Phantom-to-in vivo Correlation of TSOLD, MSOLD and SOED for Quantitative Singlet Oxygen Dosimetry in Photodynamic Therapy

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2026 JULY 19–22 VANCOUVER, BC
JOINT AAPM | COMP MEETING

ABSTRACT

Photodynamic therapy (PDT) outcome depends on the amount of reacted singlet oxygen generated in tumor tissue during treatment, a quantity that can be estimated either by direct optical detection of its 1270 nm luminescence signature or by kinetic modeling from measured drug concentration, light fluence, and oxygenation, but these approaches have rarely been cross-validated against one another for the same photosensitizer and tumor model. This study establishes such a correlation for Photofrin-mediated PDT in RIF tumor-bearing mice, comparing three approaches: time-resolved singlet oxygen luminescence detection (TSOLD), multispectral SOLD (MSOLD), and singlet oxygen explicit dosimetry (SOED). TSOLD specificity was confirmed via bandpass-filter spectral isolation and sodium azide quenching. Biexponential fitting of tissue-mimicking phantom decay curves (3–10 mg/kg Photofrin) yielded consistent singlet oxygen and triplet-state lifetimes across concentration. Applying the Stern-Volmer relationship to the photosensitizer triplet-state lifetime yielded in vivo oxygen estimates in two independent mice (71.0 ± 32.2 μM and 66.0 ± 35.2 μM) in close agreement with directly measured tissue pO_2 (0.38σ and 0.49σ, respectively). In parallel, MSOLD cumulative singlet oxygen luminescence closely tracked SOED-modeled reacted singlet oxygen dose. Together, these results demonstrate that TSOLD's photophysical lifetime parameters and MSOLD's cumulative luminescence signal both independently corroborate SOED-based dosimetry, supporting a unified, cross-validated framework for quantitative, real-time PDT monitoring.

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INTRODUCTION

Photodynamic therapy (PDT) is a cancer treatment that combines a light-activated photosensitizer with a specific wavelength of light delivered to the tumor. Upon absorbing light, the photosensitizer transitions to an excited triplet state and transfers that energy directly to ground-state molecular oxygen ($^3O_2$) generating singlet oxygen ($^1O_2$), a highly reactive species that is the primary cytotoxic agent responsible for tumor cell death in type II photosensitizers such as Photofrin. Because the therapeutic effect of PDT is governed by how much $^1O_2$ is actually generated in tissue, not by delivered light dose alone, real-time detection of $^1O_2$ itself is the most direct possible route to accurate PDT dosimetry, which is precisely what singlet oxygen luminescence dosimetry (SOLD) is designed to provide, via the characteristic 1270 nm phosphorescence emitted as $^1O_2$ relaxes back to $^3O_2$. MSOLD isolates this signal by spectrally unmixing static, per-frame spectra; TSOLD instead time-resolves the signal's decay after each laser pulse. This gives TSOLD an advantage MSOLD structurally lacks: since the photosensitizer triplet-state lifetime is itself oxygen-sensitive, TSOLD's decay curve yields a real-time, calibration-free oxygen metric.

TSOLD decay curves are fit to a biexponential model:

$$[O_2^1](t) = N\sigma[S_0]\varphi_D \frac{\tau_\Delta}{\tau_T - \tau_\Delta}\left(exp\left(\frac{-t}{\tau_T}\right) - exp\left(\frac{-t}{\tau_\Delta}\right)\right)$$

where N is the number of excitation photons per pulse, σ is the photosensitizer absorption cross-section, $[S_0]$ is the ground-state PS concentration, φ_D is the singlet oxygen quantum yield, τ_T and τ_Δ are the triplet state and singlet oxygen lifetimes respectively.

METHODS AND MATERIALS

A custom fiber-coupled TSOLD system (630 nm pulsed excitation, 130 ns pulse width, 12.82 μs PRI, InGaAs SPAD at 25% quantum efficiency, 13 μs dead time) recorded 1270 nm singlet oxygen luminescence via time-correlated single-photon counting (300 s acquisition). Decay curves were fit to a biexponential model to extract τ_Δ, and τ_T. Specificity was confirmed using discrete bandpass filters (1200–1300 nm) and sodium azide (NaN_3) quenching controls. Photofrin phantoms (3–10 mg/kg equivalent; 0.6% Intralipid + 0.002% ink in ethanol, tissue like optical properties) and in vivo measurements were acquired under identical settings.

Tissue oxygen concentration was estimated directly from τ_T via the Stern-Volmer relationship:

$$[O_2^3] = \left({}^1/_{k_2 \cdot \tau_T}\right) - \beta$$

using literature Photofrin rate constants (k_2 = 1400 $\mu M^{-1}s^{-1}$, β = 11.9 μM; Kim et al., *Phys. Med. Biol.*, 2017).

SOED was computed from a two-compartment PS/oxygen kinetic model using measured drug concentration, fluence rate, and directly measured pO_2. MSOLD cumulative signal was derived from spectral unmixing of the singlet oxygen luminescence band.

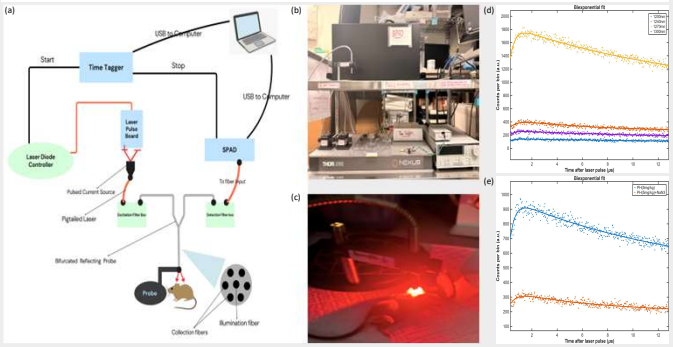

Figure 1. (a) TSOLD System schematic; (b) TSOLD Apparatus at Zhu lab; (c) In vivo measurement using 630 nm diode laser; (d) Measurement through various bandpass filters proving real singlet oxygen signal detection at 1270nm(yellow); (e) Measurement with added quencher(orange) to prove real singlet oxygen being detected.

RESULTS

Bandpass filter measurements (1200–1300 nm) confirmed a distinct 1270 nm signal peak, eliminated upon NaN_3 quenching, confirming that detected photons originate from singlet oxygen rather than background fluorescence. Biexponential fitting of phantom decay curves showed consistent lifetimes with SNR improving from 15.0 to 23.7 and R^2 from 0.85 to 0.96 with increasing Photofrin concentration (3–10 mg/kg). In vivo, fitted triplet-state lifetimes in two independent mice (Z4: 8.61 ± 3.34 μs; AM3: 9.17 ± 4.14 μs) were markedly longer than phantom values (~ 0.40–0.44 μs): the expected solution-to-tissue crossover in photosensitizer photophysics.

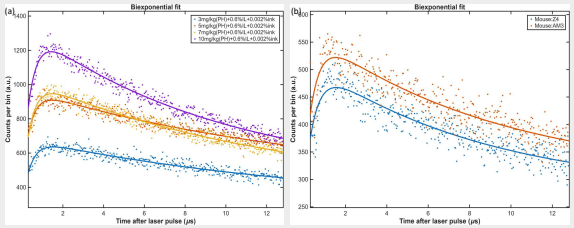

Figure 2. (a) Biexponential fits for photofrin phantoms with increasing concentration; (b) Biexponential fits for in vivo measurements for two mice

Table 1. Lifetime values for triplet state PS and singlet oxygen along with the derived molecular oxygen values

PHANTOM					
Conc.(mg/kg)	$\tau_\Delta(\mu s)$	$\tau_T(\mu s)$	$[^3O_2]$(mM)	SNR	R^2
3	14.67 ± 6.20	0.42 ± 0.10	1.68 ± 0.44	15	0.85
5	14.09 ± 4.58	0.40 ± 0.08	1.76 ± 0.41	18.14	0.9
7	12.39 ± 3.00	0.42 ± 0.06	1.67 ± 0.29	20.86	0.93
10	9.54 ± 1.43	0.44 ± 0.04	1.60 ± 0.18	23.66	0.96
INVIVO					
Mouse	$\tau_\Delta(\mu s)$	$\tau_T(\mu s)$	$[^3O_2]$(mM)	SNR	R^2
Z4	0.51 ± 0.15	8.61 ± 3.34	0.071 ± 0.032	9.45	0.82
AM3	0.49 ± 0.15	9.17 ± 4.14	0.066 ± 0.035	9.64	0.82

Applying the Stern-Volmer relationship to τ_T, TSOLD-derived oxygen estimates agreed closely with each mouse's own directly measured tissue pO_2: Z4 (71.0 ± 32.2 μM vs. 59.2 ± 24.2 μM, 0.38σ) and AM3 (66.0 ± 35.2 μM vs. 48.9 ± 0.7 μM, 0.49σ), while phantom oxygen estimates remained essentially dose-independent (1.60–1.76 mM), consistent with ethanol's known elevated oxygen solubility relative to tissue. Separately, MSOLD cumulative singlet oxygen luminescence closely tracked SOED-modeled reacted singlet oxygen dose (1.380 ± 0.091 vs. 1.382 ± 0.175 mM and 1.098 ± 0.176 vs. 1.100 ± 0.342), supporting agreement between two independently-derived dosimetry approaches despite their different underlying mechanisms.

Table 2. Validation of O_2 and $[ROS]_{1\Delta}$ SOED values with TSOLD and MSOLD respectively.

Mouse	O_2(mM): TSOLD	O_2(mM): SOED	$[ROS]_{1\Delta}$(mM): SOED	$[ROS]_{1\Delta}$(mM): MSOLD
Z4	71.02 ± 32.15	59.15 ± 24.22	1.380 ± 0.091	1.382 ± 0.175
AM3	66 ± 35.2	49.14 ± 7.15	1.098 ± 0.176	1.100 ± 0.342

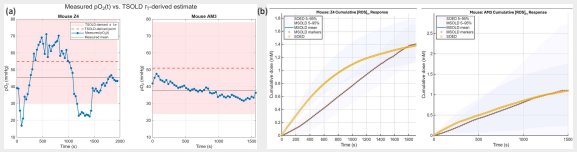

Figure 3. (a) Measured $pO_2(t)$ vs TSOLD derived estimate; (b) Measured $[ROS]_{1\Delta}$ SOED values vs MSOLD estimate.

CONCLUSION

By linking TSOLD's photosensitizer triplet-state lifetime directly to tissue oxygenation via the Stern-Volmer relationship, this study demonstrates a calibration-free route to real-time oxygen sensing during PDT, validated against direct pO_2 measurement in two independent mice. Combined with independent agreement between MSOLD and SOED, these results support a unified, cross-validated framework for quantitative PDT dosimetry and motivate continued in vivo development of TSOLD as a real-time monitoring tool.

ACKNOWLEDGEMENT AND REFERENCES

This work was funded by the U.S. National Institutes of Health (NIH) grant **R01 EB032821-01.**

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