# A Tool for Measuring Oxygen via Phosphorescence

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## ABSTRACT

Accurate measurements of dissolved $O_2$ as a function of time have numerous biological and chemical applications. Detection of $O_2$ using a Pd phosphor complex is based on the principle that the phosphorescence of this probe is inversely related to dissolved $[O_2]$. Biologic samples are flashed with red light using special instrumentation that captures emitted light and a customized software program that determines $[O_2]$ as function of time. $O_2$ concentration is calculated by fitting the decay of the phosphorescence to an exponential curve. Twelve tasks were identified, which allow full control and customization of the data acquisition, storage and analysis. The program used Microsoft Visual Basic 6 (VB6), Microsoft Access Database 2007, and a Universal Library component that allowed direct reading from the PCI-DAS 4020/12 I/O Board. A relational database was designed to store experiments, pulses and pulse metadata, including phosphorescence decay rates. This combination of experimental setup and software program can be used on wide range of experiments. The tool has now been used to conduct over 2,000 experiments during the last two years, as it permits reliable measurements of cellular $O_2$ consumption over several hours.

**Keywords:** Data acquisition; peak detection; slope calculation; phosphorescence; oxygen.

## 1. INTRODUCTION

Measurements of $O_2$ using the phosphorescence of Pd (II)-mesotetra-(4-sulfonatophenyl)-tetrabenzoporphyrin (Pd phosphor) was introduced by Wilson and colleagues [1]. An instrument (Figure 1) that measures the concentration of dissolved $O_2$ in sealed vials over several hours was constructed. The primary application was to determine rates of cellular and tissue mitochondrial $O_2$ consumption over several hours [2]. The detection method was based on the principle that $O_2$ quenches the phosphorescence of Pd phosphor; the observed phosphorescence was inversely related to dissolved $O_2$.

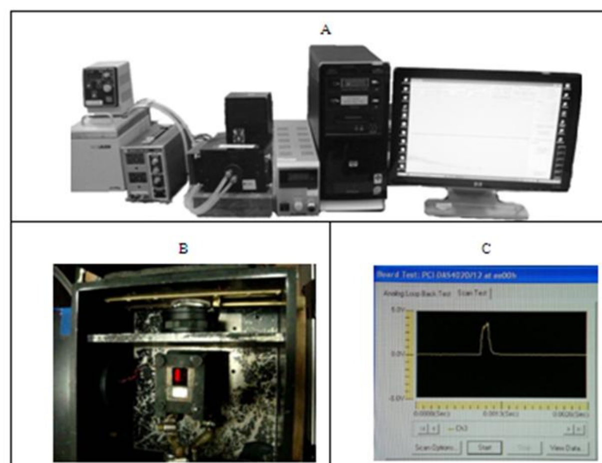


**Figure 1.** The Oxygen Analyzer. The components (panel A, left to right) include circulating water bath, power supply for the mixer, sample chamber (panel B) attached to the photomultiplier tube (PMT), high voltage power supply for the PMT, computer with PCI-DAS board, monitor with developed software running. The PMT is connected to the PCI-DAS board on the back of the computer. The board monitoring software is shown in panel C. Samples were exposed to red light flashes (10/s) from a pulsed light-emitting diode array with peak output at 625 nm (OTL630A-5-10-66-E, Opto Technology, Inc., Wheeling, IL).

## 2. BACKGROUND

Samples were flashed (10/s) from a pulsed light-emitting diode array with a peak output at 625 nm (OTL630A-5-10-66-E, Opto Technology, Inc., Wheeling, IL). Emitted light was detected by a Hamamatsu photomultiplier tube after passing through a wide-band interference filter centered at 800 nm. Amplified phosphorescence was digitized at 1-2 MHz using an analog/digital converter (PCI-DAS 4020/12 I/O Board) with outputs ranging from 1 to 20 MHz. Pulses were captured using a developed software program (described below) at 0.1 to 4.0 MHz, depending on the speed of the computer.

Representative phosphorescence pulses with exponential fits are shown (Figure 2). The reaction mixtures contained phosphate-buffered saline supplemented with 3 μM Pd phosphor, 0.5% fat-free bovine serum albumin, 50 μg/mL glucose oxidase and 0, 125 or 500 μM β-glucose. Glucose oxidase catalyzes the reaction: β-glucose + $O_2$ → glucono-δ-lactone + $H_2O_2$. Without β-glucose (air-saturated solution), the exponential fit gave a phosphorescence decay rate ($1/\tau$) of 0.019089 intensity (arbitrary units) per μs, or a lifetime ($\tau$) of 52 μs. In the presence of 125 μM β-glucose (about 55% $O_2$ depletion), the exponential fit gave a value for $1/\tau$ of 0.0056447 intensity per μs, or a lifetime ($\tau$) of 177 μs. In the presence of 500 μM β-glucose ($O_2$-depleted solution), the exponential fit gave a value

for $1/\tau_o$ of 0.0028382 intensity per μs, or a lifetime ($\tau_o$) of 352 μs.

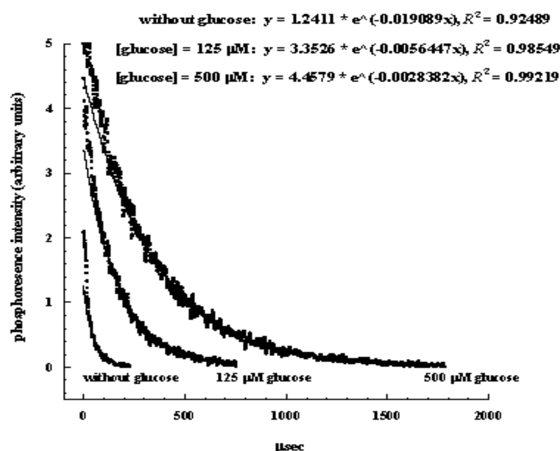


**Figure 2.** Representative phosphorescence pulses with exponential fits for reactions containing phosphate-buffered saline with 3 μM Pd phosphor, 0.5% fat-free bovine serum albumin, 50 μg/mL glucose oxidase and 0, 125 or 500 μM β-glucose.

$O_2$ concentration was calculated by fitting to an exponential the decay of the phosphorescence. The decay rates ($1/\tau$) were linear with $[O_2]$, as follows: $1/\tau = 1/\tau_o + k_q [O_2]$; $1/\tau$, the decay rate ($s^{-1}$) in the presence of $O_2$; $1/\tau_o$, the decay rate ($s^{-1}$) in the absence of $O_2$; $k_q$, the second-order $O_2$ quenching constant ($s^{-1}$ $\mu M^{-1}$), set as the slope of the plot $1/\tau$ *vs.* [β-glucose], or 97.8 $s^{-1}$ $\mu M^{-1}$.

## 3. METHODS

A program was developed using Microsoft Visual Basic 6 (VB6), Microsoft Access Database 2007, and a Universal Library component. It allowed a direct reading from the PCI card [4-5]. Needs analysis revealed developing a customized program was necessary. The following tasks were identified:

1. Experiment identification (title, date, time, sample rate, etc.).
2. Reading directly from the PCI card at the fastest possible rate.
3. Distinguish pulse data from non-pulse data.
4. Allow fuzzy detection of the pulse peak.
5. Calculate the exponential decay rate ($1/\tau$) and lifetime ($\tau$) of each pulse.
6. Store each pulse data points, along with the peak, decay and lifetime values.
7. View a representative pulse every 10 s.
8. View decay rates ($1/\tau$) in a second graph.
9. Ability to pause and place a marker with a note.
10. Ability to remove erroneous (incomplete) pulses and adjust peak values if necessary.
11. Ability to copy pulse or slope data to clipboard for further analysis.
12. Ability to access a previous experiment, review a pulse with its metadata, markers and associated notes.

## 4. RESULTS

The developed software included relational database design to store experiments, pulses and pulse metadata, including slopes (a table was used for each item). About 1,000 lines of VB6 code were developed to accomplish the required needs. A snapshot of the software's user-interface is shown in Figure 3.

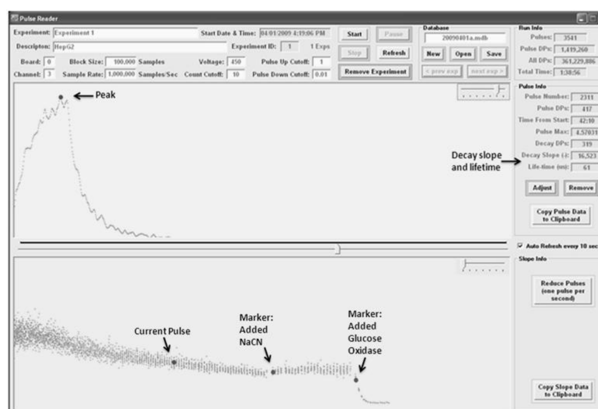


**Figure 3.** A snapshot of the software's user-interface.

Pulse detection was accomplished by finding a specific number of data points (10 phosphorescence intensities by default) above a specific cutoff value (1.0 volt by default). Peak detection was accomplished by finding the highest 10% data points of a pulse, and choosing the one in the group that is closest to the pulse's decay curve. Depending on the sample rate, a minimum number of data points per pulse was set and used as a cutoff to remove invalid pulses with too few data points.

Examination of the slope graph (bottom graph of Figure 4) shows clearly visible gaps in slope points. This is due to the fact the CPU is busy processing and storing the pulse data and slope points and unable to read from the card during that time. When the experiment lasts for several hours, this gap increases because of the increasing size of the database. However, even with experiments lasting several hours, the gaps are small enough that they do not affect the results.

The development of customized software for data management and analysis has been shown to be a top need of in biomedical research [6]. The consensus is that institutions and Informaticians should focus on developing and supporting information management service cores that assist biomedical research with unique data management and analysis needs. Our software is an example of such an attempt.

The main advantages of the developed software program over commercially available packages (e.g., DASYLab™ or TracerDAQ™) are provision of full control and customization of data acquisition, storage and analysis. The choices of VB6 and Access Database as programming and storage environments are due to their availability, simplicity, widespread use (which makes finding software developers less of a problem) and VB6 components that read directly from the PCI card.

## 5. CONCLUSION

This combination of experimental setup and software program can be used on wide range of experiments. Using the development system, it was possible to achieve accurate

measurements of dissolved $O_2$ in solution as a function of time. The tool has numerous biological and chemical applications, including studying mitochondrial dysfunction during apoptosis or other biologic processes. It allows screening for metabolic disorders associated with impaired cellular respiration (e.g., using isolated mononuclear cells from whole blood or fetal umbilical cord). The procedure is sensitive and reproducible. It is applicable to cells in suspension, adherent cells and various tissues (e.g., heart muscle, liver, spleen, pancreas and kidney). The tool has been used to conduct over 2,000 experiments in the past two years as it permits reliable measurements of cellular $O_2$ consumption over several hours.

## 6. REFERENCES


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