Direct, Reliable, and Correct Oxygen Measurement

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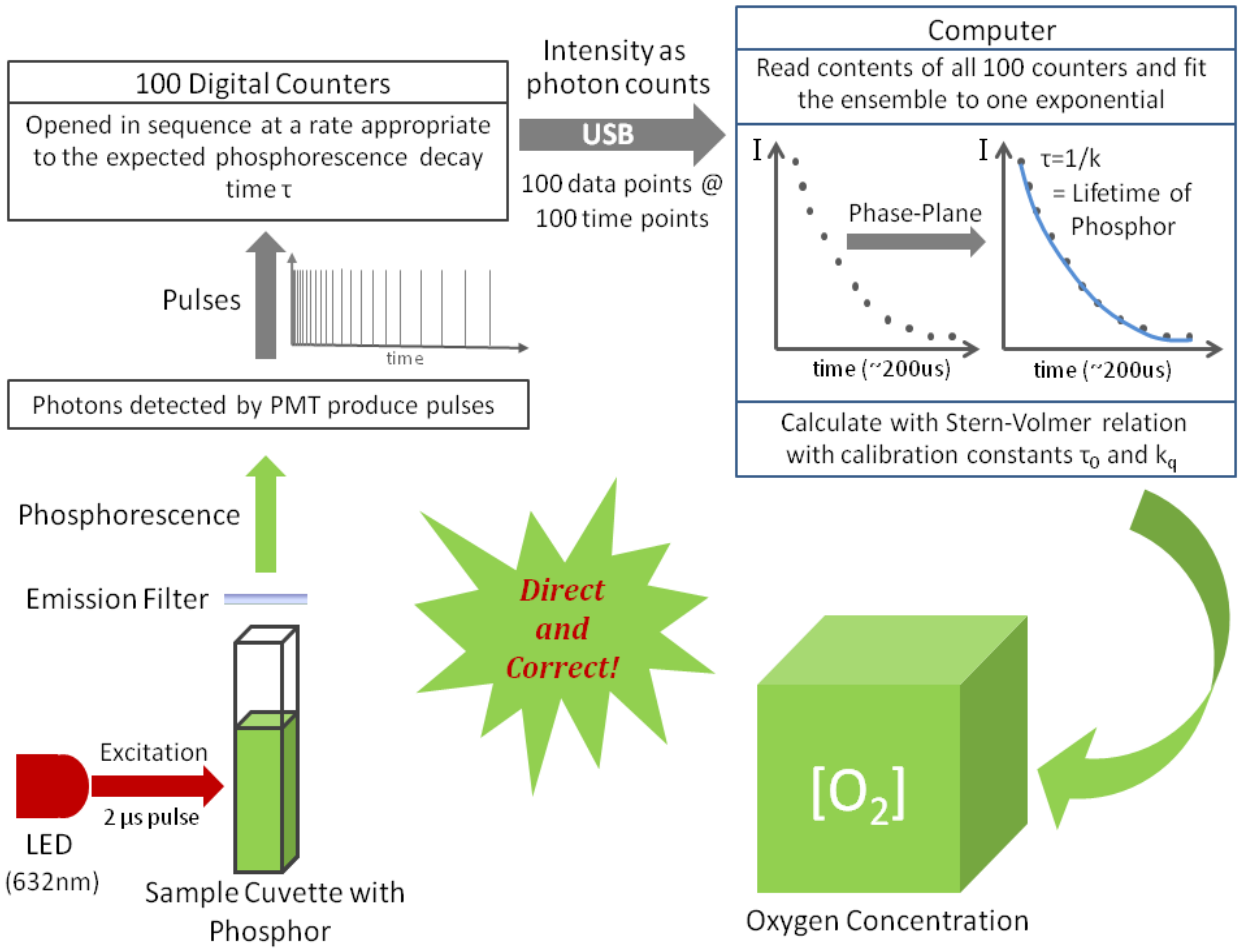
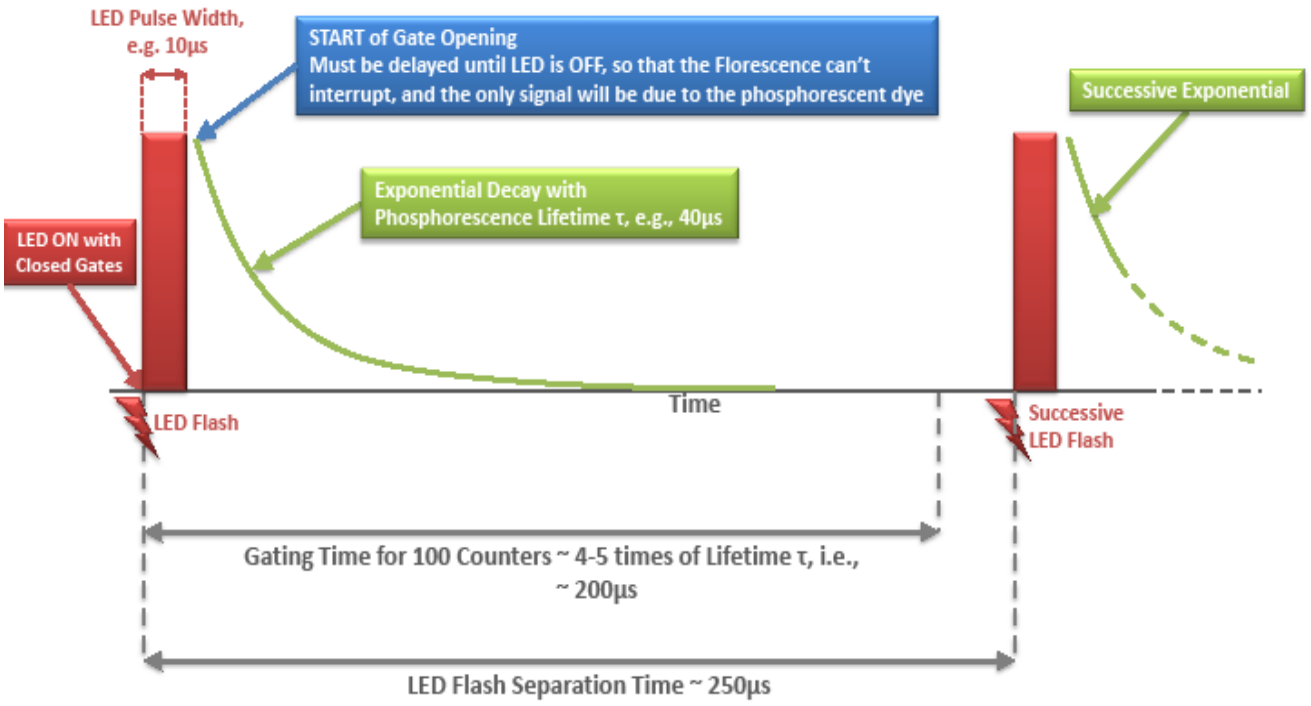
Oxygen-dependent quenching of phosphorescence has been used extensively for the last 25 years as a reliable method for measuring oxygen. Major strengths of the method are that it is noninvasive, except for the phosphor, and calibration is absolute. Because it is an optical method, oxygen can be measured through the walls of optically clear chambers. The method's absolute calibration means that there is no requirement for recalibrations at the time of the experiment with similar experimental conditions or oxygen concentrations. Phosphorescence lifetime measurements are not affected by other chromophors or fluorescence.[1]

OLIS method for Oxygen Measurement

Olis is proud to present its new technique for oxygen measurement based on oxygen-dependent quenching of phosphorescence. A schema of the OLIS method for oxygen measurement is shown as follows:



[1] I. Dunphy, S.A. Vinogradov, D.F. Wilson, Oxyphor R2 and G2: phosphors for measuring oxygen by oxygen-dependent quenching of phosphorescence, Anal. Biochem. 310 (2002) 191-198



It is well known that phosphorescence is more sensitive to oxygen measurement because it has a longer lifetime and therefore a higher quenching probability than fluorescence. The longer lifetime allows us to more easily use the pulse method to measure oxygen accurately. Supported by OLIS high resolution measurement technique and new generation phosphorescence dyes being used, the OLIS method for oxygen measurement has been forged into a powerful oxygen measurement strategy that yields both high effectiveness and efficiency with less calibration required.

I. Phosphorescence Dyes

Oxyphor 12: A second generation glutamate dendrimer of Pd- meso-tetra-(4-carboxyphenyl) tetrabenzoporphyrin.[2]

II. OLIS Instrumentation

With an all-inclusive instrument design (LED light source and controller, photon counter, ensemble of 100 digital counters, non-iterative fitting for exponential to exact lifetime of phosphorescent, and support

[2] Oxyphor 12 is a 2nd generation polyglutamic Pd-porphyrin-dendrimers, bearing 16 carboxylate groups on the outer layer. It is highly soluble in water and biological fluids soluble at pH near neutral such as blood plasma and its ability to penetrate biological membranes is very low. The maxima in the absorption spectra are at 440 and 622 nm, while an emission is near 800 nm. The calibration constants of the phosphors are essentially independent of pH in the physiological range (6.4 to 7.8). Therefore, the phosphorescence lifetimes of phosphors primarily are a function of the oxygen concentration [O2] and the temperature (Oxygen Enterprises, Ltd., http://www.oxygenent.net/phosphors.html).

software – all in one box. Just add the dye!), the OLIS oxygen measurement design incorporates two major instrumental improvements:

A LED based excitation light source excites the O_2 dye and provides a high-efficiency and very compact excitation flash of the appropriate wavelength. Its advantages include faster on-off switching, lower energy consumption, longer lifetime, improved robustness, smaller size, and low cost.

Olis photon counters are used to count photons directly to achieve high sensitivity and speed of the detection of exponential phosphorescence decays due to quenching by O_2.

III. OLIS Methodology: The Most Direct and Reliable Approach

The OLIS method for oxygen measurement measures the oxygen concentration directly by determining the phosphorescence lifetime instead of its intensity, thus increasing the accuracy greatly. This is a remarkable improvement over comparable conventional products on the market, since the OLIS method has less interference with fluorescence in the measurement of intensity and thus is more correct and reliable, more direct and effective in practical applications.

- Excitation light of the phosphor using appropriate wavelengths generated by OLIS LED is used to excite Oxyphor 12 in the oxygen-containing sample in a cuvette;
- Light incident on the PMT is converted into electronic pulses; the pulses are directed to a collection of 100 high capacity digital counters which are gated in sequence over the time of the analysis, so that the exponentially decreasing phosphorescent light is precisely represented by the resulting ensemble of counters. The 100 counters are read, producing an accurate exponential directly related to the behavior of the phosphorescence dye;
- Exponential data are then fitted by the phase-plane method, a fast, accurate, non-iterative regression algorithm, to extract lifetime (τ) (the fit to the exponential occurs in less than 0.1 millisecond CPU time!);
- Measured phosphorescence lifetime is converted to oxygen concentration ($[O_2]$) using the Stern-Volmer relation with calibration constants.

IV. Mathematical Fitting of the Lifetime τ

The phase-plane method (PP method), which is a non-iterative regression algorithm, is used to fit the lifetime characterizing the process of oxygen-dependent quenching of phosphorescence. As a non-iterative algorithm, the phase-plane method is fast, accurate, and insensitive to the nature of the noise, and thus has clear advantage over most iterative, non-linear regression algorithms commonly used in biological kinetic problems, e.g., the Marquardt-Levenberg algorithm (LMA) and χ^2 method. With its overall accuracy and forgivingness to measurement parameters, the phase-plane method is ideal for automated instruments or in measurements where making replicate determinations to optimize the fitting parameters.

V. Calibration of Constants τ_0 and k_q

Recalibration of constants τ0 and kq is needed in case of very different experimental conditions are used. The calibration can be performed either with a linear two-point calibration or with a second-order polynomial calibration.

The former requires at least two standards of known oxygen concentrations, of which one is at zero oxygen concentration and the other one is at a concentration in the high end of the concentration range. The latter requires at least three standards of known oxygen concentrations, of which two are the same as those in the linear case and the third one is between two other points. The second-order polynomial calibration provides a better curve fit and therefore more accurate data during oxygen measurements, especially when working in a broad oxygen concentration range. The linear two-point calibration is fast, direct and reliable enough for most of experimental conditions, and is implemented currently in OLIS oxygen measurement program.

VI. Signal-to-Noise Ratio (SNR)

The OLIS Oxygen system provides the possibility of very fast and reliable oxygen concentration measurements. Each measurement generally occurs in $200\mu s$, which means the excitation flash and subsequent collection of pulses due to the phosphorescence can be repeated up to 5 times per millisecond. Thus the signal-to-noise ratio (SNR) of measurement can be improved by repetition and summing the photon counts from many flashes. As discussed in Sec. III, since the conversion of incident light to pulses is very linear with low noise and the digital photon counters are essentially perfect summers of replicate bursts of pulses, the system SNR follows

$$SNR \sim \sqrt{N} \tag{1}$$

where N is the number of repetition of measurements, and $200\mu s \times N$ is the total time spent in collecting one datum.

In OLIS *SNR* test, as $\sqrt{N}$ increases with a ratio of $\sqrt{100}: \sqrt{1000}: \sqrt{4000}: \sqrt{8000}$, i.e., with a ratio of $1: 3.2: 6.3: 8.9$ (data collection time increases with $20\text{ms}: 200\text{ms}: 800\text{ms}: 1600\text{ms}$), the resulted *SNRs* increases with a ratio of $1: 3.2: 6.5: 9.2$, which agrees with the above Equation (1). This agreement affirms that the methodology of OLIS Oxygen system is very solid. Figure 1 shows one of those test results obtained at a room temperature of $25°C$ and an atmospheric pressure of $763mmHg$. Data are taken from ***OLIS Oxygen Measurement-O2Dye*** with $50nMol$ Oxyphor 12 dye in air-saturated fresh water.

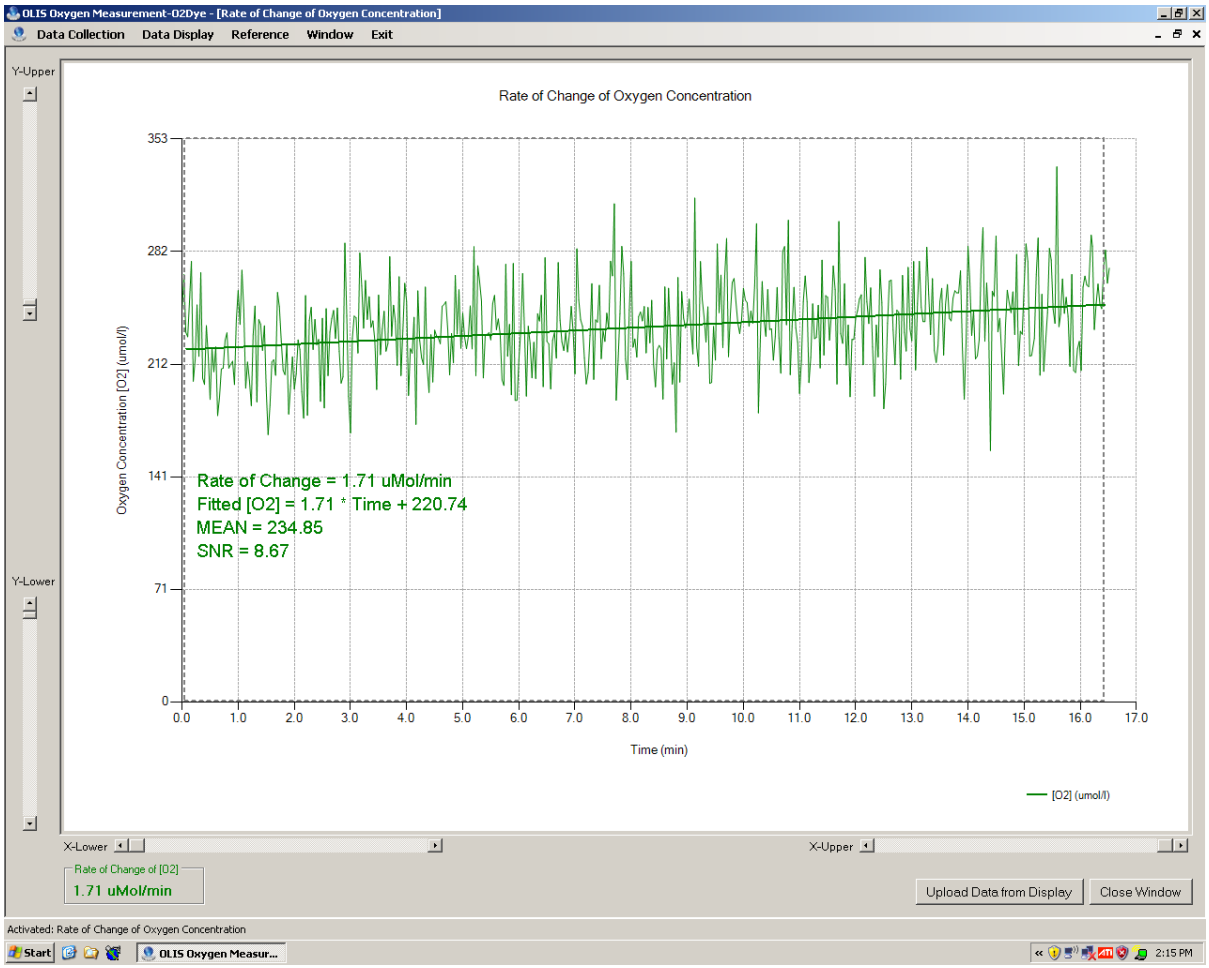


Figure 1:$SNR = 8.7\ with\ 20ms/Datum$

Appendix I: Background Theory

Quenching of phosphorescence by oxygen is determined by the frequency of collisions between excited triplet state molecules and oxygen. The phosphor, placed in the media of interest, is excited by absorbing a photon of excitation light from a light-emitting diode (LED), and returns to the ground state with emission of light (phosphorescence) or by a non-radiative transfer of energy to other quenching molecules (quenchers) in the environment. This effect (first described by Kautsky in 1939) is called “dynamic quenching”. The rate of decay of the phosphorescence (inverse of lifetime) relates to the frequency of collisions, and therefore to the concentration of quenching molecules, i.e., [O_2].

In solutions with oxygen as the primary quencher (as it is in most biological samples), the phosphorescence quenching by oxygen follows the *Stern-Volmer* relation,

$$\frac{I_0}{I} = \frac{\tau_0}{\tau} = 1 + k_q \tau_0 [O_2] \ ,$$

where I_0 and I are the phosphorescence intensity at zero oxygen and at an oxygen concentration [O_2] (in μM) respectively, τ_0 and τ are the phosphorescence lifetimes (in *s*) at zero oxygen and at an oxygen concentration [O_2] respectively, k_q, as a function of the diffusion constants for phosphor and oxygen, temperature, and phosphor environment, is the second-order Stern-Volmer constant (in $\mu M^{-1} \cdot s^{-1}$) related to the frequency of collisions of the excited-state phosphor with molecular oxygen, and [O_2] is the oxygen concentration in solution, which is proportional to the partial pressure of oxygen in the gas phase. Therefore, an alternative form of the Stern-Volmer equation is written as

$$\frac{I_0}{I} = \frac{\tau_0}{\tau} = 1 + k'_q \tau_0 pO_2 \ ,$$

where $k'_q = \alpha k_q$, where α is the oxygen solubility coefficient (in µM/mmHg), *pO2* is the partial oxygen pressure, which is proportional to the concentration of oxygen in solution.

Calibration involves determining the dependencies of τ_0 and k_q on such variables as temperature and pH under readily reproducible experimental conditions (e.g., ionic strength).