Pulsed Diode Laser-Based Monitor for Singlet Molecular Oxygen

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Abstract. Photodynamic therapy (PDT) is a promising cancer treatment. PDT uses the affinity of photosensitizers to be selectively retained in malignant tumors. When tumors, pretreated with the photosensitizer, are irradiated with visible light, a photochemical reaction occurs and tumor cells are destroyed. Oxygen molecules in the metastable singlet delta state O$_2$(^1$\Delta$) are believed to be the species that destroys cancerous cells during PDT. Monitoring singlet oxygen produced by PDT may lead to more precise and effective PDT treatments. Our approach uses a pulsed diode laser-based monitor with optical fibers and a fast data acquisition system to monitor singlet oxygen during PDT. We present results of in vitro singlet oxygen detection in solutions and in a rat prostate cancer cell line as well as PDT mechanism modeling.

Keywords: photodynamic therapy; singlet oxygen; light dosimetry

1. Introduction

Photodynamic therapy (PDT) is a rapidly developing and promising branch of oncology. In PDT, a photosensitizer, preferentially retained in tumors, is irradiated with visible to near infrared light. The photosensitizer (PS) molecules initiate a reaction that selectively kills the malignant cells to
which they are attached. FDA approval has been granted for the treatment of esophageal and certain lung cancers, and PDT is being used in clinical trials for bladder, brain, skin and other cancers.\textsuperscript{2-6} PDT is also being applied to other areas outside of cancer treatment including age related macular degeneration and actinic keratosis, a pre-cancerous skin condition. There is compelling evidence that O\textsubscript{2}(^{1}\Delta), also known as singlet molecular oxygen, is the active species in cancer cell destruction when tumors containing photosensitizers such as hematoporphyrin derivative (HPD) are irradiated with visible light.\textsuperscript{7} There are two proposed mechanisms for PDT-induced cancer cell destruction: a) direct damage to cell walls and mitochondria,\textsuperscript{8} and b) vascular constriction that starves the tumor of nutrients.\textsuperscript{9} Singlet oxygen is implicated in both mechanisms.

The fundamental, type II PDT, process is shown in Fig. 1. The PS singlet state radiates rapidly to the ground state and has characteristic emissions in the red to near infrared spectral region. This emission can be used to locate tumor tissue and there are several groups investigating this effect as a diagnostic tool.\textsuperscript{10-12} A rapid intramolecular transfer process populates a triplet state in the PS indicated by T\textsubscript{1} in Fig. 1. Collisions between the metastable PS molecules and ground state oxygen (present in the tumor) populate the singlet delta state of oxygen via an energy transfer process.

The PDT treatment involves three parameters: PS concentration, oxygen concentration, and light dosage. The singlet oxygen production depends on all the parameters affecting PDT and is a direct measure of available molecular oxygen multiplied by the PS concentration i.e. [O\textsubscript{2}(^{1}\Delta)] \propto [PS] \times [O\textsubscript{2}]. The ability to correlate the amount of singlet oxygen produced to treatment outcome could lead to more precise and effective treatment as well as a better understanding of the PDT mechanism. It would be beneficial to be able to monitor singlet oxygen in real-time during PDT treatment. Diode lasers are ideal for producing low peak power laser pulses of \textasciitilde 1-10 µs and provide
low power excitation pulses consistent with typical PDT treatment lasers. In this paper, we report the results of our initial investigation to develop a sensitive, diode laser-based monitor for singlet oxygen produced by PDT light treatment. Using a pulsed diode laser and photon counting methods, we demonstrated that we could measure the production of singlet oxygen in solutions including: water, methanol, acetone, and protein rich aqueous solutions. We also report the results of singlet oxygen detection in a rat prostate tumor cell suspension.

2. METHOD

The weak optical emission from singlet oxygen presents a significant challenge for developing an optically-based monitor. While optical filtering provides some measure of sensitivity, temporal discrimination provides a significant enhancement. For example, if one can observe the emission during periods of time when the excitation laser is off, then any prompt PS fluorescence interference is removed. The prompt PS fluorescence has a lifetime on the order of 10 ns since it is from radiatively allowed transitions. It decays much more rapidly than the emissions from the singlet oxygen (lifetime of 4 µs in aqueous media and as short as 0.1 µs in biological media). This forms the basis of our detection strategy as illustrated in Fig. 2.

Until relatively recently, the most sensitive optical sensors for singlet oxygen emission were solid state, liquid nitrogen cooled germanium photodiode detectors. While these devices can provide high sensitivity (detectivity of a detector, $D^* \sim 10^{15} \text{ cm}^2 \text{ Hz}^{1/2}/\text{W}$), transimpedance amplifiers are required to amplify the weak signals, and they operate at what is known as the "gain/bandwidth limit". For example, the highest sensitivity Ge devices have a temporal response time of 1 ms. With these sensitive but relatively slow Ge detectors, one cannot temporally isolate the singlet oxygen
emission from prompt PS emission. The detector simply cannot discriminate between laser on and laser off conditions with adequate temporal resolution. Recently, we described a solution to this problem by using a pulsed diode laser combined with a novel photomultiplier tube (PMT) with a time response < 10 ns and photon counting capabilities.\textsuperscript{15} Niedre et al.\textsuperscript{16} used a similar PMT and a frequency-doubled Q-switched Nd:YAG laser and an optional parametric oscillator\textsuperscript{17} to detect PDT-produced singlet oxygen emission both \textit{in vitro} and \textit{in vivo}.

Diode lasers are ideal for producing short pulses and have output wavelengths appropriate for PDT. Note that unlike Q-switched Nd:YAG lasers, short (\(\mu\)s) diode laser pulses do not imply significant energy compression. While the diode laser is on, its peak power is essentially equal to that when operating CW. Consequently, tissue damage is unlikely. This lack of significant energy compression in diode lasers implies low peak powers. For example, a 300 mW cw diode laser when pulsed will produce pulses which have peak powers of this value. (One can produce powers of 2 to 3 times the cw value for very short pulses and low duty cycles.) Pulses of 2 \(\mu\)s duration contain only 0.6 \(\mu\)J and each pulse has a peak power of 300 mW. In comparison, Niedre et al.\textsuperscript{17} used a Nd:YAG laser pumped optical parametric oscillator (OPO) that produced 1 mJ, 20 ns pulses (peak power \(~50\ kW\)). Our diode laser approach allows us to irradiate with much less pulse energy and peak power. We use high repetition rates to enhance our signals, an ideal approach for photon counting. Compared to Q-switched Nd-YAG lasers, diode lasers also offer improved energy efficiency, smaller size, and monolithic coupling to fiber optics. We summarize our strategy in Fig. 2.

Since we are using time resolved fluorescence to enhance the sensitivity for detecting the singlet oxygen, it is important to be able to interpret the observed signals. Earlier we discussed the overall process of singlet oxygen production in the PDT process. From Fig. 1, we can write down
the rate equations for the concentrations of the relevant species: \([S_o]\), the ground singlet state of the photosensitizer; \([S_1]\), the excited singlet state of the photosensitizer; \([T_1]\), the excited triplet state of the photosensitizer, and \([^1\Delta]\); the singlet state of oxygen. The relevant equations are:

\[
\frac{d[S_o]}{dt} = I\sigma[S_o] - \frac{[S_1]}{\tau_{S_1}} \tag{1}
\]

\[
\frac{d[T_1]}{dt} = [S_1]K_{S_1 \rightarrow T_1} - \frac{[T_1]}{\tau_{T_1}} \tag{2}
\]

\[
\frac{d[O_2(^1\Delta)]}{dt} = [T_1]K_{T_1 \rightarrow ^1\Delta} - \frac{[O_2(^1\Delta)]}{\tau_{\Delta}} \tag{3}
\]

where

\[
I = \text{excitation laser intensity in photons cm}^{-2}\text{sec}^{-1}
\]

\[
\sigma = \text{absorption cross section of the photosensitizer}
\]

\[
1/\tau_{S_1} = \text{total removal rate from } S_1
\]

\[
K_{S_1 \rightarrow T_1} = \text{intrasystem transfer rate from } S_1 \rightarrow T_1
\]

\[
1/\tau_{T_1} = \text{total removal rate from } T_1
\]

\[
K_{T_1 \rightarrow ^1\Delta} = \text{collisional transfer rate from state } T_1 \text{ of the photosensitizer to the } ^1\Delta \text{ state of oxygen, and}
\]

\[
1/\tau_{\Delta} = \text{total removal rate from the } ^1\Delta \text{ state of oxygen}
\]

\[
[O_2(^1\Delta)]_t = \text{singlet oxygen concentration in cm}^{-3} \text{ as a function of time } (t)
\]

Treating the excitation pulse as an "instantaneous" source leads to a time dependent solution for the singlet oxygen concentration after the termination of the excitation pulse:

\[
[O_2(^1\Delta)]_t = N\sigma[S_o] \Phi_{\Delta} \frac{\tau_{\Delta}}{\tau_{T_1} - \tau_{\Delta}} \left\{e^{-t/\tau_{T_1}} - e^{-t/\tau_{\Delta}} \right\} \tag{4}
\]
where \( N \) is the total photon fluence delivered to the photosensitizer and \( \Phi_\Delta \) is the yield of \( \text{O}_2(1\Delta) \) from absorption of a pump photon by the photosensitizer.

The form of Eq. (4) is important for interpreting the time resolved singlet oxygen signals. For short excitation pulses (\( \tau_{\text{ex}} \ll \tau_T, \tau_\Delta \)) after the termination of the laser pulse, the singlet oxygen signal will be described by the difference of two exponentials as shown in Eq. (4). One requires knowledge of the relative values of the two lifetimes, \( \tau_T \) and \( \tau_\Delta \), to properly interpret the rising and falling parts of the temporal evolution of the singlet oxygen emission. When \( \tau_T < \tau_\Delta \), the rising portion of the signal is dominated by the production of singlet oxygen from energy transfer from the PS triplet state and the later decaying portion of the signal is dominated by the quenching processes of the singlet oxygen. The opposite is true when \( \tau_T > \tau_\Delta \). Thus in media where the singlet oxygen is severely quenched or when the PS triplet state lifetime is increased, the correct calculation of the two lifetimes from analysis of the decay curves requires care.

As we describe below, our diode laser approach uses much longer pulses (1 to 10 \( \mu \)s) and the instantaneous excitation model is no longer valid. In our case the diode laser intensity is constant over the duration of the pulse. To gain a better understanding of this limit, we solved Eqs. (1) through (3) numerically using MathCAD. As shown below, during the diode laser pulse, the population of the PS singlet state quickly reaches a steady state value. The PS triplet state population grows and populates the oxygen singlet state. At the termination of the diode laser pulse, an equation of the form of Eq. (4) still applies. We show comparisons of this model to our data below.
3. EXPERIMENTS

The overall experimental setup is illustrated in Fig. 3. The system consisted of three major elements: 1) a fiber-coupled, pulsed diode laser module, 2) optical filters/photomultiplier tube (PMT) detection system, and 3) a data acquisition system. There are no free-space external optics that require alignment, because both light delivery and collection are fiber coupled. In one configuration a fiber bundle (bifurcated or a tight bundle of 7 optical fibers with a configuration of 6 fibers around 1 fiber) was used, and each core fiber size was 600 $\mu$m. The fiber probe was placed ~ 1mm above either the solution surface or cell suspension. For some experiments with PS solutions, a liquid light guide was used to collect the near-IR emission. The pulsed diode laser typically produced less than 1 $\mu$J/pulse. Pulse widths of 1, 2, 5, and 10 $\mu$s were used in this study.

In the beginning of the study, a liquid nitrogen-cooled Hamamatsu PMT (5509-42) was used for detection of the singlet oxygen emission with a Stanford Research System (SRS model 453) multichannel scaler as the photon counting system. This photon counting device limited the pulsed operation of 1 kHz with 5 or 10 $\mu$s pulse width. Typically we averaged 30,000 laser pulses for a scan. With the diode laser operating at 1 kHz, this represents a data acquisition time of approximately 30 seconds.

When a thermoelectrically cooled PMT (Hamamatsu Model H9170-45) became available, we improved our singlet oxygen monitor device with this compact PMT. We also integrated a fast data acquisition system with a fast photon counting board (Becker and Hickl Model MSA-300) that can handle up to a 20 kHz repetition rate with the device system configuration. With the improved fast singlet oxygen monitor, the diode laser was operated in a pulsed mode with 10 kHz repetition
rate and 5 µs pulse width. It reduced the data acquisition time to 3 seconds to acquire the same sensitivity comparing the previous system configuration.

The near-IR emission was collected with a 1.5 mm diameter light guide that transmitted greater than 80% at 1.27 µm. The output beam of the liquid light guide was collimated and sent through a pair of optical filters including a narrow bandpass filter centered at 1.27 µm. The PMT has a quantum efficiency of about 1% at the singlet oxygen emission wavelength and a temporal response of less than 10 ns. However, the capability of this detector to be used in a photon counting mode is crucial for our application. The output current pulses from the PMT were amplified with a high bandwidth amplifier whose output voltage pulses were fed to the data acquisition system. A personal computer equipped with a National Instruments GPIB card controlled the photon counting board. We wrote custom software using a National Instruments LabWindows CVI platform.

Several solvents were used to provide a variety of quenching environments. These included: acetone, methanol, and water. Since the lifetimes of singlet oxygen are known in these solvents, they provided an excellent test of our system. Photosensitizers were procured from Frontier Scientific and solvents from Fisher Scientific. PS concentrations covering the range 10^{-3} to 10^{-6} molar were prepared. All mixed PS solutions were kept in amber glass bottles to minimize any interactions with room lights. The photosensitizers studied were summarized in Table 1.

4. RESULTS

4.1 Detection of singlet oxygen in solvents

We completed a matrix of runs using the photosensitizers listed above and excitation pulse lengths of 1, 2, 5, and occasionally 10 µs. Figure 4(a) and (b) show data for chlorin e6 (C6-e6) in acetone
and water for a 5 µs excitation pulse width. The increasing production of singlet oxygen during the diode laser pulse (via transfer from the photosensitizer triplet state) and its subsequent quenching (by the solvent molecules) are very evident in these data. For the acetone solution, the quenching is relatively weak and the singlet oxygen emission is several times stronger than that from the photosensitizer while the diode laser is on. The dramatic reduction in $\tau_\Delta$ shown as Fig. 4(b) is due to efficient water quenching when compared to acetone.

Figure 5 compares the observed temporal profiles for Cl-e6 in water for three diode laser pulse widths: 1, 2 and 5 µs. The actual diode laser pulses were square waves. Thus, the increasing signal while the laser is on is due to a rising singlet oxygen concentration. Indeed, inspection of the data in Fig. 5 shows that the singlet oxygen concentration during the diode laser pulse has not reached a maximum value even after 5 µs excitation pulse. We also observed larger singlet oxygen emission signal after the longer pulse excitation. This implies that there may be an optimum diode laser pulse length for maximum production of singlet oxygen. This optimum value of the pulse length will depend upon the rate of the singlet oxygen quenching.

At the termination of the diode laser pulse, the evolution of the singlet oxygen is adequately described by Eq. (4) and one can extract the lifetimes for both the PS triplet state and the singlet oxygen level. These lifetimes are dominated by collisions between the respective excited state and solution molecules. Using analytical fits such as shown in Fig. 6, we have determined kinetic rates and lifetimes for both the PS triplet state and the singlet oxygen state. Our measured values are shown in Table 2.

As described above, we have modeled the diode laser excitation of singlet oxygen using Eqs. (1) to (3). With pulse widths between 1 and 10 µs, the instantaneous excitation model is no
longer valid. Thus, we integrated the differential equations through two time regions: a) $0 < t < t_P$ and b) $t > t_P$ where $t_P$ is the length of the diode laser pulse. In the first time regime we assume that the diode laser power is constant, $I_0$. For $t > t_P$, the diode laser power is zero and the form of Eq. (4) is then an adequate description of the singlet oxygen emission. The observed growth of the singlet oxygen emission while the diode laser was on ($t < t_P$) provides interesting insight into the excitation process.

In Fig. 7 we show the temporal evolutions of all three important excited states in the type II PDT process: singlet and triplet state of the PS and the singlet molecular oxygen. As shown in Fig. 7, during the diode laser pulse, the population of the PS singlet state quickly reaches a steady state value. The PS triplet state population grows and populates the oxygen singlet state. When the diode laser turns off, an equation of the form of Eq. (4) still applies.

Figure 8 shows comparisons of this model to our data in water with two different excitation pulse widths: 1 and 5 µs. It is important to note that for many photosensitizers there is some prompt, singlet state emission even at the singlet oxygen emission wavelength of 1.27 µm. This emission decays in a few ns after the diode laser pulse is terminated. This behavior is shown clearly both in our experiment and model. The model adequately predicts singlet oxygen production in the solution phase with PDT treatment parameters such as photosensitizer concentration, laser intensity, PS triplet lifetime, singlet oxygen lifetime, and transfer rates. This model may enable us to predict optimized conditions of PDT treatments with light delivery and pulse widths for singlet oxygen production.

In order to verify that the observed signals were indeed from singlet oxygen, we included the capability for oxygenating and de-oxygenating the liquid samples in the cuvets. A small Teflon cap
that contained a pair of stainless steel tubes was attached to the cuvet. The PS solution was initially saturated with oxygen in air. To deoxygenate the solution, nitrogen gas was slowly bubbled through the cuvet. While the PS solution was deoxygenated, we observed the singlet oxygen signal decreasing with increasing nitrogen bubbling time until the added nitrogen fully displaced the dissolved oxygen. Figure 9 shows a comparison of data from a C\ell-e6 sample in water both oxygenated and deoxygenated. The diode laser pulse width was 2 μs for these data. The deoxygenation removed both the relatively slow rising part of the emission during the diode laser pulse and the emission after the termination of the diode laser pulse. When nitrogen bubbling was stopped, the singlet oxygen signal recovered after several minutes due to diffusion of air back into the solution. These data provide unambiguous proof that the signals observed indeed arise from singlet oxygen. The emission remaining when the oxygen dissolved in the solvent has been removed is due to weak IR emission (at 1.27 μm) from the photosensitizer singlet state. Because this remaining fluorescence is from the radiatively allowed \(S_1 \rightarrow S_0\) system of the PS, its temporal evolution reproduces the diode laser emission pulse and is consistent with our model described above and illustrated in Fig. 7.

4.2 Detection of singlet oxygen in protein containing solutions

Having established a baseline sensitivity for our system, we extended the detection of singlet oxygen to an even more severe quenching environment by introducing fetal bovine serum (FBS) to the aqueous solutions of photosensitizers. The protein laden FBS has been shown to be a highly efficient quencher of singlet oxygen and has limited several previous attempts to observe singlet oxygen emission produced by the PDT process. We added FBS to some aqueous photosensitizer solutions: 2.5% and 5% concentrations, known to severely quench singlet oxygen.\textsuperscript{14-17} This visually
changed the viscosity of the solutions. Figure 10(a) and (b) show the singlet oxygen emission signals produced in aqueous Cℓ-e6 and 5% FBS added by 5 µs diode laser pulse, respectively. Analysis of these data imply $\tau_T = 5.0 \mu s$ and $\tau_\Delta = 0.30 \mu s$. Although the magnitude of the singlet oxygen signal is reduced, we still have adequate signal above the noise in the emission curves. Figure 10(c) shows a model prediction of the 1.27 µm emission pulse (5 µm laser pulse) with these $\tau_T$ and $\tau_\Delta$ values.

Even though the lifetime of singlet oxygen is severely quenched, the PS triplet state (the source of the singlet oxygen) is not. Consequently, the absolute magnitude of the singlet oxygen emission is reduced, but it persists for over 10 µs. Similar results have been reported by Niedre et al.\textsuperscript{16,17}

4.3 Detection of singlet oxygen signal in a rat prostate tumor cell line (\textit{in vitro})

We also used our singlet oxygen detector on a rat prostate tumor cell line incubated with the photosensitizer Cℓ-e6 for 4 hours. The cells were washed and palletized prior to the tests, and the cell suspension was put into a glass bottle with a magnetic stirrer. Therefore, the observed singlet oxygen signal was from intracellular singlet oxygen, not from the extra PS in the medium since Cℓ-e6 photosensitizer that was not taken into tumor cell was removed by washing.

Each spectrum was corrected by background fluorescence of cell suspension without PS, such as autofluorescence from biomolecules or any photosensitizer fluorescence. With the initial PS concentrations of 100 µM and 10 µM, we measured the singlet oxygen signal in a rat prostate tumor cell line, as shown in Fig. 11, along with fits to the data. The areas under the decay curves are proportional to the singlet oxygen produced. In preliminary experiments, we also have seen some morphological evidence of cell death after light treatment.
We recorded total singlet oxygen counts with an extended light exposure to see the singlet oxygen signal changes during light exposure in real time. We operated the 655 nm diode laser at 1 kHz with the pulse width of 5 µs. Each recorded scan was accumulated with 10000 laser shots, which is 10 seconds of data accumulation time. Figure 12 presents about 2000 seconds of operation, a little more than 30 minutes. The average laser power was 2.5 mW, which is below current treatment laser power. Figure 12 shows the singlet oxygen signal depletion in real time as a function of the light exposure time. Therefore, when there is a singlet oxygen signal level change whether due to PS photobleaching, oxygen depletion or cell death, we may be able to monitor the singlet oxygen production in real time using our singlet oxygen monitor. These capabilities will be the subject of future studies.

5. SUMMARY
We have described the results of an initial investigation to develop a sensitive, pulsed diode laser-based monitor for singlet oxygen produced during PDT. Using a pulsed diode laser and photon counting methods, we have demonstrated that we can measure the singlet oxygen production in a variety of media including protein rich aqueous solutions. These results are promising for eventual development of a system for real-time, in vivo monitoring of singlet oxygen produced during treatments.
ACKNOWLEDGEMENTS

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REFERENCES


Table 1. Photosensitizers Used in this Study

<table>
<thead>
<tr>
<th>Photosensitizer</th>
<th>Molecular Weight (g/mole)</th>
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<tbody>
<tr>
<td>Aluminum phthalocyanine tetrasulphonate (AlPcS4)</td>
<td>595</td>
</tr>
<tr>
<td>Chlorin e6 (Cℓ-e6)</td>
<td>594.6</td>
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<tr>
<td>Meso-tetra-(4-sulphonatophenyl)-porphine-dihydrochloride (T4PS-4)</td>
<td>1007.7</td>
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Table 2. Summary of Measurements

<table>
<thead>
<tr>
<th>PS</th>
<th>Solvent</th>
<th>$O_2(^1\Delta)$ Lifetime $\tau_\Delta$ ($\mu$s)</th>
<th>PS Triplet State Lifetime $\tau_T$ ($\mu$s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cℓ-e6</td>
<td>Acetone</td>
<td>49.5</td>
<td>0.4</td>
</tr>
<tr>
<td></td>
<td>Methanol</td>
<td>9.4</td>
<td>0.5</td>
</tr>
<tr>
<td></td>
<td>Water</td>
<td>3.7</td>
<td>0.9</td>
</tr>
<tr>
<td></td>
<td>Water with 5% FBS</td>
<td>0.4</td>
<td>5.0</td>
</tr>
<tr>
<td>T4SP</td>
<td>Methanol</td>
<td>9.7</td>
<td>0.7</td>
</tr>
<tr>
<td></td>
<td>Water</td>
<td>4.4</td>
<td>2.7</td>
</tr>
<tr>
<td>AℓPcS4</td>
<td>Methanol</td>
<td>10.6</td>
<td>0.6</td>
</tr>
<tr>
<td></td>
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<td>4.3</td>
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</tr>
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</table>

Note: Literature values of $\tau_\Delta$ in acetone, methanol, and water are 51 $\mu$s, 9.5 $\mu$s, and 4.2 $\mu$s, respectively (Refs. 13-15, 17-19).
Fig. 1. Mechanism for production of singlet molecular oxygen in the photodynamic therapy.

Fig. 2. Outline of the detection strategy for our singlet oxygen monitor: (a) temporal discrimination, and (b) optical filtering method (spectral discrimination).
Fig. 3. Device setup of singlet oxygen monitor.

Fig. 4. Temporal evolution of the IR emission centered at 1.27 μm following 5 μs excitation of Cℓ-e6. (a) acetone, (b) water.
Fig. 5. Comparison of the observed signal at 1.27 µm produced in Cℓ-e6 with various diode laser excitation pulse widths. (a) $\tau_p = 1$ µs; (b) $\tau_p = 2$ µs; (c) $\tau_p = 5$ µs.

Fig. 6. Temporal evolution of singlet oxygen emission after the termination of the diode laser pulse in an $10^{-4}$ M aqueous solution of A/PcS4. The dots represent photon counts and the line is a fit to Eq. (4).
Fig. 7. Model predictions showing components of the PDT process for long pulse (5 µs) diode laser excitation.

Fig. 8. Temporal profiles for the radiative emission from singlet oxygen in water with different laser pulse widths: (a) prediction using our kinetic model with $\tau_T=1\ \mu s$, $\tau_\Delta=4\ \mu s$, $\phi_T=0.4$, $\phi_\Delta=0.7$; and (b) experimental results with chlorin e6 in aqueous solution.
Fig. 9. Comparison of near-IR emission observed in Cl-e6 in water using a 2 \( \mu \)s diode laser pulse.

(a) oxygenated sample, and (b) same sample deoxygenated.

Fig. 10. Comparison of measured and predicted singlet oxygen emission in Cl-e6 in aqueous solutions with and without fetal bovine serum added: (a) Measured profile with no FBS added; (b) Measured profile with 5\% FBS added; (c) Model prediction of emission pulse at 1.27 \( \mu \)m using \( \tau_T = 5.0 \ \mu \)s, \( \tau_\Delta = 0.3 \ \mu \)s.
Fig. 11. Temporal evolution of singlet oxygen emission in a rat prostate tumor cell line using Cl-e6 photosensitizer.

Fig. 12. Real-time signal depletion of singlet oxygen as a function of the light exposure. The profiles of $[\text{Cl-e6}] = 100$ and 10 $\mu$M show a decreasing singlet oxygen signal with longer light exposure comparing to the no Cl-e6 case of no signal depletion. The laser light dose was 2.5 $\mu$J/pulse, and the average intensity was 9 mW/cm$^2$. 