Dual-channel imaging system for singlet oxygen and photosensitizer for PDT

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Abstract: A two-channel optical system has been developed to provide spatially resolved simultaneous imaging of singlet molecular oxygen (¹O₂) phosphorescence and photosensitizer (PS) fluorescence produced by the photodynamic process. The current imaging system uses a spectral discrimination method to differentiate the weak ¹O₂ phosphorescence that peaks near 1.27 μm from PS fluorescence that also occurs in this spectral region. The detection limit of ¹O₂ emission was determined at a concentration of 500 nM benzoporphyrin derivative monoacid (BPD) in tissue-like phantoms, and these signals observed were proportional to the PS fluorescence. Preliminary in vivo images with tumor laden mice indicate that it is possible to obtain simultaneous images of ¹O₂ and PS tissue distribution.

OCIS codes: (170.0110) Imaging systems; (170.5180) Photodynamic therapy; (170.3880) Medical and biological imaging.

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1. Introduction

Photodynamic therapy (PDT) is a promising cancer treatment. It involves optical excitation of photosensitizers that subsequently produces excited singlet molecular oxygen (¹ O₂) via collisional quenching by ground state oxygen molecules within the tumor tissue [1,2]. However, the tumor response to PDT treatments is highly variable depending on the photosensitizer (PS) concentration in the tumor, treatment light intensity, total light dose (fluence), and tumor oxygenation. An accurate dosimeter to optimize the individual treatment response of PDT in a clinical environment would be a valuable tool to improve the treatment outcomes. A number of groups have attempted to develop dosimeters based on the fluorescence intensity of the PS in the tumor [3–5], but the complex dynamics of oxygen-independent photobleaching of the PS as well as photoproduction may preclude this as an accurate method without careful calibration for each indication. Optical measurement of the ¹ O₂ phosphorescence produced in tissue could be a more direct way to assess PDT dose to the tissue. It is well established that the PDT process consumes oxygen in a manner which can substantially deplete the available oxygen supply within tumor tissues [6]. This effect is unfavorable for maximal treatment response, as it results in decreased ¹ O₂ production. It has been suggested that maximizing the oxygenation of tissue can enhance PDT efficiency [7]. Our imaging system may be able to provide insight into this mechanism.

During the PDT treatment, oxygen molecules in the metastable singlet delta state, O₂(a¹ Δ), are generated and are believed to be responsible for the destruction of cancer cells [8,9]. The ¹ O₂ generation is related to the product of the PS concentration [PS], the molecular oxygen concentration [O₂], and the light dosage Φ(t), i.e., [¹ O₂] = [PS(t)] [O₂] Φ(t) dτ. There has been considerable interest in developing a sensor for ¹ O₂ that could be used as a real-time dosimeter during PDT treatments [10–15]. Although the measurement of the ¹ O₂ emission centered near 1.27 µm has the potential to be an effective technique for evaluating the PDT treatment efficacy, monitoring only the ¹ O₂ production does not distinguish whether the PDT efficacy is
limited by oxygen availability or the localized tissue PS concentration. To address this issue, we developed a two-dimensional (2D) imaging dosimeter system that enables simultaneous imaging of both spatially resolved $1^O_2$ phosphorescence and PS fluorescence. In 2002, a $1^O_2$ image from a→X transition at 1270 nm with a lateral resolution of 2.5 μm was report by Andersen et al. [16]. They used a microscope with a custom-made InGaAs linear array. The sample was scanned with a movable x-y stage to generate 2D images. Several groups have reported $1^O_2$ images of a single cell and intradermal tumor model in mice by using scanning-based techniques [17–19]. Since the $1^O_2$ emission signal is very weak at 1270 nm, chemically trapped $1^O_2$ detection methods in the visible wavelength region have also been reported [20,21]. Recently, Hu et al. reported the images of the $1^O_2$ emission with only using a 1.27 μm optical filter with a cooled InGaAs CCD camera with detection limit of ~0.0181 μg/ml BPD-MA in 75% ethanol [22]. They claimed that a long-pass filter (LP1150) was sufficient to achieve spectral discrimination of the $1^O_2$ emission from other possible radiations, such as PS fluorescence, backscattered laser light, and autofluorescence from tissues in vivo.

The weak optical emission from $1^O_2$ is one of the major challenges in developing an optically-based monitor for this species. We have previously reported on an ultra-sensitive, non-imaging sensor with a near-infrared (IR) sensitive photomultiplier tube (PMT) detector for $1^O_2$ measurements both in vitro and in vivo [13–15]. In those studies, a low power, pulsed diode laser was used to produce the PDT photoreaction products and the optical emissions were detected with a fast photon-counting system. Spectral and temporal discrimination techniques were used to isolate the $1^O_2$ phosphorescence from the PS fluorescence: (1) spectral discrimination via a set of three optical filters (1.22, 1.27, and 1.32 μm), and (2) the lifetime difference between short-lived PS fluorescence (a lifetime of the order of 10 ns) and longer-lived $1^O_2$ phosphorescence (lifetime of 4 μs in aqueous media and as short as 0.1 μs in biological media) [13,14]. The 1.22 and 1.32 μm filters provide a measure of the PS fluorescence and possible tissue fluorescence in this near infrared region, while the 1.27 μm filter contains underlying continuum PS fluorescence, autofluorescence, and $1^O_2$ near-IR emission. We have successfully detected $1^O_2$ phosphorescence in tumor laden mice and healthy human skin [14,15]. In the animal study, a positive correlation was observed between the $1^O_2$ production and tumor regression.

In this study, we describe our early continuing progress in the development of the 2D imaging configuration to produce spatial maps of the $1^O_2$ phosphorescence and PS fluorescence during PDT. The ability to obtain simultaneous images of both the PS and $1^O_2$ will be valuable for assessing the relationship between PS bleaching and $1^O_2$ production. In addition, knowledge of the spatial extent of the tumor sites may help to target the tumor more effectively without damaging healthy tissues. We have previously characterized the system using in vitro samples with several photosensitizers (chlorine e6, δ-aminoleuvulinic acid-induced protoporphyrin IX, and benzoporphyrin derivative monoacid) in several media including methanol, water, and fetal bovine serum (FBS), and intralipid solutions as well as tumor laden mice [23,24]. Here, we report the results obtained with benzoporphyrin derivative monoacid (BPD-MA) photosensitizer in methanol, water, FBS, and intralipid solutions. We also present preliminary in vivo images with tumor laden mice.

2. Methods

2.1. Dual-channel imaging system configuration

Figure 1(a) shows a schematic of the 2D imaging system consisting of the excitation diode laser, near-IR sensitive camera, and visible wavelength sensitive camera. The imaging system is capable of simultaneous registration of images of $1^O_2$ phosphorescence and PS fluorescence in a time frame of a few minutes, compared with typical raster-scanning methods that take tens of minutes to map out an entire area of interest. Fast image acquisition becomes critical because the PDT treatment needs to be monitored in real-time without compromising the detection quality in a clinical environment. Figure 1(b) shows the in vitro configuration to characterize the system with deoxygenating capability. The camera viewed the solution.
samples within a few degrees of the direction of the excitation beam. To deoxygenate the solution, nitrogen is slowly bubbled through a sample bottle. This deoxygenation method was used to demonstrate that the near-IR camera measures the $^1\text{O}_2$ phosphorescence even in the presence of PS fluorescence.

A near-IR camera (MOSIR 950, 26.6 $\times$ 6.7 mm, 1024 $\times$ 256 pixels, 26 $\times$ 26 $\mu$m pixel size) was used for the $^1\text{O}_2$ phosphorescence detection. This camera uses a high quantum efficiency photocathode and an electron bombardment intensifier to provide near single-photon detection in the 1 to 1.5 $\mu$m spectral region. A visible camera (Pike F-145, 9.0 $\times$ 6.7 mm, 1392 $\times$ 1040 pixels, 6.45 $\times$ 6.45 $\mu$m pixel size) was used for the visible PS fluorescence measurement. To image entire area of ~1 $\times$ 1 cm to the detector, the magnification of the imaging system was set for $\times$ 0.57. Under these conditions, pixel resolution in the object plane is ~46 $\mu$m for the near-IR image and ~12 $\mu$m for the visible image. The focal length of the dual beam imaging system was 55 mm, and collimated light from the image area was split between the IR camera and the visible camera through a beam-splitter.

For the BPD excitation, a fiber coupled diode laser with the wavelength centered at 692 nm was operated at a repetition rate of 10 kHz with a pulse width of 5 $\mu$s with an average output power of ~230 mW during each pulse. Each pulse contained only 1.15 $\mu$J. The beam size of the excitation laser was 15 mm in diameter at the focal plane of the imager, and an optical diffuser was used to generate a uniform excitation spot. At present, the time-gating rate of the near-IR camera is insufficient for rapid data accumulation. Therefore, a non-gating mode (continuous mode) for the near-IR camera with appropriate spectral background subtraction was tested in this study. In the non-gating mode, the camera is focused on the fluorescence volume and measures the collected photoelectrons for a preset length of time.

For the $^1\text{O}_2$ detection, three spectral images were recorded in rapid succession using a computer controlled slider containing three bandpass (BP) filters centered at 1.22, 1.27 and 1.32 $\mu$m, each with a full width at half maximum (FWHM) bandwidth of 15 nm. These filters were used to spectrally isolate the $^1\text{O}_2$ emission near 1.27 $\mu$m from the long wavelength spectral background signal, such as PS fluorescence and/or phosphorescence, and autofluorescence. The emissions at 1.22 and 1.32 $\mu$m (out-of-the-band wavelengths) contain only PS fluorescence while the emission at 1.27$\mu$m contains contributions from both the $^1\text{O}_2$ and PS as shown in Fig. 2(a) obtained in vitro using BPD (1 $\mu$M in methanol). The images recorded at 1.22 and 1.32 $\mu$m were co-registered and averaged to generate a single spectral image of the PS fluorescence. This formed a first order estimate of the signal level of the PS fluorescence contribution to the 1.27 $\mu$m image. This averaged PS fluorescence image was subsequently subtracted on a pixel by pixel basis from the image obtained with the 1.27 $\mu$m filter. For the visible PS fluorescence detection, a BP filter was selected and placed before the visible CCD camera to transmit a specific wavelength region for the PS fluorescence. Each bandpass filter was selected for a particular PS to optimize the transmission and spectral discrimination.

Fig. 1. Singlet oxygen 2D imaging system. (a) The schematic of current 2D imaging system (in vivo). (b) Experimental setup of deoxygenating configuration (in vitro).
Fig. 2. Singlet oxygen detection method. (a) Temporal profiles (using PMT detector) of $^1\text{O}_2$ phosphorescence at three bandpass filter positions with 1 μM BPD in methanol. The signal during excitation light ON period (6-11 μs) is due primarily to PS fluorescence with 1320 and 1220 nm filter, or $^1\text{O}_2$ emission and PS fluorescence with 1270 nm filter. The increase in the signal with 1270 nm filter is due to increasing $^1\text{O}_2$ production during the excitation pulse. (b) Spectral features of $^1\text{O}_2$ phosphorescence and total emission intensity with 1 μM BPD in methanol. (c) The method of the $^1\text{O}_2$ image process with the three-filter operation (in vitro).

To investigate the background signal level more precisely at and near the $^1\text{O}_2$ emission band, a liquid crystal tunable filter (Cambridge Research & Instrumentation, Inc., model# LNIR-06, FWHM = 6 nm) was used to obtain a detailed spectrum. Figure 2(b) shows how the long wavelength (1.2-1.4 μm) PS fluorescence is recorded and subtracted from the entire PDT emission spectrum to provide the emission due to the $^1\text{O}_2$. In the current 2D imaging system, both $^1\text{O}_2$ phosphorescence and PS fluorescence were collected, as shown in the upper trace of the triangle symbols in Fig. 2(b) with three optical filters centered at 1.22, 1.27 and 1.32 μm. The shaded areas under A, B, and C in Fig. 2(b) represent the total light intensities that were measured for the out-of-the band baseline signals (A and C) and in-band signal of $^1\text{O}_2$ intensity and baseline contribution (B). By subtracting the average baseline signal (average value of A and C) from the signal B, the $^1\text{O}_2$ intensity was calculated. This spectral discrimination approach is essential to distinguish the $^1\text{O}_2$ emission from other long wavelength background signals, as mentioned above. Hu et al. measured the 2D images of $^1\text{O}_2$ emission using a 1.27 μm optical filter with a long-pass filter (LP1150) [22]. As shown in Fig. 2(b), a single long-pass filter with a 1.27 μm filter is not able to robustly differentiate $^1\text{O}_2$ phosphorescence from other long wavelength background signals. This is especially true for in vivo measurements where the $^1\text{O}_2$ phosphorescence is typically weak relative to the underlying fluorescence background.

Figure 2(c) shows an example of how the images of $^1\text{O}_2$ phosphorescence were acquired. The diode laser beam was directed onto the face of a 1 cm square cuvette which contained 50 μM chlorine e6 (Cl-e6) in phosphate buffer solution. The pixel by pixel averaged values of the 1.22 and 1.32 μm images was subtracted from the image recorded at 1.27 μm to produce the...
image of the $^{1}$O$_{2}$ phosphorescence. Note that it takes typically less than 30 sec with in vitro samples. In this example, dark count subtraction of each image before calculating the $^{1}$O$_{2}$ intensity is not shown. The dark count subtraction for each image is an essential step to improve S/N with in vivo image quality.

The spatial resolutions of both the visible and near-IR imaging systems were measured using a standard Air Force test pattern as shown in Fig. 3. This imaging system was developed to image the entire area of light illumination, $\sim 1 \times 1$ cm. Therefore the magnification of the imaging system considering physical size of detectors was optimized to image the entire area to the detector as $\times 0.57$ for both cameras. The respective spatial resolutions for the visible and near-IR systems were $<30$ μm and $<100$ μm empirically estimated based on a FWHM limit of a line spread function method.

Fig. 3. Image resolution of the dual-channel system. (a) Visible image. (b) NIR image.

2.2. In vitro and in vivo experiments

$^{1}$O$_{2}$ phosphorescence imaging from BPD in methanol, water, FBS, and intralipid solutions was investigated in order to determine the detection sensitivity. The solvents were used to provide a variety of quenching environments. Since the lifetimes of $^{1}$O$_{2}$ are known in these solvents, they provided an excellent test of our system. BPD solutions were procured from U.S. Pharmacopeia (Verteporfin) and solvents from Fisher Scientific. BPD concentrations covering the range $10^{-4}$ to $10^{-6}$ molar were prepared. All mixed BPD solutions were kept in amber glass bottles to minimize any interactions with room lights.

A preliminary study of $^{1}$O$_{2}$ production during PDT in tumor laden mice was also conducted. The BPD photosensitizer is commonly used for treatment of age related macular degeneration [25], and has been initiated in studies for solid pancreatic tumors [26]. However, the $^{1}$O$_{2}$ generation of BPD is not as well studied as C/-e6 or $\delta$-aminolevulinic acid-induced protoporphyrin IX (ALA-induced PpIX). We have previously reported results using these latter photosensitizers [23,24]. All animal procedures were carried out according to protocols approved by the Dartmouth College Institutional Animal Care and Use Committee (IACUC). Pancreatic tumor cells were implanted subcutaneously in 6-week-old male nude mice (~22g).

AsPC-1 cells, derived from a human pancreatic acinar cell adenocarcinoma (CRL-1682, American Type Culture Collection (ATCC), Manassas, VA 20108) were cultured in RPMI 1640 with 10% (v/v) fetal bovine serum (FBS), 1% (v/v) penicillin-streptomycin prepared for a stock solution of 10,000 IU penicillin and 10,000 g/ml streptomycin (Mediatech Herndon, Virginia), 2mM L-glutamine, 10 mM HEPES, 1M sodium pyruvate, 4500mg/L glucose, and 1500mg/L sodium bicarbonate. The cells were passed by washing twice with phosphate buffer solution (PBS) without calcium and magnesium and then incubated at 37°C with 0.25% trypsin for 5-10 minutes. When all the cells had lifted off from the bottom of the culture flask,
the trypsin was neutralized with culture medium and the cell solution was pelleted and cells suspended in complete medium at 4x10^7 cells/ml.

The cells, required for implantation, were prepared in a 1:1 mixture of cell culture medium and Matrigel® (BD Biosciences, San Jose, CA). Matrigel® was thawed on ice in a 4°C refrigerator overnight and was kept on ice for the entire implantation procedure. AsPC-1 cells were diluted in a 1:1 ratio of culture medium and Matrigel® to a final concentration of 4x10^7 cells/ml for implantation. Sterile insulin syringes (1/2 cc U-100 Lo-Dose Insulin Syringe 281/2, Becton Dickinson & Co., Franklin Lakes, NJ) were loaded with the cell-Matrigel® solution and placed and kept on ice ready for the implantation procedure.

Once the mouse was sedated using isoflurane gas (O_2 flow meter set to 1L/min; induction at 3% then reduced to 1.5-2%), the left side of the mouse’s abdomen was sterilized with an iodine solution (Povidone-Iodine, Novaplus, Irving, TX) and the cell-Matrigel® solution (1x10^6 cell in 50µl) was injected subcutaneously. The Matrigel® was allowed to set (~10 seconds) and the needle was gently removed from the injection site and swabbed with iodine to kill any stray cells in the injection site. The growth of the AsPC-1 tumors in each mouse was measured daily, using calipers, up to two weeks after implantation so that an average tumor volume of approximately 90mm^3 was reached for the \textit{in vivo} BPD study.

BPD doses of 0.5, 1, and 2 mg / body weight kg were used for this part of the study. Verteporfin for injection was obtained from QLT Inc. (Vancouver, Canada) as a gift. Verteporfin for injection is composed of a sterile liposomal formulation of BPD-MA (Visudyne, Novartis, New York). A stock saline solution of Verteporfin was reconstituted in water according to the manufacturer’s guidelines, using 2.5% as the active component. Animals were injected intravenously, via the lateral tail vein, with 75ul of Verteporfin to achieve the required dose of 0.5, 1 or 2 mg/kg body weight.

After one hour to allow for systemic tissue distribution and uptake within the tissue organs, the mouse was anesthetized for \textit{in vivo} imaging. Gas anesthesia is the preferred method of immobilization for \textit{in vivo} imaging of mice and rats and isoflurane gas is minimally metabolized (<0.17%) by the liver and therefore is less toxic to the animal’s metabolism as compared to injectable anesthetics. Once the mouse was sedated, the skin around the tumor was carefully cut and drawn back to expose the tumor tissue situated subcutaneously. The mouse was transferred to the imaging platform of the dual-channel imaging system and placed in position so that its nose was in front of the nose cone attached to the isoflurane anesthesia system. The imaging system platform has an electric heat pad integrated in order to keep animals warm during anesthesia in order to prevent hypothermia. Once the ideal position had been achieved, images of BPD fluorescence and O_2 phosphorescence were acquired using the visible and near-IR cameras respectively. Each mouse took approximately 10 minutes to image. After the tumor side was imaged, the skin on the contralateral side of the mouse was removed and normal tissue was imaged for comparison. Following the completion of imaging, the anesthetized mouse was euthanized by cervical dislocation.

3. Results and discussion

To characterize the imaging system, a series of \textit{in vitro} studies were conducted with the BPD photosensitizer in several media including protein-laden aqueous solutions that are significant quenchers of O_2. Figure 4(a) shows the spatially resolved images of both the O_2 phosphorescence (right panel) and BPD fluorescence (left panel) in methanol recorded for 10 seconds through each of the three optical filters. To verify that this signal originates from O_2, nitrogen gas was bubbled through the sample bottle to displace the dissolved oxygen with the configuration shown in Fig. 1(b). When the solution was deoxygenated as shown in Fig. 4(b), the O_2 phosphorescence signal diminished significantly. The intensities of the O_2 phosphorescence and BPD fluorescence were summed within the illumination areas (marked with a square box in the images) as shown in Fig. 4(c). The total photoelectron counts from the O_2 phosphorescence decreased more than 90% when the sample was deoxygenated. In contrast, the BPD photosensitizer fluorescence remained essentially constant. These
Fig. 4. Spatially resolved images (10 μM BPD in methanol). (a) Ambient air saturated. (b) Deoxygenated solutions (nitrogen gas purging through the solution). (c) Total BPD fluorescence and singlet O₂ phosphorescence intensities in the area of the interest marked with a square box in the images of 5 × 5 mm.

observations support our conclusion that the additional signal at 1270 nm we observed when oxygen is present is from ¹O₂ phosphorescence.

Figure 5 shows the plot of both the ¹O₂ phosphorescence and BPD fluorescence intensities (from spatially resolved images) as functions of the BPD concentration in a highly quenching FBS environment. For comparison, the signal intensity of the ¹O₂ phosphorescence in 5% FBS solutions was ~20 times lower than that in methanol solutions. These data were obtained in 40-50 seconds at each optical filter position to increase signal-to-noise level. There is a strong

Fig. 5. Plot of singlet O₂ phosphorescence and BPD fluorescence as a function of BPD concentration in 5% FBS with 5% TTX-100. Note that the intensities of PS fluorescence and singlet O₂ phosphorescence are not normalized with respect to the signal accumulation conditions of the different camera settings.
correlation between the PDT produced $^1\text{O}_2$ and the PS fluorescence. Spatially resolved images of the $^1\text{O}_2$ phosphorescence and the PS fluorescence were obtained with the BPD concentration as low as 500 nM (0.355 $\mu$g/ml) in FBS solution as well as in a highly scattering environment using 2-5% intralipid solution. These concentrations are relevant to \textit{in vivo} applications and indicated that we might observe images of $^1\text{O}_2$ phosphorescence in tumor models. For comparison, a detection limit of 100 nM (0.071 $\mu$g/ml) was determined in methanol solution (less quenching environment). These detection limits were determined with the PS baseline subtraction to estimate $^1\text{O}_2$ signal level.

Simultaneous, spatially resolved images were recorded for both the BPD fluorescence and $^1\text{O}_2$ phosphorescence with tumor laden mice. Both images from two mice with tumors implanted are shown in Fig. 6 with 0.5 and 1 BPD mg/ body kg administered one hour prior to imaging. We did not observe any selective localization of BPD photosensitizer in the AsPC-1 tumors vs. normal tissues, similar to that reported by O’Hara et al. [27]. These particular images were recorded with the skin removed at the tumor sites. The BPD photosensitizer accumulation in the AsPC-1 pancreatic model has been a challenge for these preliminary experiments because of the lack of extensive vascular structure and considerable stroma associated with this tumor model. The low concentration of BPD in tumor sites was confirmed with separate BPD fluorescence measurements. Much stronger PS and $^1\text{O}_2$ signals were observed in earlier animal studies that we reported on, using \textit{C}l-e6 photosensitizer with prostate tumor laden mice [23,24].

Even with a low $^1\text{O}_2$ production condition caused by low BPD concentration in tumor sites, we have obtained spatially correlated images of BPD fluorescence and $^1\text{O}_2$ phosphorescence using our image data reduction algorithms described in the Methods section. Some of the spatial features are common in both the PS and $^1\text{O}_2$ images. In addition, the spatial profiles of the intensities for the two species differ indicating that the $^1\text{O}_2$ and PS spatial profiles may be distinct. The noise in the near-IR $^1\text{O}_2$ image is due to the relatively small signal remaining subsequent to the data reduction algorithm that removes the near-IR PS fluorescence background. Currently, we utilize a simple linear fit to estimate the PS fluorescence baseline under the $^1\text{O}_2$ phosphorescence spectral feature. However, the linear baseline subtraction is clearly a first-order approximation. While this approximation appears to work fairly well for lower quenching and scattering environments, we have seen preliminary evidence that suggests this simple assumption may not be adequate with highly quenching/scattering environments. For example, we observed that long wavelength
background signals in intralipid solutions are better fit with a non-linear polynomial function. We will further investigate the long wavelength baseline component in this near-IR region to improve the spectral discrimination method especially within \textit{in vivo} environments. In addition, once the system is optimized for PS fluorescence and $^{1}$O$_{2}$ emission, our plan is to calibrate the entire system using a blackbody source to determine its absolute spectral radiant responsivity of each imaging channel. Recently, we also obtained spatially resolved images of $^{1}$O$_{2}$ phosphorescence and PS fluorescence using \textit{Ct-e6} with tumor laden mice in less than 3 minutes of data acquisition and these results will be reported in the near future.

4. Conclusion

We have described a dual-channel imaging system for simultaneous measurements of $^{1}$O$_{2}$ phosphorescence and PS fluorescence for PDT. The current imaging system enabled direct spatially resolved measurements of both the $^{1}$O$_{2}$ and PS concentrations produced during PDT. We performed sensitive \textit{in vitro} measurements and obtained initial simultaneous images of the $^{1}$O$_{2}$ and PS from tumors in two mice. These results are promising for the development of a real-time imaging dosimeter for PDT.

The 2D imaging system has the potential to facilitate a greater understanding of the kinetics involved in PDT mechanisms, the relationship between $^{1}$O$_{2}$ production and photobleaching of the PS, and for developing more effective treatment modalities. An accurate real-time dosimeter to characterize and optimize the individual treatment response of PDT in a clinical environment would be an important tool to improve PDT efficacy. The dual-channel imaging system may enable us not only to target a specific area of PS in tumor sites more precisely by monitoring PS fluorescence, but also to monitor the $^{1}$O$_{2}$ production which is an indication of tumor killing power during the PDT treatment. Eventually, it may even be possible to measure the local singlet oxygen dose delivered to tissue during PDT and use such dosimetry to improve therapeutic outcomes.

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