Detection system for singlet oxygen luminescence in photodynamic therapy

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Singlet oxygen ($^1O_2$) is widely considered to play a major role in photodynamic therapy (PDT), and thus an increasing attention has been focused on the direct detection of $^1O_2$ near-infrared luminescence around 1270 nm for PDT dosimetry. A new sensitive detection system is developed to directly measure the temporal and spectral resolved $^1O_2$ luminescence spectra. The triplet state and $^1O_2$ lifetimes of Rose Bengal as a model photosensitizer in different solvents are determined, and the obtained results agree well with the published data. Our detection system has the potential application in $^1O_2$ luminescence-based PDT dosimetry.

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Photodynamic therapy (PDT) is a promising therapeutic modality for both oncological and non-oncological diseases[1−3]. Most current photosensitizers used in PDT are widely believed to elicit their damage primarily through singlet oxygen ($^1O_2$)-mediated pathways. Therefore, the $^1O_2$ measurement during PDT treatment has been of great interests over the past two decades[4−6].

$^1O_2$ can be indirectly measured via absorption, fluorescence or chemluminescence by adding $^1O_2$ quenchers, such as 9,10-diphenylanthracene, $^1O_2$ Sensor Green, or fluoresceinyl cypridina luciferin analogue[7−9]. However, the observed quencher consumption may not exactly reflect the amount of generated $^1O_2$ because the localization of the quencher may differ from the $^1O_2$ generation site during PDT treatment[10]. In addition, these indirect measurements will unavoidably consume the desired cytotoxic $^1O_2$ for PDT. To date, direct measurement of near-infrared (NIR) $^1O_2$ luminescence at 1270 nm has been achieved with a high sensitive NIR photomultiplier tube (PMT) as a PDT dose metric for both *in vitro* and *in vivo* outcome evaluation[5,10,11]. As a result, considerable attention has recently been paid to the direct detection of $^1O_2$ luminescence for PDT dosimetry. 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 the treatment[12]. In this letter, a new sensitive detection system is developed to directly measure the temporal and spectral resolved $^1O_2$ luminescence.

Figure 1 shows the simplified energy-level diagram for type II PDT. Upon the absorption of a photon of appropriate wavelength by the ground state (S_0) photosensitizer, the electronic excited state (S_1) is generated. It can be de-excited by intersystem crossing to a triple state (T_1), and the T_1 state can exchange energy with the ground state molecular oxygen ($^3O_2$) to generate $^1O_2$.

The detection system developed to directly measure $^1O_2$ luminescence is shown schematically in Fig. 2. A diode-pumped, Q-switched, frequency-doubled 523-nm Nd:YLF laser (QG-523-500, Crystalaser Inc., Reno, USA) was used as the excitation light source. The solutions were placed in a standard 10-mm-pathlength quartz cuvette (Yixing Jingke Optical Instrument Co. Ltd., Yixing, China) mounted on a hotplate-stirrer unit (RH digital KT/C Package, IKA GmbH, Konigswinter, Germany). This allowed the samples to be continuously stirred with a magnetic stir bar and maintained the source-sample-detector geometry constant during measurements. The cuvette was open so that the solutions were exposed to room air at the top. The sample was excited by the laser and the $^1O_2$ luminescence signal was detected by a PMT (H10330-45, Hamamatsu Corp., Hamamatsu, Japan) in the photon counting mode. The PMT pulses were recorded by a fast multiscaler (MSA-300, Becker & Hickl GmbH, Berlin, Germany), which is triggered from the reflection laser by a photodiode (PDM-400, Becker & Hickl GmbH, Berlin, Germany). A 1000-nm long-pass filter (Omega Optical, Brattleboro, USA) was used to block out the unwanted scattering excitation light and fluorescence from the sample. The

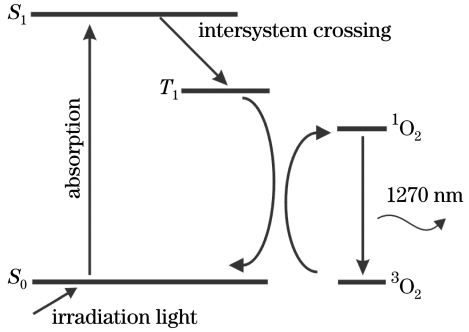

Fig. 1. Simplified Jablonski energy-level diagram for type II photosensitization.

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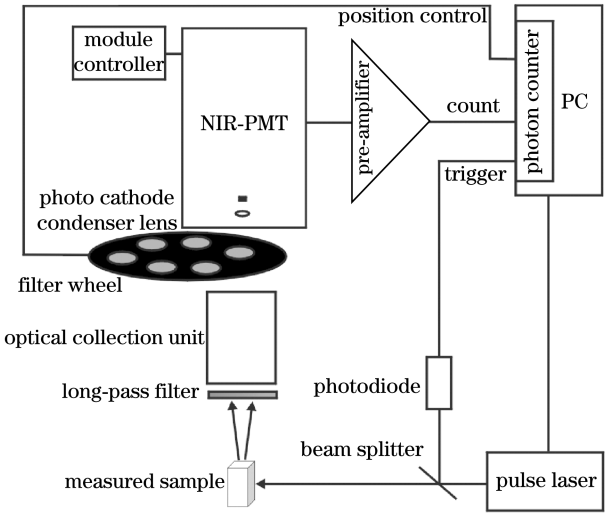

Fig. 2. Schematic of the $^1O_2$ luminescence detection system.

collected NIR luminescence reached the PMT photocathode through a custom designed optical collection unit. The operating voltage of the PMT was set to −900 V. The PMT output was amplified and converted to a voltage pulse by using a high-speed wide-band pre-amplifier (HFAC-26, Becker & Hickl GmbH, Berlin, Germany). Spectral discrimination of the detected luminescence was achieved using a set of five narrow-band filters centered at 1190, 1230, 1270, 1310, and 1350 nm (OD3 blocking, 20-nm full-width at half-maximum (FWHM); Omega Optical, Brattleboro, USA) mounted on a 6-position motorized filter wheel (FW102B, Thorlabs Inc., Newton, USA) in front of the PMT. These filters allowed sampling of the NIR $^1O_2$ luminescence spectrum. The control panel software of the detection system was designed by LabVIEW 8.5 (National Instruments Corp., Austin, USA) and operated on a personal computer (PC).

In the following studies, Rose Bengal (RB) (Sigma-Aldrich., St. Louis, USA) was used as a model photosensitizer in the solution-based measurements. In order to minimize the self-absorption effect, all samples having an absorbance between 0.062 and 0.248 (corresponding to concentrations from 2 to 8 μmol/L) at 523 nm were prepared for $^1O_2$ luminescence measurements. Figure 3 shows the time-resolved NIR luminescence spectra of the five wavelengths for 8-μmol/L RB in distilled water, and the $^1O_2$ luminescence at 1270 nm can be clearly demonstrated. In order to remove the fast fluorescence and background of the detection system, the corresponding time-integrated spectra were generated by summing the delayed luminescence for the five individual wavelengths from 1 to 25 μs, respectively. As expected, a strong spectral peak was observed at 1270 nm, as shown in Fig. 4. Furthermore, the $^1O_2$ luminescence intensity at 1270 nm was linearly dependent on RB concentration, as described in the inset of Fig. 4.

Further confirmation that the detected signal at 1270 nm is due to the $^1O_2$ luminescence has been achieved by adding sodium azide (NaN_3) (Sigma-Aldrich., St. Louis, USA), a well-known $^1O_2$ quencher, resulting in the elimination of $^1O_2$ luminescence. To investigate this, additional NaN_3 was rapidly added to the solution containing 4-μmol/L RB after $^1O_2$ luminescence

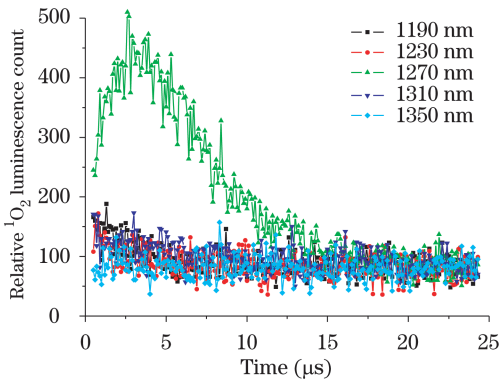

Fig. 3. Time-resolved luminescence spectra of five individual wavelengths for RB in water.

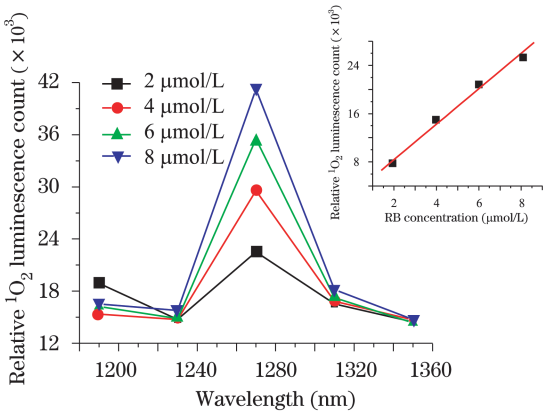

Fig. 4. Spectrum-resolved $^1O_2$ luminescence spectra of RB in water.

measurement, and then the second spectroscopy was continuously recorded for comparison. As illustrated in Fig. 5, the $^1O_2$ luminescence at 1270 nm significantly decreases after adding various amount of NaN_3. The observed depletion is attributed to the reaction of NaN_3 with photosensitized $^1O_2$.

Figure 6 indicates the normalized time-resolved $^1O_2$ luminescence spectra for 8-μmol/L RB in different solvents, including acetone, dimethylformamide (DMF), methanol (MeOH), methylsulfinylmethane (DMSO) (Sigma-Aldrich., St. Louis, USA), and distilled water. Based on the well-established photophysical model for determination of the $^1O_2$ rise and decay time from its luminescence[13], the measured $^1O_2$ luminescence data

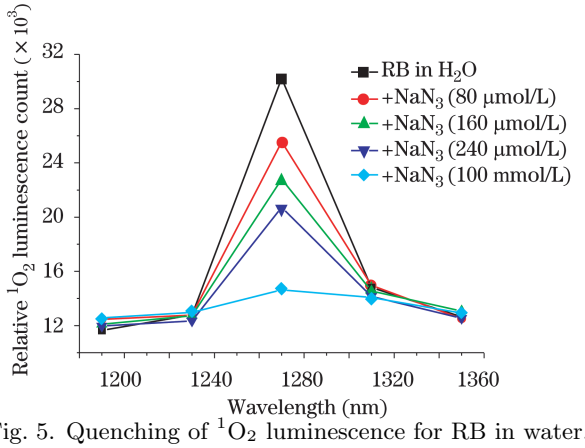

Fig. 5. Quenching of $^1O_2$ luminescence for RB in water.

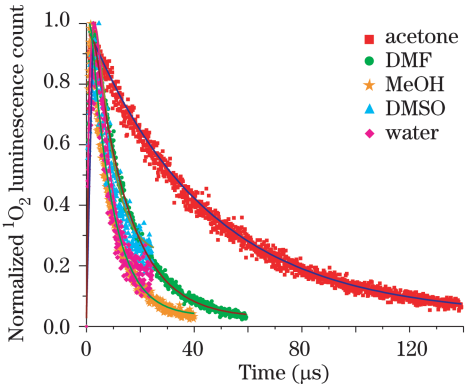

Fig. 6. Time-resolved $^1O_2$ luminescence spectra of RB in different solvents.

Table 1. Triplet State (τ_T) and $^1O_2$ (τ_D) Lifetimes of RB in Different Solvents (μs)

Solvent	Fitting Value		Published Value
	τ_T	τ_D	τ_D
Acetone	0.41±0.01	44.11±0.20	40.0±2.0
DMF	0.76±0.01	12.84±0.17	17.9±0.2
MeOH	0.40±0.01	8.15±0.09	9.2±0.1
DMSO	1.61±0.17	5.93±0.28	5.7±0.3
Water	2.78±0.34	3.06±0.26	3.0±0.6

after background subtraction can be fitted (OriginPro 7.5, OriginLab, Northampton, USA) by

$$I(t) = I_0\left(\frac{\tau_D}{\tau_T - \tau_D}\right)[\exp(-t/\tau_T) - \exp(-t/\tau_D)] + I_1, \quad (1)$$

where τ_T and τ_D are the lifetimes of the photosensitizer triplet-state and $^1O_2$, I_0 and I_1 are the intensities of $^1O_2$ luminescence and background signal from the detection system, respectively. The derived triplet state and $^1O_2$ lifetimes of RB in the investigated solvents are summarized in Table 1. The triplet state and $^1O_2$ lifetimes of RB in different solvents are in good agreement with the previously published values[13–15], demonstrating that our new system works well as expected. For instance, the triplet state and $^1O_2$ lifetimes of RB in water are 2.78±0.34 and 3.06±0.26 μs, respectively.

In conclusion, a new sensitive detection system is developed for the direct measurement of the temporal and spectral resolved $^1O_2$ luminescence spectra, and the satisfied signal-to-noise ratio (SNR) for $^1O_2$ luminescence measurement is achieved. $^1O_2$ luminescence at 1270 nm is confirmed by using $^1O_2$ quencher of NaN_3. Moreover, the triplet state and $^1O_2$ lifetimes of RB in different solvents are determined, and the $^1O_2$ lifetimes agree well with the published data. Compared with the previous PMT modules used for $^1O_2$ luminescence detection[5,11], the new H10330-45 PMT module in the present study consists of a compact NIR PMT contained in a thermally insulated, sealed-off housing evacuated to a high vacuum. The internal thermoelectric cooler eliminates the requirement for liquid nitrogen and cooling water, which is particularly beneficial for developing the miniaturized $^1O_2$ detection system in PDT clinical applications. As we have discussed previously[13], the $^1O_2$ lifetime may depend on the micro-environment, which would alter the measured $^1O_2$ luminescence signal. Therefore, the integrated $^1O_2$ luminescence counts should be normalized by the $^1O_2$ lifetime. Using this system, the temporal and spectral resolved $^1O_2$ luminescence spectra can be simultaneously detected, and the integrated $^1O_2$ luminescence counts can be directly obtained for each measurement. Furthermore, the $^1O_2$ luminescence around 1270 nm for measured samples can be corrected for background by subtracting the luminescence from other control filters. The evaluation of PDT-$^1O_2$ dosimetry for cell suspension and animal model is now in progress.

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