# A Phosphorescence Imaging System for Monitoring of Oxygen Distribution in Rat Liver under Ischemia and Reperfusion

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## Abstract

Oxygen-dependent quenching of phosphorescence has been validated as a useful and essentially noninvasive optical method for measuring oxygen both *in vitro* and *in vivo*. The capability of imaging oxygen distribution based on phosphorescence quenching provides a highly useful tool for investigation of oxygen delivery in tissues at the microvascular level. We demonstrated the implementation of a phosphorescence imaging system incorporating an intensified, gated, monochrome CCD camera which equips a red sensitive Gen III cathode and power supply. By selecting of the delay time sequence for phosphorescence imaging in accordance with the quenching constant and lifetime at zero oxygen of used phosphor, a series of phosphorescence intensity images corresponding to the oxygen pressures within the region of interest would be acquired and digitized. The phosphorescence lifetimes were converted form the digitized intensity levels pixel by pixel upon the assumption of single exponential decay of phosphorescence. Thus, the oxygen distribution of interested area could be calculated from the phosphorescence lifetime map in virtue of well-defined Stern-Volmer equation. Two new phosphors of Generation 2 polyglutamic Pd-porphyrin-dendrimers were used in present study, which have high water solubility and intend to stay in blood circulation without extravasating through the vascular membrane. A Pd-meso-tetra-(4-carboxyphenyl) porphyrin based phosphor termed Oxyphor G2 was used in testing of the instrument. The changes of hepatic oxygen pressure under the portal triad clamping model (PTC model) of ischemia and subsequent reperfusion were real-time monitored by phosphorescence imaging. The images indicated a transient recovery of hepatic oxygen level during the reperfusion following the PTC ligation. The lack of complete restoration of oxygen level has been implicated to the similar pattern of hepatic blood flow observed during reperfusion in previous reports. The other phosphor Oxyphor G2, derived from Pd-meso-tetra-(4-carboxyphenyl) tetrabenzoporphyrin, was recognized by its near infrared spectral characteristic. The calibration of Oxyphor G2 illustrated the independence of quenching constant to pH in the physiological range (6.4 to 7.8) and as such to make it a suitable phosphor for oxygen measurements, especially for those at depth in tissue.

*Keywords*: Phosphorescence lifetime imaging, Hepatic oxygen measurement, Ischemia and reperfusion
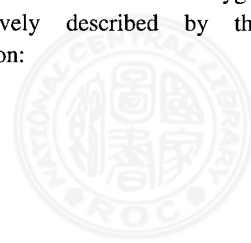
## Introduction

Development of quantitative real-time imaging system is underlying that it can not only provide physical structure of the imaged object but also include physiological information that can be further referred to planning of clinical therapeutics and assessment of curative efficacy. Oxygen-dependent quenching of phosphorescence has become an important method for measuring oxygen concentration both *in vitro* and *in vivo* [1-11]. The characteristic of phosphorescence being a non-invasive optical measurement of oxygen has distinguished itself for real-time live tissue imaging. The principle of this method has been described previously [12]. In brief, oxygen quenches phosphorescence emitted by phosphor molecule in the excited triple state in terms of non-radiative energy transfer, and which appears as the major quenching agent in biological systems as well. Increase of oxygen concentration therefore increases the decay rate of phosphorescence, resulting in a shorter lifetime of phosphorescence and a decrease in total phosphorescence intensity. With the designed phosphor which characterizes single exponential decay, the relationship between phosphorescence lifetime and oxygen concentration can be quantitatively described by the well-defined Stern-Volmer equation:

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$$PO_2 = \left(\frac{1}{k_Q}\right)\left[\left(\frac{1}{\tau}\right)-\left(\frac{1}{\tau^0}\right)\right] \qquad (1)$$

, where $\tau^0$ is the lifetime in the absence of oxygen, $\tau$ is the lifetime at an oxygen pressure $PO_2$, and $k_Q$ is a constant describing the frequency of quenching collisions between the phosphor molecules in the triplet state and molecular oxygen. $k_Q$ is a function of the diffusion constants for phosphor and oxygen, temperature and phosphor environment [11]. The calibration involves determination of both $\tau^0$ and $k_Q$. This process called absolute calibration indicated in Lo et al (1996) means that no recalibration is needed upon the experiment and measurements of phosphorescence lifetimes of designed phosphors are independent on other chromophors in the sampling volume. This facilitates its application on measurements of tissue oxygenation *in vivo*.

For measuring oxygen concentration using phosphorescence quenching, one of the most readily available and easily used phosphors is Pd-*meso*-tetra (4-carboxyphenyl) porphyrin. However, it has to be prepared in the form associated with albumin to increase its water solubility while applying it to biological systems. Wilson and co-workers have been developing phosphors based on metal chelates of porphyrins and extended porphyrins [13-16]. Two new phosphors have been constructed with the periphery of each molecule being modified with a layer of 8 glutamates, i.e. bearing a total of 16 carboxylate groups on the outer layer. These are the Generation 2 polyglutamic dendrimers containing Pd-meso-tetra-(4-carboxyphenyl) porphyrin and Pd-meso-tetra-(4-carboxyphenyl) tetrabenzoporphyrin, designated as Oxyphor R2 and Oxyphor G2, respectively [17]. The water solubility of both phosphors at physiological pH is high and which are suitable for *in vivo* oxygen measurements.

Oxygen is one of the major metabolites governing bioenergetics and physiological functions. It directly correlates ischemia and reperfusion injury, which has been acknowledged as a common theme underlying a wide spectrum of pathophysiological conditions, such as stroke in central nerves system and atherosclerosis in cardiovascular system. In the present study, we used Oxyphor R2 to investigate the oxygen distribution of rat liver throughout the complete course of temporal ischemia and reperfusion. An imaging system based on oxygen-dependent quenching of phosphorescence has been implemented to perform such a real-time monitoring of changes in hepatic oxygenation. The liver offers an interesting model for the study of oxygen delivery, since hepatic blood circulation is unique in sense that the supply comes from both venous blood from the portal vein and arterial blood from the hepatic artery [18]. The former contributes 70-80% of the total hepatic blood flow and the latter contributes 20-30%. The inverse relationship between the portal blood flow and arterial blood flow is known as the hepatic arterial buffer response. Kazuo et al (1998) indicated different recovery patterns of blood flow from the hepatic artery and portal vein after temporal ischemia, and concluded the final total hepatic
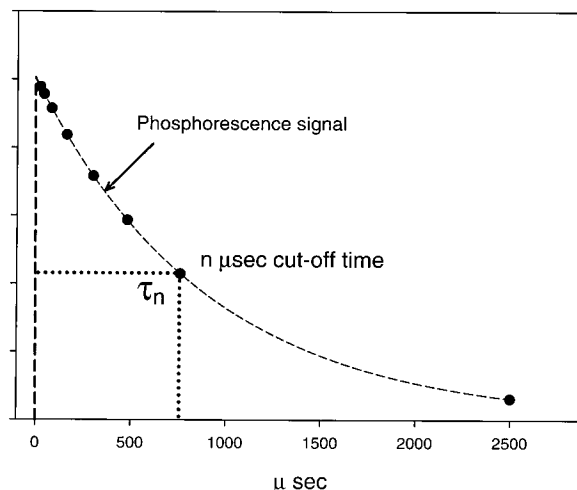


Figure 1. A typical phosphorescence single with single exponential decay. The delay time sequence which was set to acquire seven images, plus background image, for the phosphor Oxyphor R2 is: 20 μsec, 40 μsec, 80 μsec, 160 μsec, 300 μsec, 480 μsec, 760 μsec, and 2500 μsec.

blood flow does not reach the preischemia level. We, on the other hand, demonstrated here using the constructed phosphorescence imaging system that the oxygen transport in the hepatic microvasculature after ischemia and reperfusion displays a pattern similar to that of total hepatic blood flow. Moreover, the physical properties of near infrared phosphor, Oxyphor G2 were characterized in the present study as well.
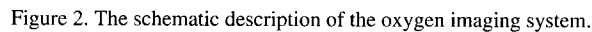
## Materials and Methods

### Basics of the Phosphorescence Imaging

The oxygen concentration distribution of the tissue is determined in a two step process: 1. collection of phosphorescence intensity images and 2. conversion of phosphorescence intensity images into lifetimes and oxygen concentrations within the region of interest based on Stern-Volmer equation. To acquire the phosphorescence images, the system triggers the flash lamp at time zero to excite the phosphor molecules preinjected and circulated in the vasculature, the phosphorescence is collected by an intensified CCD camera after a predefined delay time $t_1$ and an intensity image $I_1$ is acquired and stored in the computer memory buffer. A sequence of similar process is performed to generate a series of intensity images $I_n$ denoted with different delay time $t_n$. The default value of the intensifier gating pulse duration is 2500 μsec. However, this value can be preselected according to the estimation of phosphorescence lifetimes and the $\tau^0$ of the phosphor used. The produced serial intensity images In usually contain 5-12 images. The intensity of each pixel is given by the integral:

$$I_n = \int_{t_n}^{\infty} P(t)dt$$

$$= \int_{t_n}^{\infty} P_0 \cdot \exp(-\frac{t}{\tau})dt = P_0 \cdot \tau \cdot \exp(-\frac{t_n}{\tau}) \qquad (2)$$

Figure 2. The schematic description of the oxygen imaging system.

, where P(t) is the phosphorescence intensity over time. We can further compute logarithm of each image as:

$$\log(I_n) = \log(P_0 \cdot \tau) - (\frac{1}{\tau}) \cdot t_n \qquad (3)$$

, the dependence of log $(I_n)$ on time $t_n$ is a linear function with the slope reciprocal of $\tau$. Thus lifetime $\tau$ for each pixel can be determined by linear 2-fitting of the set $\{\log(I_n)\}$. Upon determination of the phosphorescence lifetime image, the oxygen distribution image can be calculated using the Stern-Volmer equation.

In our experiment, the delay time sequence which was set to acquire seven images, plus background image, for the phosphor Oxyphor R2 is: 20 μsec, 40 μsec, 80 μsec, 160 μsec, 300 μsec, 480 μsec, 760 μsec, and 2500 μsec (Figure 1). This time sequence was selected in consideration of calibration constants for Oxyphor R2 at 38°C and pH 7.4, e.g. $k_Q$ and $\tau^0$ (430 mmHg$^{-1}$s$^{-1}$ and 640 μsec respectively) [17], and the expected dynamic range of liver oxygenation under the protocol of ischemia and reperfusion.

### Instrumentation

The scheme of the oxygen imaging system is shown in Figure 2. The data acquisition and processing are controlled by a microcomputer with Pentium4, 1 GHz processor, 256 MB RAM. A fully programmable counter-timer board, which serves as a source and detector of digital TTL compatible signals, controls the timing of the flash lamp and several functional modules of the camera and PCI imaging board. The phosphorescence emission is collected by an intensified, gated, monochrome CCD camera (Xybion Electronic Systems Corporation, San Diego, CA, USA) with a 55-mm telecentric lens (Computar Corporation, Japan). This camera equips a

red sensitive Gen III cathode and power supply. This camera can be gated in less than 100 nsec. Multiple excitation cycles can be summed on chip by suppressing the video readout. The intensifier therefore provides an electronic shutter between the lens and a CCD array. Optical filters are used to make the camera selectively to phosphorescence, blocking the excitation light in order to avoid possible saturation of the intensifier during the excitation flash. For Oxyphor R2, a long pass filter with 630 nm cutoff wavelength at 50% is mounted directly on the camera lens. The flash lamp with a flash less than 2-5 μsec at half maximal intensity is used as a light source and it operates on a rising edge of a TTL signal and is able to produce flashes with frequency 100-200 Hz. The optical system has been designed to project the image of flash lamp arc on the surface of the illumination ring light guide through the excitation light filter. This optical coupling system contains an excitation light filter, a typical ∅ 18 mm bandpass filter (540 ± 35 nm), which covers the Q-band of Oxyphor R2 absorption (525 nm) indicated in Figure 3A. The excitation light is filtered and conducted to the sample through a Chiu R-90, focusable 8-point ring light. This ring light provides uniform illumination and can be focused on the sample at distances from 4 to 12 inches from the ring and enables homogenous illuminated area from ∅ 10 mm to ∅ 35 mm.

### Phosphors

Pd-*meso*-tetra (4-carboxyphenyl) porphyrin-dendrimer (Oxyphor R2) (Figure 3(a)) and Pd-*meso*-tetra (4-carboxyphenyl) tetrabenzoporphyrin-dendrimer (Oxyphor G2) (Figure 3(b)) were synthesized [14] and well calibrated for their applications in oxygen measurements [17]. Both Oxyphor R2 and Oxyphor G2 can be obtained through Oxygen Enterprises (Philadelphia, PA, USA). The 16 carboxylate
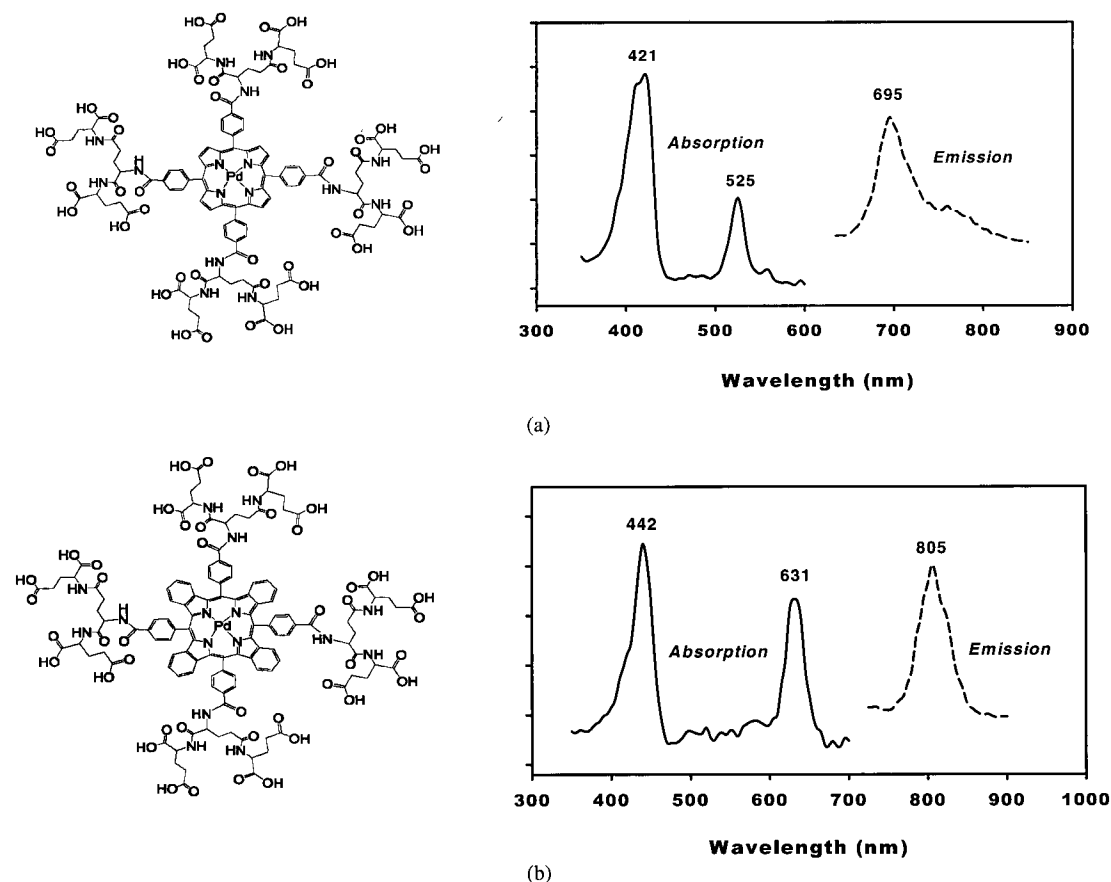
(a)



(b)

Figure 3. The chemical structure and absorption and emission spectra of (a) Pd-*meso*-tetra (4-carboxyphenyl) porphyrin-dendrimer (Oxyphor R2) and of (b) Pd-*meso*-tetra (4-carboxyphenyl) tetrabenzoporphyrin-dendrimer (Oxyphor G2).

groups located on the outer layer of the dendrimers render net 16 negative charges at near neutral pH, making both phosphors highly water soluble and impedimental to extravasate from blood vessels. These properties make both phosphors very suitable for measuring tissue oxygenation which represents the oxygen concentrations in the microvasculature of tissue. Pd-*meso*-tetra (4-carboxyphenyl) porphyrin-dendrimer (Oxyphor R2) has two absorption maxima in the visible spectrum, Soret-band at 421 nm and Q-band at 525 nm (violet and green), and emission of phosphorescence at 695 nm (Figure 3(a)). As indicated in [17], for Oxyphor R2, the extinction coefficient for the Q-band is approximately 19 $mM^{-1}cm^{-1}$ and the quantum efficiency of phosphorescence is approximately 10%. The molecular weight of Oxyphor R2 with or without the carboxylate groups being protonated with $Na^+$ salt is 2794 or 2442, respectively. The amount needed for oxygen measurements is in the range of 15-60 mg/kg body weight.

Pd-*meso*-tetra (4-carboxyphenyl) tetrabenzoporphyrin-dendrimer (Oxyphor G2) on the other hand has Q-band absorption at 631 nm and phosphorescence emission at 805 nm (Figure 3(b)). These near infrared spectral characteristics make Oxyphor G2 prominent for measurements of tissue

oxygenation that require photon penetration into depth of tissue. Its Q-band extinction coefficient is approximately 50 $mM^{-1}cm^{-1}$ and the quantum efficiency of phosphorescence is approximately 12% [17]. The molecular weight of Oxyphor G2 with or without the carboxylate groups being protonated with $Na^+$ salt is 2994 or 2642, respectively. The amount needed for oxygen measurements is in the range of 1-5 mg/kg body weight (around 1 μmol/kg body weight).

*Animal Preparation*

Male Sprague-Dawley rats (260-350 gm) were deprived of food and had free access to water for 12 h before the experiments. All animal experiments were conducted under National Health Research Institutes guidelines for the care and use of laboratory animals. Rats were anesthetized with urethane (1.2 g/kg body wt i.p.) and allowed to breathe spontaneously. The body temperature was maintained at 37 °C with a heating pad. Polyethylene catheters were inserted into the femoral artery for the administration of Oxyphor R2 phosphorescence dye. In the present study, the anesthetized rat was administered intravenously (bolus) with 50 mg/kg body weight of Oxyphor R2 for the hepatic oxygenation measurements.
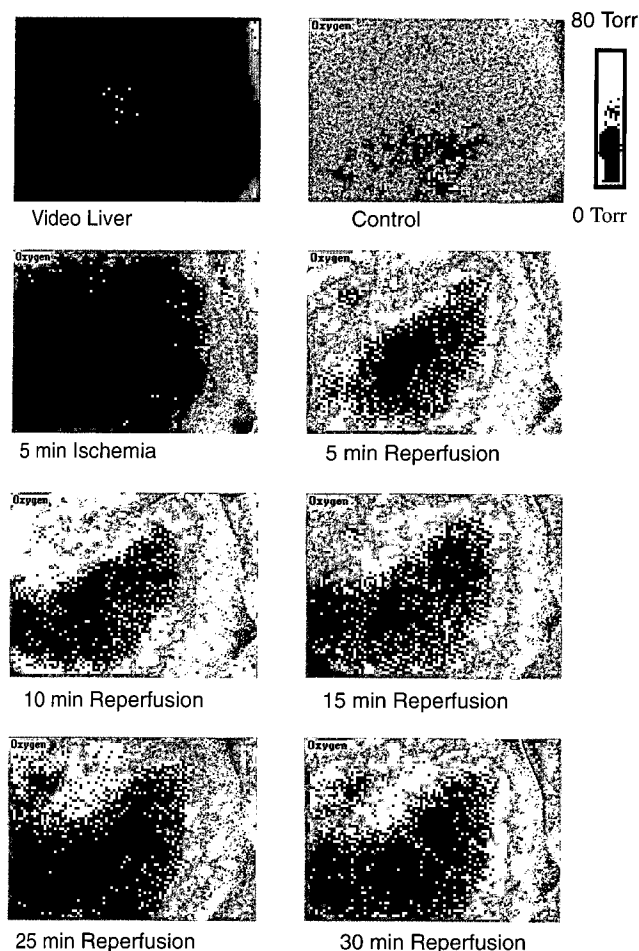
Figure 4. The changes of oxygen distribution in the hepatic micro-vasculature under the PTC model of ischemia and reperfusion measured using phosphorescence imaging. The images were calculated with the maximal oxygen pressure of 80 torr.

After a transverse upper abdominal laparotomy, the liver was mobilized and isolated by dividing all of its attachments with negligible bleeding. Temporal ischemia was introduced with the procedure similar to the portal triad clamping model (PTC model) that used total ligation of the vessels (hepatic artery, portal vein, and bile duct) in the hepatoduodenal ligament.

## Results

### Oxygen Distribution of Hepatic Microvasculature under Ischemia and Reperfusion

The changes of oxygen distribution in the hepatic microvasculature were mapped under the course of ischemia and reperfusion using the PTC model (Figure 4). The imaged area of rat liver was first taken using reflected room light under live video mode of ICCD. This picture of rat liver can be superimposed on the oxygen pressure maps to correlate the measured oxygen pressures with morphological features of the liver. Before the PTC mode ligation, the oxygen pressure distribution measured by the phosphorescence imaging system was heterogeneously over the liver from 30 to 55 mmHg. In contrast, the image taken at 5 min after the onset of PTC mode ligation showed the decrease of oxygen pressures to the range of 20 to 40 mmHg, with distinct areas of oxygen pressure marked even below 10 mmHg. During reperfusion, the oxygen level of the liver heterogeneously increased to 60-70 mmHg in some parts at 10 min after reperfusion and then gradually decreased to a stable level of 20-40 mmHg. All the oxygen distribution maps of rat liver were acquired from the procedure of sequential conversion from original intensity image and its derived lifetime image, as delineated in Materials and Methods. As demonstrated in Figure 5, for the liver undergone 20 min reperfusion, a series of intensity images were captures with a specific delay time sequence selected for Oxyphor R2: 20 μsec, 40 μsec, 80 μsec, 160 μsec, 300 μsec, 480 μsec, 760 μsec, and 2500 μsec. The prominent ischemic area of the liver was indicated and contoured by the emerging phosphorescence intensity after the 300 μsec intensifier gating delay, for which it corresponded to the liver area with oxygen pressure around 20 mmHg. The image collected after a delay of 2500 μsec was used as the background and subtracted from each of the other phosphorescence images to prevent any light intensity arisen from non-phosphorescence sources into the integrated images.

### Quenching Properties of Oxyphor G2: A Near Infrared Phosphorescence Probe

In addition to the Oxyphor R2 used as the phosphorescence probe for current study, there is a Pd-*meso*-tetra (4-carboxyphenyl) tetrabenzoporphyrin- dendrimer called Oxyphor G2 ready for measurements of oxygen-dependent quenching of phosphorescence. As described in Materials and Methods, Oxyphor G2 has a near infrared spectral characteristic which advances its application to the oxygen measurement at depth in tissue. Based on the same parent porphyrin molecule, another near infrared phosphor of Pd-*meso*-tetra (sulfophenyl) tetrabenzoporphyrin, termed Green 2W, has been use for oxygen distribution imaging of tumor using transillumination through thickness of body tissue [19]. To further advance Oxyphor G2 to similar applications, the quenching properties of it need to be determined. In Figure 6, it showed the relationship of the phosphorescence lifetime ratio ($\tau^0/\tau$) to the oxygen pressure for Oxyphor G2 in phosphate buffer solution at various pH (A) and temperature (B). The quenching constant was obtained form the best fit of the data [17]. For Oxyphor G2 at 38°C, the quenching constant showed no significant dependence on pH from 6.4 to 7.8, encompassing most of the pH range in mammalian systems where the oxygen measurement may engage (Figure 6(a)). On the other hand, at pH 7.4, the quenching constant of Oxyphor G2 increased as temperature increased from 23°C to 38°C, with the slope about 2.5%/°C (Figure 6(b)).
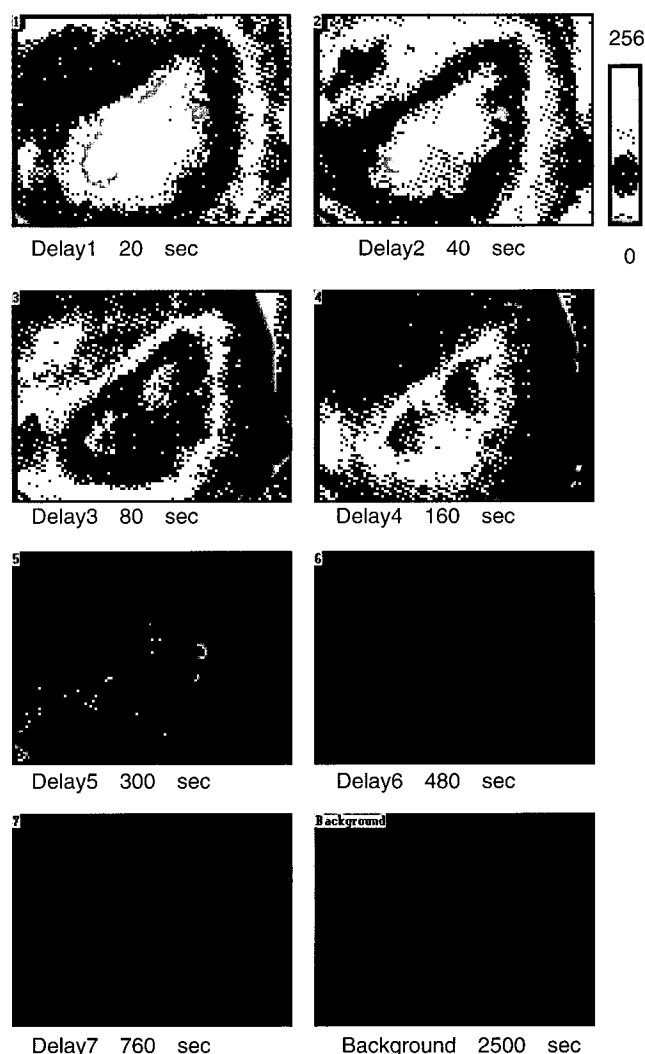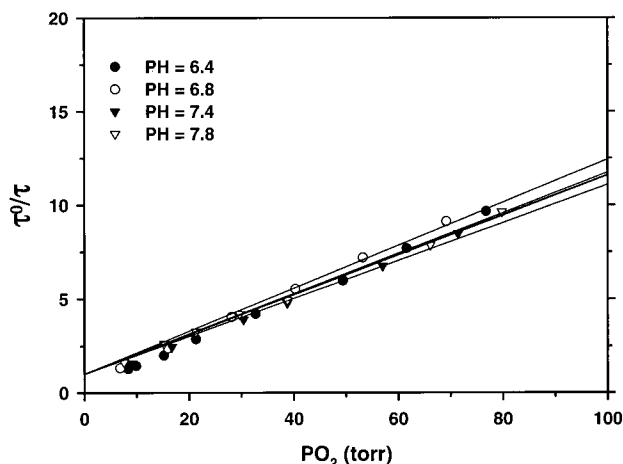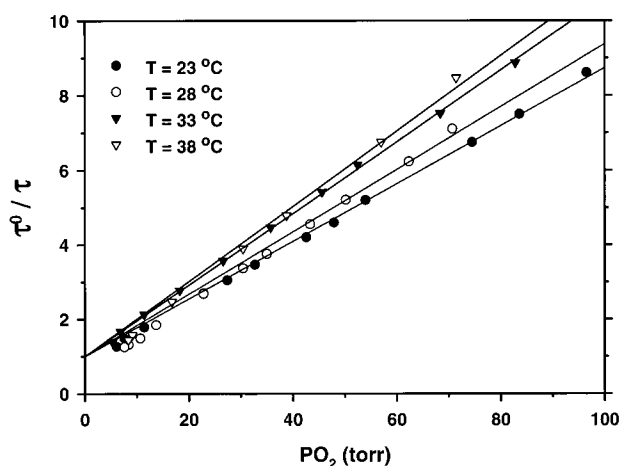
Figure 5. A demonstration of serial intensity images taken with the delay time sequence arranged specifically for Oxyphor R2. The ischemic area of the liver was prominent with the image taken after 300 μsec delay time. The palette represented the 256 intensity levels (8 bits) digitized by the image processor board.



Figure 6. The relationship of the phosphorescence lifetime ratio ($\tau^0/\tau$) to the oxygen pressure for Oxyphor G2 in phosphate buffer solution at various pH (a) and temperature (b).

## Discussion

Measurement of phosphorescence lifetime is a better indicator of quencher concentration than is measurement of phosphorescence intensity because light intensity measurements can be affected by several processes that otherwise does not affect the lifetime measurements. The most common difficulty with intensity measurements is that: 1. They are influenced by other chromophors in the system that absorbs either the excitation or emitted (phosphorescence) light. 2. The intensity of phosphorescence cannot be distinguished from fluorescence emitted in the detected wavelength range. Neither of these processes affects phosphorescence · lifetime measurements. Lifetime measurements are concerned only with the rate of decrease of the light emission following a flash of excitation light, and are not dependent on the absolute intensity of the emitted light. It should be also pointed out that the phosphorescence intensity may deviate from Stern-Volmer equation while certain quencher (e.g. oxygen) appears at high concentration. Because this high concentration of quencher may increase intersystem crossing, it results in the higher phosphorescence intensity than the prediction value. But the measurement of lifetime of the triplet state is not affected by this phenomenon [12].

In order to calculate the oxygen pressure, the quenching constant and the lifetime in the absence of oxygen must be accurately known. These two constants are dependent on the parameters which determine the molecular environment of the phosphor, including the solvent, ionic strength, ion composition, temperature and intermolecular associations of the phosphor with other molecules [11]. Determination of the dependence of $k_Q$ and $\tau^0$ on these physiologically important parameters is essential to obtaining useful calibration of the phosphor.

As a method for measuring oxygen, phosphorescence

quenching has several desirable characteristics. First, it is a noninvasive optical method. When phosphors are dissolved in the blood, phosphorescence can be used for oxygen measurements in living tissue. Second, the response time is very short, around milliseconds, and thus the measurements can be made in real time and are capable of following even very rapid transients in oxygen pressure. Third, if the phosphorescence quenching is well behaved, the dependence of phosphorescence lifetime on oxygen pressure follows a well-defined physical relationship, which can readily be expressed in a linear equation. This greatly facilitates data analysis. Fourth, in biological systems phosphorescence quenching is highly specific for oxygen.

A fifth, and very valuable, characteristic of this method is that the calibration is dependent only on the phosphor and its molecular environment. When the environmental parameters are held constant, the calibration is absolute. This means that, unlike oxygen electrodes and most of the other available methods for measuring oxygen, once the calibration constants are determined they can be generally applied, independent of the operator or, within limits, the preparative procedures [20]. The calibration constants including the quenching constant and lifetime in the absence of oxygen need to be determined for physiological ranges of pH, temperature and ionic strength. They can therefore be used by anyone wishing to make oxygen measurements under similar experimental conditions, including measurements of the oxygen pressure in blood *in vivo*. These values may also be used for verification of the performance of the phosphorescence measuring device and/or instrument settings.

The phosphorescence lifetime map was calculated from the change in intensity with time after the flash and as such is independent of the absolute value of the phosphorescence intensity. The calculations require, however, that the phosphorescence intensity is sufficiently large that the digitized values for at least three delay times allow reliable mathematical operations (the image process board digitizes only on 256 intensity levels). As calculations are made of regions with lower phosphorescence intensities, the values for the lifetime progressively become noisy (poor correlation coefficients). When the phosphorescence values become very low, the lifetime values are no longer meaningful.

The measurements have been limited to the surface layer of the tissue because the absorption bands of the phosphor, Pd-*meso*-tetra-(4-carboxyphenyl)-porphyrin, or its derivatives such as Oxyphor R2, were all at wavelengths less than about 540 nm. At these wavelengths, absorption of the excitation light by chromophores in the tissue, notably hemoglobin, myoglobin, and cytochrome, limits the depths in tissue to which the excitation light can penetrate (and thereby phosphorescence measurements) to about 1 mm or less. The emitted phosphorescence, in contrast, has a maximum at 695 nm, where the tissue chromophores absorb much less, and can penetrate much greater thicknesses of tissue. Vinogradov and Wilson (1995) have reported synthesis of a group of oxygen sensitive phosphors (tetrabenzoporphyrins) with absorption maxima near 636 nm and phosphorescence maxima near 800

nm. Its derivative such as Oxyphor G2 has been developed as well [17], and whose physical properties have been investigated in our present study. By using near infra-red phosphors, it is possible to image tissue oxygen distribution through centimeter thicknesses of tissue (such as through the abdomen of an adult mouse). Thus, phosphorescence quenching can be further used both to detect the presence of small regions of hypoxia embedded in much larger volumes of normal tissue and to quantify the oxygen pressure in such hypoxic tissue volumes.

The oxygen distribution of rat liver under ischemia and reperfusion was used as an imaging model to demonstrate the capability of phosphorescence imaging. The real-time monitoring of hepatic oxygenation showed its responses to ischemia below 10 mmHg and to following reperfusion as a transient increase from approximate 60-70 mmHg back to 20-40 mmHg. This observation was consistent to the previous report that the postischemic hepatic blood flow could not fully restore to the level as that before ischemic insult, no matter of varying periods of ischemia [18]. Nevertheless, the differential contributions from hepatic artery and portal venous blood flow need to be further identified.

## Conclusion

In the present study, we provided the implementation of a real-time oxygen imaging system that is especially suitable for monitoring of tissue oxygenation varying with fast changes of pathophysiological conditions, such as ischemia and reperfusion injury. Both phosphors of Generation 2 polyglutamic Pd-porphyrin-dendrimers, Oxyphor R2 and Oxyphor G2 that bearing 16 carboxylate groups on the outer layer have been used for oxygen distribution imaging of rat liver and calibrated for important physical properties, respectively. The near infrared spectral characteristic of the latter implicates its future application on non-invasive oxygen measurement through thickness of body tissue. With the current imaging system based on oxygen-dependent quenching of phosphorescence, the real-time investigation of correlation of oxygen distribution in tissue microvasculature and local or systemic physiological disturbance can be detailed analyzed.

## Acknowledgement

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# 磷光造影系統監測老鼠肝臟氧含量於缺血及再灌流之變化

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## 摘　　要

　　氧焠冷磷光為一非侵襲式之光學方法，並可應用於活體之氧濃度量測。而應用此一原理發展二維之氧濃度分佈造影系統，對研究組織中微血管系統氧氣之輸送動力學有極大的助益。我們藉由高速之 ICCD 攝影機，考慮特定使用之磷光分子對氧焠冷之動力常數與其在無氧狀態時之磷光生命期，設定 ICCD 攝影機之取光樣的延滯啟動時間順序，以完整擷取造影區中每一影點在不同 ICCD 啟動時間相對應之磷光強度。由數位儲存之磷光強度影像，於每一影點以單指數衰退計算，將磷光強度轉換為生命期。最後，以 Stern-Volmer 方程式為基礎，由磷光生命期計算出相應之氧濃度分佈影像。我們以兩種多 glutamate 樹狀修飾之鈀紫質為研究使用之磷光分子。並使用其中一種命名為 Oxyphor R2 之可見光激發的磷光分子注射於老鼠體內，以磷光造影系統監測其肝臟氧含量在缺血及再灌流狀況下之變化。實驗所得之即時氧氣分佈影像顯示，肝臟中氧含量在缺血後之再灌流期間，只有暫時性的回復，並不能完全回復到缺血前之氧含量；此結論與缺血及再灌流下肝臟血流回復狀態相似吻合。另一種名為 Oxyphor G2 之磷光分子，因其具備近紅外光激發之光譜特性，同時其對氧焠冷之動力常數不受環境酸鹼度的影響，這些特性驗證其應為一相當適合活體組織氧濃度測量之磷光分子，而近紅外光對組織之高穿透性的性質，更使 Oxyphor 2 特別適用於組織深部之氧濃度的造影與分析。

**關鍵詞**：磷光生命期造影、肝臟氧含量測量、缺血及再灌流

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