Optimal discrimination threshold for the detection of singlet oxygen luminescence

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Abstract. Direct detection of singlet oxygen ($^{1}$O$_{2}$) luminescence around 1270 nm is the golden standard of $^{1}$O$_{2}$ identification. In this study, the effect of the discrimination threshold on the detection of $^{1}$O$_{2}$ luminescence that generated from the photoirradiation of Rose Bengal (RB) was evaluated by using a self-developed photon-counting detection system. The obtained results show that the discrimination threshold for photon counting has a significant impact on the intensity and shape of the measured $^{1}$O$_{2}$ luminescence, which resulted in the variation of $^{1}$O$_{2}$ lifetimes. The optimal discrimination threshold is determined to be about -0.0412 V, and the corresponding $^{1}$O$_{2}$ lifetime in air-saturated distilled water is 4.26±0.06 μs.

1. Introduction

Photodynamic therapy (PDT) is a rapidly developing treatment modality that has been successfully used in the treatment of oncologic and non-oncological diseases [1,2]. Robust individualized dosimetry for PDT is vital for achieving a precise and effective PDT treatment [3,4]. Singlet oxygen ($^{1}$O$_{2}$) is widely known to be the major mediator for the destruction of target tissue during PDT [2-4]. Therefore, the quantitative measurement of $^{1}$O$_{2}$ during PDT treatment is clinically meaningful for PDT dosimetry. Most recently, with the advances in photon counting techniques and high sensitive photomultiplier tube (PMT) for near-infrared luminescence detection, the direct detection of $^{1}$O$_{2}$ near-infrared luminescence around 1270 nm has attracted growing attention for PDT dosimetry [5,6]. The advantage of this photophysical technique for PDT dosimetry is that it can circumvent the complicated interactions between the photosensitizer, light and molecular oxygen during PDT treatment.

The most challenging work for $^{1}$O$_{2}$ luminescence detection is to achieve a satisfied signal to noise ratio (SNR) and to obtain the convincing data. In particular, the measured $^{1}$O$_{2}$ lifetimes in aqueous environments have been previously reported in the range from 3.0 to 4.4 μs [7,8], and there seems still to be so much uncertainty among these values. As we know, the measurement of $^{1}$O$_{2}$ luminescence was mainly affected by the excitation light source, the optical collected unit, the photon counting technique, and the data processing approach. In this study, the effect of the discrimination threshold for photon counter on the detection of $^{1}$O$_{2}$ luminescence that generated from the photoirradiation of Rose Bengal (RB) was evaluated by using our self-developed photon-counting detection system [9].

2. Materials and methods
2.1. Theoretical model

Based on the well-establish photophysical model for determination of the \( ^1O_2 \) rise and decay times from its luminescence, a simplified four-parameter photophysical model was proposed for fitting the measured \( ^1O_2 \) luminescence data [9]:

\[
I(t) = A \left( \frac{\tau_D}{\tau_T - \tau_D} \right) \left( \exp\left(\frac{-t}{\tau_T}\right) - \exp\left(\frac{-t}{\tau_D}\right) \right) + B \tag{1}
\]

where \( A \) and \( B \) are the intensities of \( ^1O_2 \) luminescence and background signal of the detection system, respectively. \( \tau_T \) and \( \tau_D \) are the lifetimes of the triplet-state photosensitizer and \( ^1O_2 \).

2.2. Chemicals

Rose Bengal (RB) (Sigma-Aldrich., St. Louis, USA) was selected as a model photosensitizer for \( ^1O_2 \) generation. A 100 \( \mu \)M stock solution of RB was made up in distilled water and stored at 4 °C in the dark. The fresh air-saturated samples were prepared from this stock solution prior to the measurements. Preparations were done in near dark conditions.

2.3. Experiment setup

The detection system used to measure time-resolved \( ^1O_2 \) luminescence is illustrated schematically in Figure 1. The present system has been described in detail elsewhere [9], with the exception that the 6-position motorized filter wheel has been replaced by a narrow-band filters centered at 1270 nm (OD3 blocking, 20-nm full-width at half-maximum (FWHM); Omega Optical, Brattleboro, USA) and the optical collection geometry has been re-optimized, which has a much larger numerical aperture for luminescence collection.

![Figure 1. Schematic of the \( ^1O_2 \) luminescence detection system](image)

Briefly, a diode-pumped, Q-switched, frequency-doubled Nd:YLF laser (QG-523-500; Crystalaser Inc., Reno, NV, USA) with an emission wavelength of 523 nm was used for excitation. The pulse repetition rate is set to 12 kHz while average output power of pulse was adjusted to 20 mW by using a neutral-density attenuator. The 2.5 mL samples were held in a standard 10 mm pathlength quartz cuvette mounted on a hotplate-stirrer unit. This allowed the samples to be continuously stirred with a magnetic stir bar and maintained the source-sample-detector geometry constant between experiments. The cuvette was open so that the solutions were exposed to room air at the top. The exciting luminescence was collected through a 1000 nm long-pass filter (Omega Optical, Brattleboro, USA), the optical collected unit and a narrow-band filter that placed in front of the PMT (H10330-45, Hamamatsu Corp., Japan). The working voltage of the PMT was set to -900 V, which is provided by a high voltage module controller. The luminescence counts were recorded by a fast photon counter.
((MSA-300, Becker & Hickl GmbH, Berlin, Germany). In order to achieve a sufficient SNR, the $^1$O$_2$ luminescence counts were summed over 285000 laser pulses for the following measurements.

2.4. Discrimination threshold for $^1$O$_2$ measurements
The MSA-300 integrated a discriminator, which has an adjustable pulse-height discrimination threshold from -0.5000 to 0.5000 V. In our detection system, the pulses from the pre-amplifier were negative pulses. The discriminator compares the input pulses with the preset discrimination threshold to divide them into two groups: one group is lower and the other is higher than the preset discrimination threshold. The higher pulses are shaped to have a constant level to be counted, while the lower pulses will be eliminated during counting [10].

2.5. Data processing
All the measured data were processed and graphed using OriginPro8.0 software (OriginLab, Northampton, MA, USA). Data are means plus-minus the standard deviation (SD) of three independent experiments each performed in duplicate.

3. Results and discussion

3.1. Time-resolved $^1$O$_2$ luminescence spectra
The representative time-resolved $^1$O$_2$ luminescence spectra of RB were shown in Figure 2, which were measured with the discrimination threshold ($V_{th}$) of -0.1800 and -0.2000 V, respectively. As indicated in Figure 2, the discrimination threshold has significant impact on both the intensity and the shape of $^1$O$_2$ luminescence.

Figure 2. Time-resolved $^1$O$_2$ luminescence spectra of 6 μM RB.

When the discrimination threshold was set to -0.2510 V, the intensity of the $^1$O$_2$ luminescence was extremely low and this value just allowing counter to count the $^1$O$_2$ luminescence. In the following measurements, the time-resolved $^1$O$_2$ luminescence spectra of 6 μM RB were measured, in which the discrimination threshold was continually increased from -0.2510 to -0.0059 V in ca. 0.010 V step. The $^1$O$_2$ lifetimes can be derived from the fitting curves by using the equation (1), as shown in Figure 3. There has a turning point for the $^1$O$_2$ lifetime with the discrimination threshold value of -0.1800 V, as marked by the red arrow. It can be clearly seen that $^1$O$_2$ lifetimes have great variability when the discrimination threshold is lower than -0.1800 V, while the $^1$O$_2$ lifetimes are most same once the discrimination threshold is higher than -0.1800 V.
3.2. Optimization of the discrimination threshold

In order to determine the optimal discrimination threshold, the time-resolved \textsuperscript{1}O\textsubscript{2} luminescence spectra of 6 μM RB were measured with the discrimination thresholds that was initially set from -0.1800 V and then continually increased from -0.1800 V to -0.0059 V in ca. 0.01 V step. According to the time-resolved characteristics of the \textsuperscript{1}O\textsubscript{2} luminescence spectra in Figure 2, the background counts can be clearly found in the early time points (ca. 0~1 μs) and beyond ca. 20 μs. Therefore, we can define the integration signal counts from 1 to 20 μs in the time-resolved spectra with and without irradiation as “signal and background” and “background”, respectively. Figure 4 shows the integration counts of the “signal and background” and “background” with different discrimination thresholds for each measurement. The optimal discrimination threshold can be determined to be about -0.0412 V from the gentle slope portion, which is indicated by red arrow in Figure 4. In this case, the unwanted background counts can be maximally suppressed [10].
3.3. Determination of the $'\text{O}_2$ lifetime with an optimal discrimination threshold
When the optimal discrimination threshold was set to -0.0412 V, the time-resolved $'\text{O}_2$ luminescence spectra of RB were measured with different concentrations. After each measurement, the time-resolved $'\text{O}_2$ luminescence spectrum was mathematically fitted by using the equation (1) to yield the $'\text{O}_2$ lifetime. As shown in Figure 5, there is no significant difference among the obtained $'\text{O}_2$ lifetimes, as expected. The $'\text{O}_2$ lifetimes were determined to be about 4.26±0.06 μs, which is in good agreement with the previously published values [8]. However, further comparative experiments for different photosensitizers or solvent environments should be performed.

![Figure 5](image.png)

Figure 5. The $'\text{O}_2$ lifetimes against the RB concentrations from 1 to 6 μM for $'\text{O}_2$ luminescence measurements.

4. Conclusions
The effect of the discrimination threshold on the detection of $'\text{O}_2$ luminescence that generated from the photoirradiation of photosensitizer RB was investigated by using our self-developed photon-counting detection system. Our preliminary results show that the discrimination threshold for photon counting impacts both the intensity and the shape of $'\text{O}_2$ luminescence, which resulted in the variation of $'\text{O}_2$ lifetimes. In order to develop the $'\text{O}_2$ luminescence dosimetry for PDT treatment, the discrimination threshold is a crucial parameter for the $'\text{O}_2$ luminescence detection system that needs to be optimized.

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Erratum

Figure 1 was incorrect in the original PDF, the correct figure 1 is given below.

![Diagram of ¹O₂ luminescence detection system]

Figure 1. Schematic of the ¹O₂ luminescence detection system