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Temperature Compensation of Oxygen Sensing Films Utilizing a Dynamic Dual Lifetime Calculation Technique

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Abstract

With advances to chemical sensing, methods for compensation of errors introduced by interfering analytes are needed. In this work, a dual lifetime calculation technique was developed to enable simultaneous monitoring of two luminescence decays. Utilizing a windowed time-domain luminescence approach, the response of two luminophores is separated temporally. The ability of the dual dynamic rapid lifetime determination (DDRDL) approach to determine the response of two luminophores simultaneously was investigated through mathematical modeling and experimental testing. Modeling results indicated that lifetime predictions will be most accurate when the ratio of the lifetimes from each luminophore is at least three and the ratio of intensities is near unity. *In vitro* experiments were performed using a porphyrin that is sensitive to both oxygen and temperature, combined with a temperature-sensitive inorganic phosphor used for compensation of the porphyrin response. In static experiments, the dual measurements were found to be highly accurate when compared to single-luminophore measurements—statistically equivalent for the long lifetime emission and an average difference of 2% for the short lifetimes. Real-time testing with dynamic windowing was successful in demonstrating dual lifetime measurements and temperature compensation of the oxygen sensitive dye. When comparing the actual oxygen and temperature values with predictions made using a dual calibration approach, an overall difference of less than 1% was obtained. Thus, this method enables rapid, accurate extraction of multiple lifetimes without requiring computationally intense curve fitting, providing a significant advancement toward multi-analyte sensing and imaging techniques.

Keywords

rapid lifetime determination; luminescence; dual sensors; error compensation

I. Introduction

LUMINESCENCE-BASED SENSING has become an area of interest in a variety of industries due to the high sensitivity and measurement flexibility offered over other measurement methods including electrochemical [1, 2]. Oftentimes, multiplexed sensors or

multi-sensors capable of measuring several analytes are needed for diagnostic and/or compensation purposes [3]. For example, temperature compensation is often needed of luminescent oxygen sensors [4-15]. A key issue of multiplexing, however, is the separation of the individual sensor responses [3]. In the past, sensors capable of monitoring multiple analytes or multi-sensors were measured using intensity measurements; however, spectral cross-talk or overlap must be avoided [3]. Finding luminophores with adequate sensitivity, selectivity, and stability while still maintaining this spectral distinction may be difficult [16]. Even if appropriate luminophores are found, additional optical hardware will be required in order to separate the emission signals and/or excite the respective luminophores. In addition, intensity measurements are susceptible to errors resulting from photobleaching of the luminophore, optoelectronic drift, or variations in luminophore concentrations from sensor-to-sensor [17, 18]. Luminescence lifetime measurements are able to overcome these problems by determining the rate of luminescence decay using temporally-resolved measurements in the time- or frequency-domain (TD and FD, respectively). Advances in technology in recent years have allowed these types of measurements to become more common [18].

Several methods have already been reported which temporally-resolve the response of multiple sensors with distinct lifetimes. Although multi-sensor lifetime measurements can be made by optically filtering the signal from spectrally-distinct luminophores, this again requires spectrally-distinct luminophores [13]. As an alternative, many TD and FD methods have been developed which utilize luminophores with distinct decay times [1, 9, 13, 14]. These and other approaches that utilize temporal-based measurements have been recently reviewed elsewhere; only lifetime calculation techniques applicable to multiple lifetime determinations will be discussed herein [19].

Multi-lifetime calculations in the frequency-domain (FD) require phase or modulation measurements at multiple frequencies. Non-linear least-squares analysis (NLLS) is then used to estimate the frequency dependent response data and determine the lifetimes [20]. Fittings, however, are computationally intense and dependent on the initial guesses which can be an issue for highly-sensitive luminophores where the lifetime and intensity are expected to have a wide dynamic range [21]. The increased computational intensity can affect the speed of calculation and thus the real-time measurement capabilities. In addition, FD calculations can suffer from errors *in vivo* due to scattered excitation light and tissue autofluorescence. TD measurements, however, can easily remove these short-lived signals (typically <10 ns) by delaying the lifetime calculation of the longer-lived luminophores until after these events have decayed to zero.

In the time-domain, the decay data obtained are typically fit to a multi-exponential curve:

$$I(t) = I_1(t) + I_2(t) = k_1 e^{-t/\tau_1} + k_2 e^{-t/\tau_2} \quad (1)$$

where k_1 and τ_1 refer to the initial intensity and lifetime of the response of the luminophore with a shorter lifetime, L_1 . Similarly, k_2 and τ_2 describe the response of the luminophore with the longer lifetime, L_2 . An example of a multi-exponential decay can be seen in Fig. 1.

As with mono-exponential lifetime calculations, several methods have been developed to simplify multi-exponential lifetime measurements. This can be done by separating the response of L_2 from the combined response allowing mono-exponential calculations to be used for each response assuming the contribution from L_2 is constant during the decay of L_1 . This technique has been demonstrated with a windowed calculation technique (Dual Lifetime Determination) and NLLS [1, 9, 13, 14]. However, this particular approach may not be practical in cases where the lifetimes are within three orders of magnitude, as this will result in the calculated τ_1 being dependent on the response of L_2 . A similar method developed by Sharman *et al.* utilizes four equal width windows, either contiguous or overlapping, to calculate both lifetimes without separation of the signal [22]. However, the accuracy of their method has been shown to be highly dependent on signal level [22, 23]. The requirement of equi-width windows for both lifetime measurements also means lifetimes must be similar in value ($\tau_2/\tau_1 \approx 3$ to 4) to ensure that the window width is optimal and accurate lifetimes can be calculated.

Due to the limitations of these approaches, a new method for dual lifetime calculations was investigated. This method combines the Dynamic Rapid Lifetime Determination (DRLD) demonstrated previously and a decay correction which will allow accurate lifetime calculations of both dyes [24].

II. Theory

As an alternative to least-squares analysis, there have been several methods developed which utilize window-sums to determine the lifetime responses [22, 25-35]. Window-sums are calculated by performing integration or adding the data found in each windowed segment. Of the various methods reported, DRLD is of particular value for dual lifetime calculations because of its speed and simplicity while ensuring accuracy [24]. This method calculates lifetimes using the traditional Rapid Lifetime Determination (RLD) equation:

$$\tau = \frac{\Delta t}{\ln(W_1/W_2)} \quad (2)$$

where τ is the lifetime, t is the window width, and W_1/W_2 is the ratio of the window sums from the respective windows which are contiguous in this case [26, 27]. In addition, a key aspect of the approach is an algorithm that is used to dynamically select the appropriate window width. This is approximated by adjusting the window widths in real time until the window sum ratio is within a defined range related to the range of optimal window widths ($1/\tau - t - 2/\tau$). Without such a selection algorithm, the window widths and start times remain constant during an experiment, leading to sub-optimal sampling parameters and inaccuracies in many cases [22, 26, 28, 34]. For example, windows that are too large will lead to sampling data that contains very little signal while windows that are too small will be more greatly affected by the signal noise. However, by optimizing the window width, the accuracy of lifetime calculation can be improved over a wider dynamic range—this is essential for applications where the lifetime is expected to vary by more than a few percent (*e.g.* oxygen sensitive luminophores). In addition, this method retains the speed of other

approaches that utilize window sums when compared to the speed of traditional NLLS calculations [24].

To calculate the lifetimes of *two* luminophores, a *pair* of windows is needed for *each* luminophore (four windows total). DRLD is first utilized to calculate τ_2 with the second pair of windows which is delayed until L_1 decays to zero (Fig. 2). In order to determine the contribution of L_2 to the measured (combined) signal, the initial intensity is also calculated by using:

$$k_2 = \frac{W_3}{\tau_2 f_s (1 - W_4/W_3) e^{-t_d/\tau_2}} \quad (3)$$

where τ_2 is the lifetime calculated using (2), f_s is the sampling frequency used during luminescence measurement, t_d is the time delay incorporated before sampling, and W_n is the respective window sum for each window (see Fig. 2). The time delay was incorporated to remove unwanted scattering and instrument response. After k_2 is determined, the response of L_2 can be subtracted from the measured signal. The resulting decay will then be representative of L_1 and is used to calculate τ_1 with DRLD. Adjustment of the window delay of the second set of windows to 5 times τ_1 will allow improved accuracy of τ_2 and subsequently improved accuracy of τ_1 . This delay was chosen because the signal from L_1 will be less than 1% of the original signal level k_1 and thus assumed to be negligible during calculation of τ_2 .

Dual lifetime calculations are performed recursively until the window delay is no longer changing. The initial window delay time for the second set of windows for both calculation algorithms is initially set to a value greater than ten times the maximum expected lifetime of τ_1 in order to ensure that there is not any contribution from L_1 in the initial τ_2 calculation. In addition, the maximum number of iterations set for each algorithm is 10 in order to prevent infinite loops in the software where lifetimes bounce back and forth between two values. However, in most cases less iteration are needed. A simplified algorithm for this approach, which will be referred to as dual DRLD (DDRDL), can be found in Fig. 3. An example of the MATLAB code used for DRLD and DDRDL calculations can be found in the Supplementary Information. It should be noted that for the algorithm to work properly, the decay of L_2 must behave mono-exponentially. If it is not mono-exponential, it will be more difficult to distinguish the individual responses of each luminophore leading to less accurate lifetime calculations.

III. Materials and Methods

To evaluate the ability of this method to accurately calculate the lifetime response of two luminophores simultaneously, an oxygen and temperature sensing system was employed. These analytes were chosen because of their biological significance and due to the need for temperature compensation of oxygen sensitive luminophores [4-15]. Porphyrins are attractive for luminescent oxygen sensing due to their high sensitivity, large Stokes' shift, good photostability, long lifetimes, and emission in the red to NIR wavelengths [36-44]. Platinum(II) octaethylporphyrin (PtOEP) was chosen because of its excitation and emission

peaks close to 400 and 650 nm, respectively [8, 45]. The lifetime of this dye in the absence of oxygen is also expected to be approximately 100 μ s [45]. An inorganic phosphor, manganese(IV)-doped magnesium fluorogermanate (MFG), was utilized for temperature sensing because of its long lifetime (>3 ms) and insensitivity towards oxygen [46]. This phosphor also has excitation and emission peaks similar to PtOEP at 400 and 665, respectively [46, 47]. These luminophores were chosen because they both showed a large Stokes' shift and can be excited with a single LED at 400 nm. The large difference in lifetime will also reduce the chance of inaccuracies occurring as was seen with similar approaches [22]. As previously mentioned, the overlap in emission spectra is not a concern because the response from each luminophore is resolved temporally.

A. Modeling of Dual Exponential Decays

Due to the large difference in lifetimes expected from PtOEP and MFG, dual exponential decays were first simulated using M_{ATLAB} to test the ability of DDRLD to distinguish decays from one another for a range of lifetimes and pre-exponential factors. This was done by setting k_1 and τ_1 to 1 and 10 μ s, respectively, while k_2 and τ_2 were systematically varied from 0.01 to 100 and 15 to 100 μ s, respectively. This allows for the accuracy to be estimated as a function of the ratios k_2/k_1 and τ_2/τ_1 . For each possible combination of decay parameters, ten decays were modeled using a sampling frequency of 2 MHz and a signal-to-noise ratio (SNR) of 10 in order to simulate real decays obtained. Lifetimes were then calculated using DDRLD with the first window delay set to 1 μ s and the second window delay set to five times τ_1 (50 μ s) which is where it will ideally remain due to algorithm restrictions (Fig. 3). Initial widths of each window pair were set to 10 μ s. From these data, the relative standard deviation (RSD) of each lifetime was determined for each set of decay parameters by dividing the standard deviation by the average lifetime calculated. Absolute percent difference (APD) was also investigated by comparing the mean calculated lifetime at each k and τ value to the values modeled. These parameters characterized the precision and accuracy of lifetime calculations, respectively, and allowed analysis of the results to determine the optimal relative pre-exponential factors and lifetimes similar to previous reports [22, 26-28, 34].

B. Sensor Formulations

PtOEP was purchased from Frontier Scientific and MFG was obtained from Global Tungsten & Powders Corp. (GTP Type 236). Similar to previously reported oxygen sensors, polystyrene (PS, Sigma, $M_w=280,000$) was utilized to slow oxygen diffusion through the matrix leading to higher lifetimes of the oxygen-quenchable dye [39, 42]. Toluene (Macron Chemicals) was used to dissolve PtOEP and PS. A Sylgard 184 silicone elastomer kit (Dow Corning) was used to make polydimethylsiloxane (PDMS) films for support of each phosphor during testing.

Temperature sensing gels were made by mixing 3 mg of MFG in 2 mL of PDMS precursor and 200 μ L of PDMS initiator. The solution was sonicated and then placed under vacuum until all bubbles were removed and then poured on a glass wafer. The wafer was then placed on a hot plate at 100°C to facilitate PDMS curing. The resulting film was approximately 4 cm in diameter. Although films were thoroughly mixed before curing, aggregation of the

phosphor was visible after curing and resulted in inhomogeneities in the film. The oxygen sensing film was made using a similar procedure. Fifteen μL of PtOEP solution (1 mg/mL of toluene) and 700 μL of polystyrene solution (250 mg/mL of toluene) were mixed with 1 mL of PDMS precursor and 100 μL of PDMS initiator. After vacuuming and heat curing, the resulting film was again approximately 4 cm in diameter. For individual film testing, a 2.5 mm biopsy punch was used to remove samples from each film for testing. Dual sensor measurement was performed by cutting the previously tested films in half and placing MFG and PtOEP films side by side. This arrangement allowed simultaneous illumination and collection of emission from a single fiber bundle.

C. Instrumentation and Measurement

The luminescence response of each of the sensors was measured using a custom TD measurement system similar to one described previously [24]. Excitation of the dye was performed using an LED with a peak wavelength of 405 nm (LED 405E, Thorlabs). Square wave excitation with a frequency of 10 Hz and a duty cycle of 0.2 was utilized. Luminescence was detected using a photo-multiplier tube module from Hamamatsu (H10721-20) after the light was passed through a longpass filter (3rd Millennium 620 nm, Omega Optical) using spherical lenses (LA-1951B). Decays were recorded for 15 ms with a sampling frequency of 2 MHz after the LED was turned off. For MFG and dual film experiments, the PMT control voltage was set to 0.85 V. Due to the wide range of intensities observed, a lower control voltage, 0.75 V, was used with PtOEP films to prevent saturation of the detector. During measurement, one hundred raw decays were summed to improve SNR and the resulting decay was used to calculate the lifetime.

The gas-phase response of the sensors was measured using a custom reaction chamber [24, 48]. The sensing films were immobilized in the reaction chamber by placing them on a glass slide which was then placed inside of an incubator (Torrey Pines Scientific, Echotherm IN35) used to control the temperature (25 to 65°C). The sensors were exposed to gas of various oxygen concentrations (0 to 21%) by controlling compressed air and nitrogen influx using mass flow controllers (MKS Instruments, 1179A) and a digital power supply (MKS Instruments, PR4000). The total flow rate was held constant at 2000 standard cubic centimeters per minute while the contribution from each gas was varied. All parts of the test-bench were automated and controlled using custom LabVIEW software (National Instruments).

For this work, lifetime calculations for calibration purposes (single and dual films) were performed after data collection with MATLAB (MathWorks, Inc.). DRLD was used to calculate the lifetimes for individual films while lifetimes of dual films were calculated using DDRLD. NLLS analysis with a mono-exponential fit was used to calculate lifetimes for individual films for comparison purposes. Real-time calculations utilizing DDRLD were performed with LabVIEW during a dynamic response experiment. In order to calculate lifetimes, windows used for MFG and PtOEP were set to an initial width of 200 and 5 μs , respectively. All single film experiments had an initial window delay of 1 μs while dual film experiments had 1 μs delay for the first pair of windows (used for PtOEP lifetime

calculation) and an initial delay of 1000 μ s for the second pair of windows (used for MFG lifetime calculation).

D. Film Testing and Analysis

Initially, individual PtOEP and MFG films were each tested in triplicate. For each test, ten lifetimes were averaged for each environmental condition (*i.e.* oxygen concentration and temperature) tested. The response of the three individual films was then averaged to determine the expected response from dual film measurements. For MFG films, the lifetime response of MFG films was recorded at five different temperatures (25 to 65°C in 10° increments) using an oxygen concentration of 21%. Linear regression was then used to fit the averaged film responses and obtain a calibration for the temperature sensitive MFG response.

To keep testing time to a minimum, calibration of the PtOEP films was performed at only three temperatures (25, 45, and 65°C) but for a range of oxygen concentrations at each temperature. These concentrations (0, 2.625, 5.25, 10.5, and 21% oxygen) were skewed towards lower oxygen levels where sensitivity of the porphyrin is higher. The response at each temperature was fit with NLLS using a two-site Stern-Volmer equation:

$$\frac{\tau_0}{\tau} = \left(\frac{f}{1 + K_{SV1}[O_2]} + \frac{1-f}{1 + K_{SV2}[O_2]} \right)^{-1} \quad (4)$$

where τ_0 is the lifetime in the absence of the quencher (in this case oxygen O_2), τ , is the lifetime at each oxygen concentration ($[O_2]$), f is the fractional contribution from each site, and K_{SVn} is the respective Stern-Volmer constant [37, 40, 49, 50]. After initial fittings, the fractional contribution was averaged because it is assumed that this value remains constant despite temperature changes. The averaged f was used to re-fit the data to allow more accurate temperature-dependent trends to be determined. The temperature-dependent trends of the parameters (τ_0 , K_{SV1} , and K_{SV2}) in (4) were then determined using linear regression.

Dual film responses were measured using the same measurement system with a side by side approach where one half of each of the previously-tested oxygen- and temperature-sensing films were utilized. Testing of the dual film response was performed similar to testing of PtOEP films. The validity of DDRLD was investigated by comparing the lifetimes obtained from the dual films with the response of the individual films determined using DRLD. A bi-exponential NLLS fit was also used to calculate the dual lifetime response for comparison to DDRLD.

A single dynamic experiment was also performed with a dual film to demonstrate the ability of DDRLD to measure the response of a dual luminescent system in real-time. This was done by exposing the dual films to random, un-calibrated temperatures and oxygen concentrations. When temperature was changed, the experimental setup was held constant (*i.e.* temperature and oxygen was not changed) for 2 hours to ensure the temperature throughout the reaction chamber reached equilibrium. Changes in oxygen concentration were only held for 15 minutes because the changes within the reaction chamber were almost immediate. Dual lifetime measurements were recorded for the duration of this experiment

and converted to predictions of oxygen and temperature levels after the experiment. Although predictions were not made in real-time, doing this would only require conversion of the lifetime values through application of a calibration equation; dynamic tracking abilities could still be assessed with post-experiment application of the calibration.

IV. Results and Discussion

A. Results of Modeled Dual-Exponential Lifetime Calculations

From the modeling results, the calculated RSD and APD revealed that the lifetime accuracy for both luminophores appears to be highest when k_2/k_1 is between 0.1 and 1 and when τ_2/τ_1 is greater than 3 (see Supplementary Information). When the relative decay parameters fall outside of this region, DDRLD suffers from a drastic reduction in accuracy for modeled decays with an SNR of 10. A higher SNR will likely lead to wider optimal regions, but these results clearly indicate the limitations of this method under normal working conditions. These results should be utilized to select luminophores with the appropriate lifetimes for use in dual monitoring systems. Intensity is less of a concern when selecting luminophores because it is not an inherent property of the luminophore and can be adjusted by changing the concentration of each luminophore, excitation wavelength, and/or emission filters.

B. Calibration of Individual Film Responses

Following modeling, *in vitro* testing of individual films was performed to validate the expected behavior. The response of individual MFG films can be seen in Fig. 4. Although the response from film to film was statistically different, each response follows the same trend. Variability may be a result of variations in the signal-to-noise ratio due to non-uniform dispersal of dye in the silicone. The averaged response, however, showed linearity ($R^2 > 0.99$) with a slope of $-8.28 \mu\text{s}/^\circ\text{C}$ and an intercept of $3647.8 \mu\text{s}$. The lifetimes calculated using DRLD were not significantly different than averaged lifetimes calculated using NLLS ($\alpha = 0.05$, see Supplementary Information), proving the accuracy of the approach.

In contrast to the MFG response, PtOEP films showed much less variability (Fig. 5A). The accuracy of DRLD was very high when compared with NLLS, with an average difference of only $0.77 \mu\text{s}$; however, because of the high SNR of the measurements, even this small difference was found to be statistically different ($\alpha = 0.05$, see Supplementary Information). The difference is most likely due to the multi-exponential nature of the decays for PtOEP, which has been reported to be dependent on the immobilization matrix [49, 51, 52]. Similar to reports of other oxygen sensitive luminophores, the oxygen sensitivity was higher at lower oxygen concentrations and increases in temperature lead to decreases in the lifetime response [5, 9, 13]. Due to the non-linear nature of the oxygen response as well as the temperature dependency, calibration of the PtOEP films required more thorough analysis in order to predict oxygen concentrations accurately (Fig. 5B). It is also important to note that for the oxygen and temperature values tested the ratio of the lowest lifetime obtained for MFG and the highest lifetime obtained for PtOEP is much larger than 3. This suggests that these luminophores are suitable for DDRLD calculations.

For calibration, the Stern-Volmer plot for the response at each temperature was first determined using the appropriate values for τ_0 . After initial fitting using (4), the mean fractional contribution, f , was found to be 0.278. This value was used to re-fit the Stern-Volmer responses and the resulting trends of K_{SV1} and K_{SV2} , as a function of temperature (T) were found. These values, along with the temperature dependent values for τ_0 , are presented in Fig. 6. The linear fits used for calibration of τ_0 , K_{SV1} , and K_{SV2} all had an R^2 value greater than 0.99. When these fitted parameter values were entered into (4) and compared to the original data in Fig. 5B, R^2 remained greater than 0.99. The characterization of these trends will allow oxygen concentrations to be determined for all temperatures within (or near) the tested range as long as the ambient temperature is accurately predicted.

C. Dual Film Responses

Following calibration of the individual film responses, the lifetimes of dual films calculated using DDRLD were compared to the response of individual films where the lifetimes were calculated using DRLD. As can be seen in Fig. 7A, the MFG response calculated using DDRLD for dual film measurements was not significantly different than the average DRLD response for any condition tested. Larger delay values for the second set of windows used for τ_2 calculation, which occurs for higher τ_1 values, results in a slight shift in the calculated lifetime towards higher values. Although this is not statistically significant, improvements in SNR throughout the decay are expected to reduce this issue.

As expected, PtOEP lifetimes calculated using DDRLD for dual film measurements followed the same trends as individual film data and displayed low variability (Fig. 7B). The average percent difference for the dual film lifetimes was only 2% above values expected from individual films, suggesting DDRLD can generate highly accurate determinations. Again, because of the high SNR of the measurements, even at this very low error level the lifetimes calculated for dual films were still statistically different than results calculated for individual films. The largest percent difference, 8.41%, occurred at 21% oxygen and 25°C where the SNR of the response of L_1 is the lowest compared to the response of L_2 . Overall, lifetimes calculated using DDRLD for dual film measurements were in excellent agreement with the response of individual films containing MFG and PtOEP. In addition, dual lifetime calculations made using a bi-exponential NLLS fit showed similar trends to individual film responses where MFG lifetimes were not significantly different than DDRLD and PtOEP lifetimes showed a slight difference due to lower variability ($\alpha = 0.05$, see Supplementary Information).

D. Dynamic Testing

Using the calibration curves obtained above, a dynamic experiment was performed where ambient oxygen levels and temperature were predicted as each was varied independently. The real-time temperature prediction from the MFG response compared to the temperature programmed into the incubator can be seen in Fig. 8, and as expected, the MFG response is able to track the ambient changes in temperature. Calculating the percent difference for $n = 10$ predictions at each concentration and temperature, the average percent difference for all concentrations and temperatures was found to be 0.72% demonstrating highly accurate prediction of temperature. It is important to note that the slow response time observed for

MFG is due to the long equilibration time of the incubator and reaction chamber and not the sensor film.

Oxygen predictions performed with compensation showed a similar ability to track oxygen levels as temperature predictions; however, predicted oxygen values tended to underestimate actual levels (Fig. 9A). At 15.75% oxygen, the relative error for compensated predictions was found to be 8.77% but at the lowest levels tested (2.1% oxygen), the response was off by only 0.63%. As before, this improved accuracy at lower oxygen levels is due to an increase in intensity from the PtOEP resulting in a higher SNR. In addition, the reduced accuracy in oxygen prediction at higher oxygen concentrations can also be attributed to the highly sensitive exponential oxygen response curve and the difference in PtOEP lifetimes calculated using DRLD and DDRLD. For example, the expected response of τ_1 at 25°C and 21% oxygen is 22.3 μ s; however, if τ_1 was measured to be 1 μ s lower than expected, the predicted oxygen concentration would be 22.8%. Similarly, if the expected response of τ_1 at 25°C and 0% oxygen (89.3 μ s) was low by 1 μ s, the predicted oxygen concentration would be 0.04%. These differences in predicted and actual oxygen levels indicate the greater effect inaccurate lifetime calculations have at higher oxygen concentrations. As noted above, improvements in SNR can help overcome these errors and lead to more accurate predictions.

In an effort to improve the accuracy of lifetime predictions and show the utility of this dual sensing approach, calibration curves of the PtOEP response using DDRLD data from Fig. 7b were determined similar to calibrations performed for individual films (data not shown). The lifetimes obtained from the original dynamic test were then used to obtain oxygen predictions using these new, more accurate calibration curves. As can be seen in Fig. 9B, these results show a higher accuracy at higher oxygen levels than predictions made using calibrations obtained from individual film responses. The relative error at 15.75% oxygen decreased from 8.77% to 2.33% and an overall mean percent difference of 0.78% was obtained. Again, this improvement is a result of the difference in the lifetime response calculated for individual and dual film measurements using DRLD and DDRLD, respectively. It is also interesting to note that in either case the oxygen predictions do not appear to be temperature dependent despite the long transition times when temperature is changed.

V. Conclusions

A dual dynamic rapid lifetime determination (DDRLD) algorithm was investigated. It was shown to enable simultaneous determination of the lifetime response of two temporally-distinct luminophores. This dual lifetime calculation approach has a limited range of operation, where the multi-exponential luminescence decays must have sufficient temporal separation (τ_2/τ_1 greater than 3) and the relative intensity of the emission should be relatively closely matched, but the short-lifetime decay should be more intense (k_2/k_1 between 0.1 and 1). When evaluating the approach *in vitro*, temperature compensation of an oxygen-sensitive porphyrin was found to be effective after calibrating an oxygen sensing film at three different temperatures and determining the linear temperature dependency of the calibration fit parameters. Overall, the approach yields highly accurate and statistically

equivalent lifetimes for dual films using DDRLD compared to average lifetimes calculated for individual films.

Individually, each of the techniques demonstrated (a compensation algorithm and a method for measuring two lifetimes simultaneously) can be applied to a variety of sensing applications. Temperature compensation, applied here with DDRLD to demonstrate real-time, dynamic, simultaneous predictions of oxygen and temperature, is also a valuable advance. This capability is critical for oxygen measurements, for example, because of the high temperature-dependent lifetimes of common oxygen-sensitive phosphors. The simplicity of the approach will also lend its usefulness to other applications where compensation for variation in one analyte is needed to improve the accuracy of measurements of another analyte (such as oxygen compensation of enzymatic glucose sensors). DDRLD can also be utilized with a variety of biomedical, environmental, and food industry applications due to its ability to perform real-time measurements without sacrificing accuracy.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Fig. 1. Diagram showing the combined time-dependent response of a shorter-lived luminophore, L_1 , and a longer-lived luminophore, L_2 .

Fig. 2.

Depiction of a dual-exponential decay and the window sums utilized to calculate the lifetime response using DDRLD. The black dashed line represents the combined response, while the blue and red dashed lines represent response of L_1 and L_2 respectively. Window sums utilized for lifetime calculations are shown in the shaded regions.

Fig. 3.
A diagram showing a simplified version of the DDRLD algorithm.

Fig. 4.

Raw and averaged lifetime response of three individual MFG films (Runs 1-3). The red dotted line represents the linear fit of the averaged response. Error bars represent the 95% confidence interval with $n = 10$ for individual films and $n = 3$ for the averaged response.

Fig. 5.

A) Response of individual PtOEP films to oxygen and temperature. Error bars represent the 95% confidence interval with $n = 10$ for individual films (Runs 1-3) and $n = 3$ for averaged data. B) Multisite Stern-Volmer plot for the PtOEP films. The red line represents the fit obtained using (4) which was used for calibration purposes.

Fig. 6.

A) The fractional contributions obtained for the oxygen response each temperature. The red line represents the mean value that was used to obtain K_{SV1} and K_{SV2} B), C), and D) Temperature-dependent trends for τ_0 , K_{SV2} , respectively.

Fig. 7.

Results of a dynamic, dual film test showing programmed and predicted temperatures where DDRLD was utilized to monitor the lifetime responses.

Fig. 8.

A) Comparison of the MFG lifetime responses for individual and dual film experiments is shown where lifetimes were calculated using DRLD and DDRLD, respectively. Error bars represent the 95% confidence interval for $n = 3$ films. B) Comparison of lifetimes calculated for dual film experiments using DDRLD and individual film experiments where lifetimes were calculated using DRLD. Error bars represent 95% confidence intervals for $n = 3$ films.

Fig. 9.

A) Oxygen concentration predictions are shown for a dynamic, dual film test where DDRLD was utilized to monitor the lifetime responses. Calibration from individual film responses was utilized. B) Oxygen predictions are shown using the same lifetime calculations but utilizing calibrations obtained from dual film responses.