



Real-time optimal combination of multifrequency information in phase-resolved luminescence spectroscopy based on rectangular-wave signals

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ABSTRACT

A method for optimally combining multifrequency information in phase-resolved luminescence spectroscopy using rectangular-wave signals is proposed to improve the accuracy in the determination of the analyte concentration. From the rectangular-wave signal, phase-shift- and modulation-factor-based apparent lifetimes are estimated at each harmonic independently, together with their corresponding standard errors. Both the lifetimes and their standard errors are estimated “on-the-fly” from the Fast Fourier Transform (FFT) of the excitation and emission signals and applying error propagation theory. Independent determinations of the analyte concentration and their standard errors are then optimally combined in order to obtain an improved determination of the analyte concentration. The combination, formulated in a statistical framework, is a weighted average of different determinations proportional to the inverse of the variance of each independent determination. The proposed method has been applied to an oxygen measuring system to evaluate its accuracy and demonstrate its applicability in real-time measuring instruments.

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1. Introduction

To date, most measurement schemes based on phase-resolved luminescence spectroscopy [1] make use of sine- or square-wave modulated excitation sources [2,3], and phase detection is performed at one or several modulation frequencies by use of commercial lock-in amplifiers [4–6] or either simple analog [7,8] or digital [9,10] implementations.

In a recent article [11], we demonstrated some advantages of using short duty-cycle rectangular-wave modulated excitation signals in phase-resolved luminescence spectroscopy. Among others, the use of rectangular-wave signals allows to determine the analyte concentration independently for the different harmonics of the modulated signal. In that work, we also demonstrated that the combination of the information from different harmonics can significantly improve the accuracy of an oxygen measuring system. In

a statistical formulation, the optimal combination of several independent estimates of analyte concentration is a weighted average of individual estimates, where such weights are inversely proportional to the variances of the estimates (i.e., an estimate with high uncertainty will have a lower weight than an estimate with low uncertainty). One of the problems encountered in the referenced work was the definition of a procedure for computing the variance of each estimate. In this work, the variances were determined from a collection of measurements recorded in the laboratory (i.e., from the same set of measurements used to calibrate the oxygen measuring system). The experiments using the variances computed with this procedure demonstrated that, theoretically, if we had an accurate estimation of the variances, the information from different harmonics could be combined to improve the accuracy of an oxygen measuring system. However, the procedure proposed for computing the variances cannot be implemented in a real instrument: On the one hand, it makes no sense recording N measurements for improving the accuracy of just one measurement, since it would increase the measuring time in a factor N . On the other hand, the variances cannot be previously estimated as a part of the calibration procedure, since the experimental conditions could strongly differ from the operation conditions [12].

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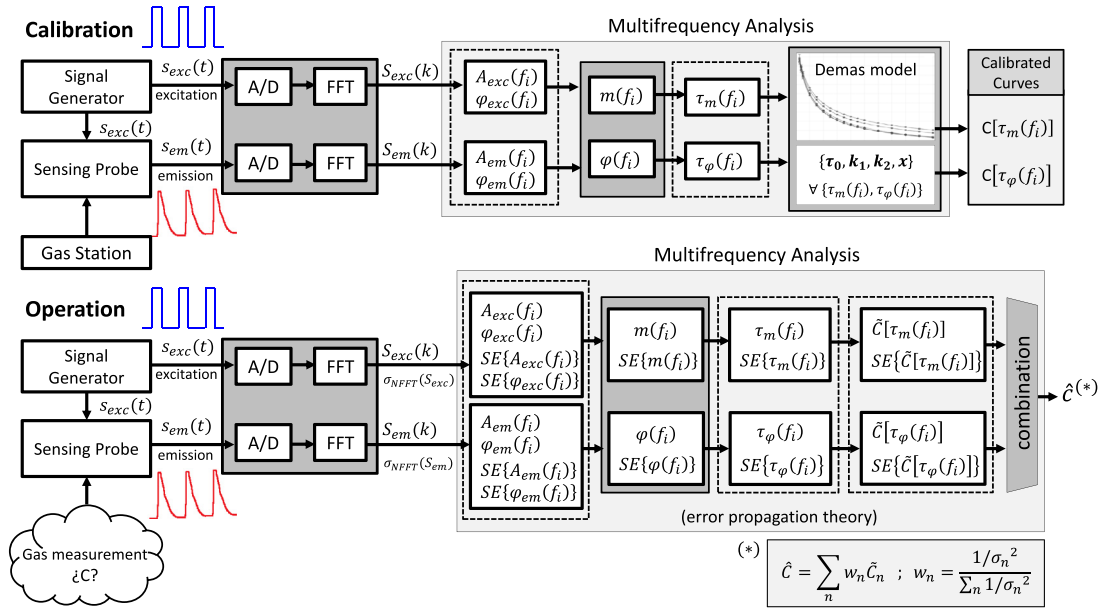


Fig. 1. Block diagram describing the calibration process in phase-resolved luminescence spectroscopy and determination of the analyte concentration in real operating conditions by combining multifrequency information using rectangular-wave signals to improve the accuracy in the determination of the analyte concentration.

In another recently published study [13], we demonstrated that an algorithm based on the Fast Fourier Transform (FFT) [14] of the luminescent signal can be used to obtain the uncertainty associated with the phase-shift or modulation-factor measurements from a single recorded emission signal. This way, the uncertainties can easily be obtained from the recorded signal in real-time and under the operating conditions. Therefore, by applying error propagation theory, the uncertainty associated with the estimated apparent lifetimes or analyte concentration determinations can be estimated.

Taking into account the contribution of the two previous works [11,13], in this letter we propose the application of the FFT-based estimation of the uncertainty (extended to the case of multifrequency signals) to the optimal combination of multifrequency information in phase-resolved luminescence spectroscopy based on short duty-cycle rectangular-wave signals. This way, the combination of multifrequency information improves the accuracy in the analyte determination using exclusively the recorded signal involved in the current measurement, which provides a procedure that can be implemented in a real instrument and can operate in real-time. To the best of our knowledge, this is the first time that a multifrequency phase-modulation method with combination of information on-the-fly from more than one harmonic has been used in luminescence spectroscopy. The proposed approach has been applied to an oxygen measuring system based on phase-resolved luminescence, and experiments show that this method is able to optimally combine information from different harmonics in real-time (i.e., without requiring a calibration in the operating conditions).

2. Theory

2.1. Estimation of apparent lifetimes $\tau_m(f_i)$ and $\tau_\varphi(f_i)$ for each harmonic f_i

The use of short duty-cycle rectangular signals for excitation provides several harmonics with enough amplitude for allowing simultaneous measurements of phase-shift (φ) and modulation-factor (m) at several frequencies. For multifrequency analysis of the response, we apply an analog-to-digital conversion and

subsequent processing of the recorded digital signals, which can easily be implemented via software in an ordinary computer or a microcontroller [4,10,11].

Let us suppose a signal $s_e(t)$, where the subindex e stands for excitation (exc) or emission (em) signals (see Fig. 1). If analysis of the digitized signal $s_e(m)$ is performed on a finite interval of M samples containing an integer number of cycles, then the amplitude (A_e) and the phase (φ_e) of the signal can be obtained for each harmonic f_i from the FFT [14]:

$$S_e(k) = \text{FFT}\{s_e(m)\} = \sum_{m=0}^{M-1} s_e(m) \exp\left(\frac{-j2\pi km}{T}\right) \quad (1)$$

$$A_e(f_i) = \sqrt{[\text{Re}\{S_e(k_i)\}]^2 + [\text{Im}\{S_e(k_i)\}]^2} \quad (2)$$

$$\varphi_e(f_i) = -\arctan\left[\frac{\text{Im}\{S_e(k_i)\}}{\text{Re}\{S_e(k_i)\}}\right] \quad (3)$$

where k_i is the frequency component associated with the i th harmonic f_i . Taking into account the amplitude and phase of both signals (exc/em), the modulation-factor (m) and the phase-shift (φ) can be estimated at each harmonic f_i as [2]:

$$m(f_i) = \frac{A_{em}(f_i)}{A_{exc}(f_i)} \quad (4)$$

$$\varphi(f_i) = \varphi_{em}(f_i) - \varphi_{exc}(f_i) \quad (5)$$

The modulation factors and the phase shifts can be used to estimate modulation-factor- and phase-shift-based apparent lifetimes ($\tau_m(f_i)$ and $\tau_\varphi(f_i)$) from each harmonic f_i as [11]:

$$\tau_m(f_i) = \tau_0 \frac{m(f_i)/m_0(f_i)}{\sqrt{1 + (2\pi f_i \tau_0)^2 \cdot (1 - (m(f_i)/m_0(f_i))^2)}} \quad (6)$$

$$\tau_\varphi(f_i) = \frac{\tan[-\varphi(f_i)]}{2\pi f_i} \quad (7)$$

where τ_0 is the apparent lifetime at null concentration of the analyte and $m_0(f_i)$ is the modulation-factor at null concentration and frequency f_i .

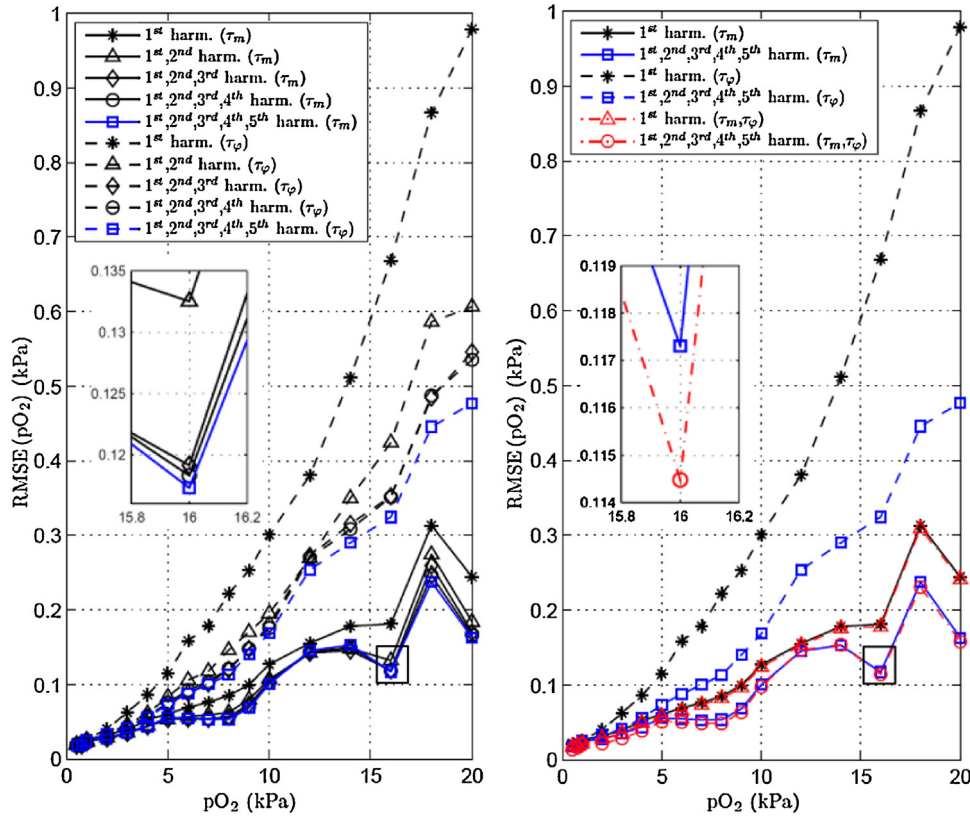


Fig. 2. RMSE in determination of \tilde{pO}_2 between 0.5 and 20 kPa by use of phase-shift-based apparent lifetime (τ_φ), modulation-factor-based apparent lifetime (τ_m), and the combination of both (τ_φ, τ_m), with the proposed multifrequency phase-modulation method combining estimations from different harmonics (from one to five harmonics).

Table 1

Average relative error (Avg. RE, in %) in determination of \tilde{pO}_2 (considering all tested concentrations between 0.5 and 20 kPa) when some information from the 5 first harmonics is combined with the multifrequency phase-modulation method proposed in this work (method A: based on FFT). These results are compared with those obtained with the multifrequency phase-modulation method previously published by our research group (method B: based on statistics from calibration data) [11].

Harmonics	Average relative error, Avg. RE (%)					
	(A) SE(\tilde{pO}_2) (FFT) (Real time combination)			(B) SE(\tilde{pO}_2) (calib. data) (No real time combination)		
	τ_φ	τ_m	τ_φ, τ_m	τ_φ	τ_m	τ_φ, τ_m
1	3.119	1.651	1.486	3.119	1.651	1.375
2	3.159	2.492	1.924	3.159	2.492	1.878
3	4.121	4.365	2.854	4.121	4.365	2.864
4	6.602	9.379	5.010	6.602	9.379	5.117
5	8.232	13.133	6.560	8.232	13.133	6.570
1,2	2.230	1.422	1.247	2.157	1.424	1.166
1,2,3	2.010	1.382	1.195	1.890	1.392	1.131
1,2,3,4	2.018	1.376	1.187	1.816	1.389	1.126
1,2,3,4,5	1.932	1.371	1.178	1.761	1.388	1.123

2.2. Analyte determination and combination of analyte estimates

In optochemical sensors based on phase-resolved luminescence, the determination of analyte concentration can be performed by use of calibration curves appropriately fitted from calibration datasets involving different analyte concentrations and the corresponding measured parameters at one or several modulation frequencies (i.e., data $m(f_i)$ or $\varphi(f_i)$), or usually the apparent lifetimes $\tau_m(f_i)$ and $\tau_\varphi(f_i)$ estimated from them as described in Eqs. (6) and (7) [3,11]. Likewise, from a single signal recording [$s_{exc}(t)$ and $s_{em}(t)$], different independent determinations of the analyte concentration can be estimated. From N unbiased and statistically

independent determinations of the analyte concentration \tilde{C}_n (each one based on an estimated parameter x_n with $n = 1, 2, \dots, N$), a more accurate estimation \hat{C} can be obtained as the weighted average of all individual estimates [11]:

$$\hat{C} = \sum_{n=1}^N w_n \tilde{C}_n \quad w_n = \frac{1/\sigma_n^2}{\sum_{n=1}^N 1/\sigma_n^2} \quad (8)$$

where w_n and σ_n (that could also be noted as $SE\{\tilde{C}_n\}$) are, respectively, the normalized weight and the standard error associated with the n th measurement \tilde{C}_n . Since the analyte concentration \tilde{C}_n is estimated from the parameter x_n by using the corresponding calibration curve (where x_n is a modulation- or phase-based apparent lifetime, i.e., $\tau_m(f_i)$ or $\tau_\varphi(f_i)$), the standard error $SE\{\tilde{C}_n\}$ can be estimated according to error propagation theory as:

$$\sigma_n = SE\{\tilde{C}_n\} = \left| \frac{\partial \tilde{C}_n(x_n)}{\partial x_n} \right| SE\{x_n\} \quad (9)$$

where $\tilde{C}_n(x_n)$ is the calibration function describing the analyte concentration as a function of the parameter x_n , and $SE\{x_n\}$ is the standard error associated with the measured parameter x_n . The value of $SE\{x_n\}$ could be estimated in two ways: (1) statistically, from several measurements of x_n acquired in the operation conditions or even from the calibration data (calibration-based conventional method) [11], or (2) from the FFT of a single recorded signal (approach proposed in this work).

In a previous work [13] we proposed a method for accurately estimating the variance of the noise in the FFT domain (σ_{NFFT}^2) for both emission or excitation signals. In that work we obtained the standard error of the modulation-factor or the phase-shift at the

modulation frequency. In the case of multifrequency spectroscopy, these standard errors can be obtained for each harmonic f_i as:

$$SE(m(f_i)) \approx \frac{\sigma_{NFFT}(S_{em})}{A_{exc}(f_i)} \quad (10)$$

$$SE(\varphi(f_i)) \approx \frac{\sigma_{NFFT}(S_{em})}{A_{em}(f_i)} \quad (11)$$

where we assumed that the contribution of $\sigma_{NFFT}(S_{exc})$ can be neglected, since excitation signal has greater amplitude and smaller noise level than emission signal. These standard errors can be used to obtain $SE\{\tau_m(f_i)\}$ and $SE\{\tau_\varphi(f_i)\}$ or $SE\{\tilde{C}_n\}$ according to error propagation theory.

Fig. 1 shows a block diagram describing the calibration process of the sensing probe and the determination of the analyte concentration in real operating conditions using our method. In calibration, the sensing probe is excited using a rectangular-wave signal where the analyte concentration is modified in a controlled way using a gas station. For each concentration value, the excitation and emission signals ($s_{exc}(t)$ and $s_{em}(t)$) are digitized and FFT is applied to obtain $S_{exc}(k)$ and $S_{em}(k)$. The amplitude and phase of both signals for each harmonic ($A_e(f_i)$ and $\varphi_e(f_i)$) are calculated from the FFT components ($S_{exc}(k_i)$ and $S_{em}(k_i)$) using Eqs. (2) and (3). From them, the modulation factor ($m(f_i)$) and the phase-shift ($\varphi(f_i)$) and the corresponding apparent lifetimes ($\tau_m(f_i)$ and $\tau_\varphi(f_i)$) are calculated using Eqs. (4)–(7). The calibration curves ($C[\tau_m(f_i)]$ and $C[\tau_\varphi(f_i)]$) are independently obtained for each harmonic f_i by applying an appropriate model (e.g., Demas model [3]) and curve fitting with different analyte concentrations.

In operating mode, the analyte concentration is determined by exciting the sensing probe with a rectangular-wave signal and processing the excitation and emission signals $s_{exc}(t)$ and $s_{em}(t)$. As in calibration process, the excitation and emission signals are digitized to calculate its amplitude and phase from the FFT at each harmonic f_i . In this case, the variance of the noise ($\sigma_{NFFT}^2(S_{exc})$ or $\sigma_{NFFT}^2(S_{em})$) is also calculated to obtain the standard error of the amplitude and phase for both signals ($SE\{A_e(f_i)\}$ and $SE\{\varphi_e(f_i)\}$). From them, and applying Eqs. (10) and (11) and error propagation, the apparent lifetimes ($\tau_m(f_i)$ and $\tau_\varphi(f_i)$) and the corresponding standard errors ($SE\{\tau_m(f_i)\}$ and $SE\{\tau_\varphi(f_i)\}$) are also calculated. Independent determinations of the analyte concentration using calibrated curves ($\tilde{C}[\tau_m(f_i)]$ and $\tilde{C}[\tau_\varphi(f_i)]$) and their standard errors ($SE\{\tilde{C}[\tau_m(f_i)]\}$ and $SE\{\tilde{C}[\tau_\varphi(f_i)]\}$) are optimally combined in order to obtain an improved determination of the analyte concentration (\hat{C}) using Eq. (8). This way, the proposed method can be applied to combine the information obtained from $\tau_\varphi(f_i)$ and $\tau_m(f_i)$ at different harmonics when the system is excited with a short duty-cycle rectangular-wave signal. The estimation of standard errors based on FFT allows the combination of multifrequency information directly from the recorded signal involved in the measurement.

3. Experiments and discussion

The proposed method was evaluated using an optical fiber oxygen measuring system based on phase-resolved luminescence excited with a 10% duty-cycle rectangular-wave signals with fundamental frequency 1715 Hz and 200 ms in duration (343 cycles of signal). The sampling frequency was 500 kHz. The sensing fiber is a plastic-clad silica optical fiber of which 2 cm of the plastic cladding was removed and cleaned. The oxygen-sensitive sensing film [platinum(II) meso-tetra-(pentafluorophenyl)-porphine in polystyrene, PtTFPP/PS] was coated on the cladding-free portion of the fiber. A schematic diagram of the experimental setup used in this work is presented in Supporting Information (see Section 2 in SI), and all the details of the oxygen measuring system can be found in Ref. [11].

In calibration process, 625 excitation and emission signals were analyzed considering 17 values of oxygen concentration (pO_2) ranging between 0 and 20 kPa. By applying the proposed method, modulation-factors and phase-shifts ($m(f_i)$ and $\varphi(f_i)$) were calculated at the 5 first harmonics of the rectangular-wave signal (i.e., 1715, 3430, 5145, 6860 and 8575 Hz) at different oxygen concentrations. The estimated average lifetimes ($\tau_\varphi(f_i)$ and $\tau_m(f_i)$) obtained at different oxygen concentrations and frequencies were used to fit calibration curves according to the Demas two-site model with a criterion of minimization of the relative error in pO_2 [3]. In this work, 10 calibration curves (5 for $pO_2[\tau_m(f_i)]$ and 5 for $pO_2[\tau_\varphi(f_i)]$) were independently used for the determination of the pO_2 . Mean values for m , φ , τ_φ and τ_m at different concentrations and frequencies, model parameters and calibration curves are shown in Supporting Information (see Section 3 in SI).

In operation mode, 625 signals (exc/em) (identical to those used in calibration, but different from them) were recorded for each pO_2 ranging between 0.5 and 20 kPa, and also the first 5 harmonics were considered. Therefore, by applying the corresponding calibration curves, 10 independent estimations of the oxygen concentration (5 for $pO_2[\tau_m(f_i)]$ and 5 for $pO_2[\tau_\varphi(f_i)]$) were available from the apparent lifetimes. Noise characterization, $\sigma_{NFFT}(S_{em})$, was performed from a set of spectral components around each harmonic f_i (see Fig. SI-3 in SI). Additionally, a correction using percentile 70 in the noise analysis was used to obtain unbiased estimations of the noise variance in the presence of interferences [13] (see Section 1 in SI for details). Using $m(f_i)$, $\varphi(f_i)$ and $\sigma_{NFFT}(S_{em})$ we can calculate $\tilde{pO}_2[\tau_m(f_i)]$ and $\tilde{pO}_2[\tau_\varphi(f_i)]$ together with their corresponding standard errors. The individual pO_2 determinations can be combined using Eq. (8). Since $\sigma_{NFFT}(S_{em})$ is estimated in real time for the operation conditions, the proposed method provides an optimal combination of the individual pO_2 estimates, improving the accuracy in the final pO_2 determination.

Evaluation of accuracy of the proposed method was based on the root-mean-square error (RMSE) and the average relative error (Avg. RE), with the concentration measurements from the mass-flow controllers used as reference. Detailed experimental results obtained with the proposed oxygen measuring system, including concentrations estimated from τ_m , τ_φ and the combination of both, and also combining estimations from the five first harmonics, are shown in Supporting Information (see Section 4 in SI). Fig. 2 and Table 1 summarize these results. Table 1 shows a comparison of accuracy (Avg. RE(%) in determination of pO_2) between (A) the multifrequency phase-modulation method proposed in this work, where weights in Eq. (8) are calculated from the FFT of the recorded emission signal (i.e., in real operating conditions), and (B) the method previously published by our research group [11] (where such weights are previously calculated from statistics of the calibration data). The experimental results obtained with both methods (noted as A and B in Table 1) are very similar, showing the same trend. The combination of both analytical signals (τ_m and τ_φ) improves the accuracy provided by either τ_m or τ_φ alone. In addition, the procedure proposed for combining information from different estimators has also been successfully applied to combine τ_m and τ_φ information from a number of harmonics. Results show that as more information is included, the error in determination of pO_2 decreases, obtaining an Avg. RE as low as 1.178% for method A and 1.123% for method B when τ_m and τ_φ from the five first harmonics are combined. It is noteworthy that although B is slightly better than A in results, B requires estimating $SE\{\tilde{C}_n\}$ at each concentration as part of calibration (and it is conditioned by the experimental conditions of the calibration and therefore not applicable in a real instrument), while in A, $SE\{\tilde{C}_n\}$ is directly estimated in real-time from the recorded signal involved in the measurement.

4. Conclusions

The method proposed in this work can be easily implemented in existing photoluminescence instruments, since modifications just involve the part of excitation of the light source (a short duty-cycle rectangular-wave signal, which usually implies a simplification of the electronics) and digital signal processing of samples after the transducer (that can be easily implemented in software). But, without any doubt, the main advantage of the proposed method is that it allows the on-the-fly estimation of uncertainties associated with measurements, which provides optimal combination of individual analyte determinations (using the uncertainties corresponding to the operating conditions).

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Appendix A. Supplementary Data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.snb.2016.07.046>.

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