured, analyzed, and stored on a computer. Fig. 6 (top) shows a
front view of the final implemented aggregation unit with 16 connection ports Fig. 6 (bottom right).

- Electronics: Detail of the proprietary circuitry that is housed within the aggregation unit is depicted in Fig. 7. The aggregation unit consists of a 32-channel data acquisition unit (National Instruments, Mfr.#USB-6353) with a total of 128 (32 × 4) software-controlled analog and digital input/output pins [54] operating at a maximum sample rate of 1 MHz. These pins are connected to the internal aggregation unit circuitry via four 17 × 2 vertical male header pins (Harwin Inc, Mfr.#M805003442) and connect to the sub-blocks “Bias Supply”, “LED Current Source”, “Reader Drivers”, and “+5 V Supply Generator”. To comply with medical safety standards (IEC60601), the aggregation unit supplies power to the computer - Fig. 6 (bottom left).

The Bias Supply block (Fig. 7, top right) consists of a switching voltage regulator (Analog Devices, Mfr.#LT3571) that converts the +5 V DC to approximately +30 V to reverse-bias the photodetectors at required levels. The CTRL pin of LT3571 is controlled by the software via the DAQ, enabling adjustment of the internal PMT gain set by \( V_{APD} \). The peripheral passive components are chosen as the data sheet for the switching voltage regulator suggests.

To independently drive the excitation LEDs in the sixteen different reader heads, a “LED Current Source” block is introduced. The block consists of eight dual motor driver carriers (Texas Instruments, Mfr.#DRV8835DSSR) that are held in the “In/In” mode by connecting their eleventh pin to ground. As such, the input pins “A1-PH” and “A2-PH” are directly transferred to the output pins “AO1” and “BO1”, respectively (Fig. 7, mid right). Each of these motor drivers carriers individually sets the anode voltage of two of the sixteen different excitation LEDs. The excitation LED cathodes are then all driven via a programmable current source (Fig. 7 (mid right)). The input analog voltage signal “ILED_PGM” is divided between the resistors \( R_1, R_2, \) and \( R_4 \) in the “LED Current Source Block” to set the voltage of the negative pin of the OpAmp (Linear Technology, Mfr.#LTC6255CS6). This sets the base voltage of \( Q_1 \), such that the current through \( R_5 \) remains constant and equals the voltage across it by its value. The programmable value of the signal “ILED_PGM” translates to a programmable drive current value between 0 to 200 mA. The input signal “nLED_Pulse”, when activated, sets the positive pin voltage of the OpAmp to zero, effectively disabling the excitation LEDs of all sixteen reader heads. Other passive elements such as the capacitors, are added to the circuit to stabilize the transient responses of the sub block.

Finally, the Reader Driver block sets all the signals that activate the reader heads and also collects the data produced by the reader heads and sends it to the computer. This sub block consists of a dual motor driver carrier that is held in the “PH/EN” mode by connecting its eleventh pin to a +5 V DC voltage. In this mode, the two signals “COIL_DIR” and “COIL_ENA” set the polarity of the two signals “COILP” and “COILN” which drive the filter flipper actuators. The three other input signals “RED”, “GRN”, “LED_ENA” are fed to a digital configuration that sets the values of the signals “RED_LED” and “GRN_LED” to determine the color of the dual-color LED within the readers as described above (Fig. 7, bottom left). The “Reader Driver” sub block receives the three input signals “LED_C”, “LED_A”, and “V_BIAS” from the “LED Current Source” and “Bias Supply” blocks, respectively. The reader head outputs are sent back to the Reader Drivers sub block via the HDMI cable, which are subsequently transferred through the DAQ to the computer software.

3) POWER CONSUMPTION
The aggregation unit and the reader heads consume electrical power when under operation. To measure the amount of power that the system consumes, a power meter (P3 International Mfr.#P4400 [55]) was plugged into the city power line. When no reader heads are turned on, the aggregation unit consumes 8.8 W. The majority of this power is consumed by the DAQ unit and its peripheral component interconnect express (PCIe) lines. When connected and on, the reader heads draw around 110 mA, from a 5 V power supply mainly to turn on their excitation LED. As such, each reader head when turned on, consumes an additional 550 mW.

B. SOFTWARE
A comprehensive instrument control program was developed using LabVIEW to produce all necessary signals that drive
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Figure 7. Aggregation unit internal circuitry. (Top left) Top-level diagram of the connections. (Top right) Circuit to produce adjustable DC voltage for reverse bias of the photodetectors. (Mid right) Circuit to provide source current to excitation LEDs in all sixteen reader heads. (Bottom left) Single driver circuit used for each reader head. (Bottom right) Circuit to supply voltage for the entire aggregation unit and all reader heads.

The software also performs real-time signal processing and data analysis to extract information crucial for user to make decisions during operation, such as measures of the relative intensity of short and long lifetime components detected by the photodetector [expressed in Volts] as well as the phosphorescence emission decay lifetime [S]. The following sections elaborate the software configuration and the algorithms used to produce the driving signals and analyze the measured emission decay.

An experiment setup window (Fig. 8, top left) allows an operator to enter details for recording in the log with the experimental data. These details include a name for the test article/subject, the experiment name, and the output file name and path. The software uses hardware signals that sense the channels of the aggregation unit to which a reader head is connected, and automatically gives the operator the option to choose to activate each channel by clicking on the corresponding circle icon. This opens a configuration panel for the respective reader head, described below. Once configured, the circle turns green to indicate successful activation. After the properties of the experiment are completed and readers are configured, the operator clicks on the “Start Experiment” button to initiate data collection. It should be noted that the experiment may be paused at any time to change reader configuration, add or remove readers, add notes, etc.

The reader configuration window (Figure 8, top right) allows the operator to set the reader head properties including the normalized LED power, excitation LED on-time, and the duration over which to collect the decay signal after the LED is turned off. To avoid erroneous estimation of lifetime, it is important that the operator set the decay duration such that a sufficient fraction of the phosphorescence emission be captured by the software. The operator may also change settings to adjust for the quality of the observed signal as well as desired frequency of data collection (number of acquisitions, frames/measurement, measurement intervals). The number of acquisitions ($N$) sets the number of consecutive decays.
FIGURE 8. Software user interface (LabVIEW front panel). (a) Welcome window, with entry fields for experimental description and data file storage location, and also indicating the status of each channel, including active and connected reader heads. (b) Individual reader head configuration window. (c) The localization mode and real-time results window. (d) Experimental results window, displaying key parameters and measurement results for all sixteen different channels; the current view shows only the results for measurements on Channel 2 (as an example), which were captured over the course of half an hour, one data point per minute.

that are summed before calculations of relative intensity and curve-fitting for lifetime estimation are performed. Similarly, the frames/measurement sets the number of times the values from each $N$ acquisitions are calculated and then averaged to produce a single value. The operator may also choose to configure the details of emission filters (assumed to be bandpass) by entering the appropriate center wavelength and full-width at half-maximum bandwidth. To add a second filter for the readers that have filter changers, the “Feasibility Probe” button as indicated in Fig. 8, top right) may be selected and a second column for all of the above settings may be added. It should be noted that collecting data at two different wavelengths involves mechanically changing the filter position and then changing the acquisition settings to be specific to that filter. Thus, the operator may adjust the settings to account for different signal strengths observed when the two filters are in place.

The operator may use the “Start Localization Mode” to determine the quality of the signal captured by the reader by observing the measured signals in real time. In this mode (Fig. 8, bottom left), the operator may, for example, confirm that the characteristic waveform is present with sufficient intensity, the detector is not saturated, and the lifetime is calculated with sufficient signal-to-noise ratio for the current channel configuration settings. For phosphorescence measurements, the waveform should consist of a rising exponential function that corresponds to the excitation of the biosensor and an exponential decay that corresponds to the phosphorescence emission. The lifetime value, which is an indicator for the existence and state of the target biomarkers, is calculated by analyzing the phosphorescence intensity decay in real time as described below. This mode is useful in “locating” an implant embedded in tissue and placing the reader in a location to make continuous measurements over the course of the planned experiment. Thus, the operator should ensure that the signals and calculated lifetimes meet expectations for the experiment once the reader head is mounted in place. Once the location of the reader head is fixed, the operator clicks on the “Complete Configuration” button to return to the main window to either configure additional reader heads or start the experiment.

Once an experiment is started, the in-progress experimental overview window depicted in Fig. 8 (bottom right) opens and the data acquisition begins. At this point, the software starts storing the data to the files indicated in the experimental setup as described above. In this last window, the operator may monitor, among other things, the lifetime, long lifetime, and the short lifetime corresponding to different reader heads that are connected to different channels of the aggregation unit. The view may be arranged by the user to display any of
the acquired parameters in any of the four panels. Further, each of the panels may be independently set up to show any subset of the configured channels. All displays may also be changed by the user to auto-scale or to only show fixed range of Y-axis values for a fixed time interval. The user may enter text notes to be logged in the spreadsheet with the data using the pane in the bottom left. Finally, the user may pause the experiment to check readers and change configuration, as necessary, and resume afterwards or may terminate the experiment using the buttons in the bottom right corner of the window.

The software executes a measurement loop wherein it first sets the actuator to position the desired filter in the emission path, measures the ambient temperature of the readers, and sets the voltage of the cathode of the photodetector (PMT). The software then measures and stores the PMT output over the full configured period (LED on-time + decay duration), analyzes it and extracts the lifetime, long lifetime intensity, and the short lifetime intensity via the algorithm depicted in Fig. 9 (right). Briefly, the entire phosphorescence illumination intensity, including the excitation exponential rise as well as the emission decay, is saved in a vector. The background signal, which represents the DC shift of the phosphorescence illumination, is first calculated and deducted from all values in the vector. The illumination is then split in two vectors, one representing the excitation rising signal and the other representing the phosphorescence emission decay. Using the intrinsic LabVIEW algorithm for Levenburg-Marquardt non-linear least-squares fitting [56], an exponential decay signal (1) is fit to the phosphorescence emission decay with the amplitude (long lifetime intensity) and decay constant (lifetime) used as the fitting parameters. In the event that the exponential fitting results in a residual error (RMSE) that exceeds a predefined threshold, the algorithm presents the number zero for the values of lifetime and the long lifetime intensity. The default setting for this noise threshold corresponds to a long lifetime intensity SNR of <10; this value is user-changeable to any arbitrary level, providing opportunity for adjustments as needed while ensuring users are making intentional decision to operate under conditions where measurements will have low SNR.

The “short lifetime intensity” is the relative contribution of short-lived background emission, such as those that arise from autofluorescence or other signal contaminants (assuming the system is intended for long-lifetime phosphorescence measurements). It is present in the signal when the LED is ON. The time-dependent signal during this period immediately following initial application of the LED driving current may be formulated as a rising exponential,

$$I = I_0(1 - e^{-t/\tau})$$

(2)

which is an exponential function that starts from zero and asymptotically approaches the value of $I_0$. To calculate the
short lifetime intensity, \( V \) is deducted from the excitation rising signal, without background correction. The remaining function resembles a DC voltage and is used as a measure of the short lifetime intensity \([V]\) \([49]\).

### C. MATERIALS FOR TESTING

Reference, oxygen, and glucose-sensitive phosphorescent hydrogels were prepared following established protocols to evaluate the performance of the instrument during \textit{in vitro} and \textit{in vivo} experiments. Briefly, for the \textit{in vitro} experiments, four oxygen-insensitive phosphorescent test articles were synthesized. These articles are referred to as Reference materials, and are indicated by “\textit{TA:Ref}1,” “\textit{TA:Ref}2,” “\textit{TA:Ref}3,” and “\textit{TA:Ref}4.” These materials have been reported to have stable lifetimes with negligible dependence on the local oxygen level \([42], [57]\). The first two of these articles contained phosphors that may be excited by green light, while the second two are excited by red light. In addition, three types of oxygen-sensitive test articles were prepared. The first “\textit{TA:O}_{2}1,” used a green-excited phosphor and was synthesized only for \textit{in vitro} testing \([58]\). For the \textit{in vitro} experiments, two different types of red-excited oxygen-sensitive test articles - “\textit{TA:O}_{2}2” \([32], [59]\) and “\textit{TA:O}_{2}3” \([59]\) - were synthesized and embedded to measure the oxygen concentrations in biological environments; these comprised of red-excited phosphors in two different types of hydrogels. Finally, glucose-sensitive red-excited test articles (“\textit{TA:BG}1”) were also produced for the \textit{in vitro} studies \([59]\). The phosphorescence lifetime of the glucose-sensitive test articles is a function of the glucose concentration in the proximity of the phosphor \textit{in vivo}. The glucose oxidase enzyme embedded in the glucose-sensitive particles yields a higher consumption of oxygen molecules for a higher concentration of glucose, which in turn yields a reduced level of oxygen quenching of the phosphor. For the interested reader, details of all the above materials preparation are provided as supplementary information. The Institutional Animal Care and Use Committee protocols established by Texas A&M University were followed during the course of \textit{in vivo} experiments (Texas A&M IACUC, AUP #2012-213, approved 12/7/2012).

### D. EXPERIMENTS

1) \textit{IN VITRO EXPERIMENTS}

- Basic performance characterization: To evaluate the basic performance and accuracy of the system in determining known lifetime values, four different oxygen-insensitive test articles with fixed but different emission lifetimes (\textit{TA:Ref}1 - \textit{TA:Ref}4) were used. Measurements of each article were performed with the prototype system, that is described herein, as well as with a commercial optical lifetime measurement tool (Ocean Insight, NeoFox) \([45]\). While a total of one hundred measurements were made by the prototype system to calculate the confidence interval (CI), a phosphorescent decay waveform was captured via the NeoFox system; an exponential decay was fit on the waveform. The fit exponential accuracy, compared to the decay waveform, was evaluated by calculating the coefficient of determination \(R^2\).

- Oxygen Sensing: To illustrate the ability to track lifetimes that change in response to oxygen levels using the prototype system, the oxygen-sensitive test articles (\textit{TA:O}_{2}1) were placed into a custom testing system previously reported elsewhere \([33]\). Briefly, a Delrin flow cell with a channel of (dimensions: 0.76 cm \(\times\) 18 cm) cross section was connected by peristaltic pump to two Erlenmeyer flasks containing the buffer solution and providing a constant recirculating flow of phosphate-buffered saline (PBS, pH 7.4). The flow cell comprised four ports to enable testing of four sensing materials in a single experiment with one individual reader head interfaced to each sample. Using mass flow controllers (MKS Instruments, Inc, 1179A), custom ratios of nitrogen and air were bubbled into the reservoir to adjust the dissolved oxygen concentrations in a step-wise fashion to achieve 20%, 10%, 5%, 2% and 0% dissolved oxygen in the PBS (confirmed with an oxygen electrode, Unisense Part No. OX500). For this experiment, highly sensitive green-excited phosphors and reader heads with green excitation LEDs (Table 1) were used to interrogate the test articles, which included

| Test Article | NeoFox Measurement | Prototype Multi-channel System |
|--------------|-------------------|--------------------------------|
|              | Est. Lifetime Value [\(\mu s\)] | \(R^2\) | Est. Lifetime Min. Lifetime Max. Lifetime 95% Confidence Interval [\(\mu s\)] |
| Ref\(_1\)    | 45.55 | 0.93 | 46.65 | 46.09 | 47.52 | (46.58, 46.71) |
| Ref\(_2\)    | 221.13 | 0.91 | 227.3 | 220 | 236 | (226.7, 227.8) |
| Ref\(_3\)    | 48.24 | 0.98 | 42.34 | 42.08 | 42.72 | (42.32, 42.36) |
| Ref\(_4\)    | 249.64 | 0.99 | 257.5 | 256.4 | 258.5 | (257.4, 257.6) |
oxygen-sensitive materials. The samples were periodically illuminated for 500 µs, followed by a period of no excitation for collection of emission decay. The raw light intensity values were captured and saved in a comma separated values (CSV) format. The same experiment was performed using ten distinct reader heads and aggregation unit channels and the CSV files were saved. Using MATLAB, the saved raw light intensity profiles were analyzed to capture the lifetime values of the test articles used as described below.

2) IN VIVO EXPERIMENTS
To further evaluate the performance of the introduced system to collect lifetime measurements to track oxygen in vivo, oxygen- and glucose-sensitive test articles (TA:(O₂)₂, TA:(O₂)₃, TA:(BG)₁₁) were inserted in rat subjects. For the in vivo studies, phosphors excited by red light were chosen to enhance efficiency of light transmission through tissue. Figure 10 illustrates the location on the back of the rat subject where the different phosphors are inserted. Briefly, the insertion and monitoring experimental procedures were as follows. Animals were anesthetized using 2% isoflurane carried by (100%) oxygen. For TA:(O₂)₂ and TA:(BG)₁₁, hydrogel slabs were prepared and then diced into 0.5 × 5 mm strips, which were then loaded into hypodermic needles for subcutaneous insertion. For TA:(O₂)₃, the hydrogel precursors were mixed to initiate the slow gelation reaction then injected subcutaneously in liquid form to enable in situ gelation. Each animal received 12 injections in total, in a 4 × 3 grid pattern as illustrated in Fig. 10. Once the test articles were implanted, up to 4 reader heads (4) were then placed over implant sites for simultaneous monitoring; the software was used in localization mode to ensure adequate signal strength and proper configuration and then the reader heads were fixed in place.

The Number of Acquisitions was set to 512 in the LabVIEW code to ensure sufficient SNR. Data was then collected for at least five minutes to establish a baseline lifetime, after which the inspired oxygen level was decreased to 12% for a maximum of about 10 minutes by mixing with nitrogen. After 10 minutes, or when all signals stabilized (whichever came first), the oxygen level was returned to 100%. Data acquisition was continued until signals reached steady-state levels. All animal procedures followed protocols approved by the local IACUC.

V. RESULTS AND DISCUSSION
A. IN VITRO RESULTS

- Basic performance characterization: Test articles were first tested via the reference measurement system (NeoFox) to produce and save emission decays. An exponential decay was then fitted on the recorded signal using the Non-linear Least Squares fitting method (NLLS) method. The fitted exponential was used to estimate the lifetime associated with emission from each. One hundred consecutive measurements of the lifetime values were captured. For each of the four test articles (Ref₁₋₄), the estimated average, minimum, and maximum lifetime values were calculated, along with the corresponding confidence intervals, as reported in Table 2. The estimated lifetime values for the four different test articles (Ref₁₋₄) that are obtained via the commercial product (NeoFox) match closely with those obtained by the prototype system presented in this paper. Estimated lifetime values that are obtained from the commercial product (NeoFox) and the prototype system are within 5% of one another in all but one case (Ref₅).

These results support the validity of the measurements acquired by the new multi-channel system. In contrast with the NeoFox system, the presented system is versatile, has a much higher throughput and can work at multiple different wavelengths. Unlike the proposed system that can interrogate sixteen different phosphorescent samples at the same time, the NeoFox system can only measure phosphorescent signals from a single channel. However, the multichannel capability of the prototype system does have some engineering tradeoffs. For one, it is bulkier and heavier than the NeoFox system, so it is not as portable. Further, the total power consumption of the prototype unit (8.8 W) is nearly four times greater than the NeoFox system at standby (2.5 W). The system produces lifetime values at a lower rate than the NeoFox (0.2 Hz vs. 10 Hz), which could limit the types of physiological biochemical targets that could be measured, although it is notable that concentration levels tend to not change in time spans less than a second. Finally, the prototype unit has a lower lifetime measurement limit of 40 µs compared to 2 µs for the NeoFox system; thus, the current system is not amenable for use with short-lifetime phosphors such as ruthenium complexes.

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measurement comes at the cost of a more complex circuitry design, due to inclusion of lock-in amplifiers and phase-locked loops.

- **In Vitro Oxygen Sensing Assessment:**
  The logged CSV files associated to the TA: \((O_2)_1\) oxygen-sensitive test articles were analyzed with post-processing. Consecutive cycles of excitation and emission illumination decay values were averaged (Number of Acquisitions, \(N\)); as expected, larger \(N\) increased congruence among estimated lifetimes between the ten different channels. In addition to the lifetime, the standard deviation of the lifetime values from the ten different channels was calculated and the SNR was then defined to be the mean estimated lifetime value of the ten different channels divided by their standard deviation. Figure 11 provides a graphical view of the calculated lifetime and SNR values for the TA: \((O_2)_1\) particles. It is clear that the expected response to oxygen was observed: the lifetime values to decline as the oxygen concentration increases (Fig. 11, top). In addition, because of the increased quenching, the measured intensity drops and the SNR is correspondingly reduced at higher oxygen levels. It is also evident from Figure 11 (bottom) that the system exhibits increased SNR for higher \(N\). A key point is that the user-configurable setting for \(N\) allows the adjustment of data acquisition parameters to match the experiment and ensure that the SNR meets minimum requirements over the full dynamic range of response for a given test article.

**B. IN VIVO RESULTS**

Figure 12 presents the measurements of emission lifetime collected from implanted luminescent biosensors during the *in vivo* experiment in which inspired oxygen levels were varied. As Fig. 10 suggests, twelve different phosphorescent samples were implanted in twelve different locations of the rat’s body. The differences in responses from sensors are attributed to several factors, including differences in materials (oxygen only vs. glucose - and oxygen - responsive), variability in tissue, the *in vivo* sensor orientation, and the body’s acute response to the implant. As depicted in Fig. 10, sites 1-1, 2-2, 2-3, 3-1, 3-2, 4-1, 4-2, and 4-3 indicate locations where sensors sensitive only to oxygen are implanted. The other sites (1-2, 1-3, 2-1, and 3-3), are locations where glucose-sensitive implants were placed. It is important to note that the glucose sensors are also sensitive to oxygen, as they contain oxygen-sensing porphyrins combined with glucose oxidase - an enzyme that consumes oxygen in the presence of glucose. It is also noteworthy that oxygen and glucose perfusion can vary significantly at different anatomical locations. Further, histology reports revealed that the phosphorescent sensors were implanted at different depths of the tissue and also took on slightly different shapes. Finally, it is noteworthy to mention that there is a dynamic injury and foreign body response activated upon implantation of the phosphorescent sensors, such that the local tissue environment is not in steady state on Day 0. Hence, while all of the sensors clearly exhibited a change in phosphorescence as inspired oxygen was
modulated (Figure 12), it was also completely expected that sensors located at different sites would yield responses that vary in magnitude and rate. In particular, the noise present on the data from Day 0 and sites 1-3 and 3-3 is attributed to the non-steady-state tissue environments at the time of test. Fig. 12 (top) contains plots of lifetime values for such an experiment as performed on the same day as test article insertion; then Fig. 12 (middle) and Fig. 12 (bottom) show the lifetime values measured during identical experiments that were performed 14 and 21 days post implantation, respectively. Phosphorescent decay signals from different sites (as suggested by Fig. 10) were captured. The measurement profiles clearly show that the lifetime values increase as the ambient oxygen levels decrease and also decrease as more oxygen is reintroduced. It was expected that there would be differences in responses from implants in different anatomical locations and that a brief lag period would be observed after changes as the inspired oxygen must be transferred from the lungs to blood and eventually to the tissue via the circulatory system. It is clear from these proof-of-concept studies that the instrument was able to locate the test articles after insertion, make high-quality measurements, and track the changes in test article phosphorescence in response to physiological conditions. It also should be noted that the glucose sensors are enzymatic and are in essence sensitive to the concentration values of oxygen in the proximity of the sensor. As such TA:($BG$)$_1$ articles also show sensitivity to the inspired oxygen levels in the rat’s body.

VI. Conclusion

This article introduces a versatile multichannel instrument for the measurement of luminescence light emitted from diverse materials, enabling parallel monitoring of many samples both in vitro and in vivo. The internal opto-electrical design of the reader heads that excite optical biosensors and collect their emission light was designed to using off-the-shelf electronic and optical components assembled with simple, robust mechanical parts that be machined or printed at low cost. The flexibility to configure the reader head hardware (e.g. different LEDs and filters) and real-time software control enables analysis over various spectral bands using both intensity and phosphorescence lifetime modes. Further, the details of the hardware design and the operating software of a multichannel aggregation unit that runs sixteen parallel reader heads was elaborated to enable simultaneous real-time monitoring of many luminescent materials at once. Compared to existing commercial products, the proposed system is versatile, customizable, and has a significant higher throughput. The system was tested using articles with well-known properties and compared to commercial reference instrument to prove the accuracy of the full system for lifetime measurements. Finally, the practical value of the system is demonstrated by performing multiple parallel oxygen measurements in both in vitro and in vivo testbeds, revealing potential for use in evaluating new materials, studying physiology of animal (or human) subjects, as well as monitoring other biological systems wherein tracking of dynamic changes of several different parameters is desired.

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