Noninvasive investigation of blood oxygenation dynamics of tumors by near-infrared spectroscopy

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The measurement of dynamic changes in the blood oxygenation of tumor vasculature could be valuable for tumor prognosis and optimizing tumor treatment plans. In this study we employed near-infrared spectroscopy (NIRS) to measure changes in the total hemoglobin concentration together with the degree of hemoglobin oxygenation in the vascular bed of breast and prostate tumors implanted in rats. Measurements were made while inhaled gas was alternated between 33% oxygen and carbogen (95% O₂, 5% CO₂). Significant dynamic changes in tumor oxygenation were observed to accompany respiratory challenge, and these changes could be modeled with two exponential components, yielding two time constants. Following the Fick principle, we derived a simplified model to relate the time constants to tumor blood-perfusion rates. This study demonstrates that the NIRS technology can provide an efficient, real-time, noninvasive means of monitoring the vascular oxygenation dynamics of tumors and facilitate investigations of tumor vascular perfusion. This may have prognostic value and promises insight into tumor vascular development. © 2000 Optical Society of America

1. Introduction

The presence and the significance of tumor hypoxia have been recognized since the 1950’s. There is increasing evidence that tumor oxygenation is clinically important in predicting tumor response to radiation, tumor response to chemotherapy, overall prognosis, or all three. Hypoxic cells in vitro and in animal tumors in vivo are documented to be 3 times more resistant to radiation-induced killing compared with aerobic cells.1 Recent studies show that hypoxia may have a profound impact on malignant progression and on responsiveness to therapy.2,3 Numerous studies on tumor oxygen tension (pO₂) measurements have been conducted in recent years by use of a variety of methods, such as microelectrodes,2 phosphors,4 electron paramagnetic resonance,5 or magnetic resonance imaging6 (MRI). Comparing needle-based, oxygen-sensitive electrodes or electron paramagnetic resonance and MRI for measuring pO₂ shows that the latter two offer the advantage of facilitating multiple repeated measurements to map pO₂ noninvasively. However, magnets are large, and the methods are not readily portable. A versatile method for monitoring intra-tumor oxygenation rapidly and noninvasively is therefore very desirable for tumor prognosis and tumor treatment planning.

In the near-infrared (NIR) region (700–900 nm) the major chromophores in tissue are oxygenated hemoglobin and deoxygenated hemoglobin, which differ in their light absorption. Measurements of the absorption of light travelling through the tissue under study allow us to evaluate or quantify blood oxygenation, such as the concentrations of oxygenated hemoglobin (HbO₂), and deoxygenated hemoglobin (Hb) and the hemoglobin saturation SO₂. In the past decade, three forms of NIR spectroscopy (NIRS) that use pulsed-laser light in the time domain, amplitude-modulated laser light in the frequency domain, and cw light in a dc form were developed for blood oxygenation quantification in tissue.7 Significant investigations in both laboratory and clinical settings by use of NIRS were conducted for noninvasive, quantitative measurements and imaging of cerebral oxygenation8–12 and blood oxygenation of exercised
muscle\textsuperscript{13–17} \textit{in vivo}. Although NIR techniques were used extensively in conjunction with cryospectrophotometry to investigate tumor blood-vessel oxygenation in biopsies,\textsuperscript{18} only a few reports\textsuperscript{19–22} were published on using the NIR techniques for monitoring tumor oxygenation \textit{in vivo}. In principle, the theoretical model, i.e., the diffusion approximation to the photon transport theory, works well for only large and homogeneous media.\textsuperscript{23,24} Accurate quantification of tumor oxygenation by use of the NIR approach is limited because of the considerable heterogeneity and the finite sizes of tumors.

It is understood and documented\textsuperscript{25} that the NIR technique used for blood oxygenation monitoring is sensitive to vascular absorption in the measured organ. The NIR method is not limited to measurements of blood oxygenation in arteries (c.f., pulse oximetry) or in veins but interrogates blood in the entire vascular compartment, including capillaries, arterioles, and venules, i.e., the vascular bed. A variety of terms like cerebral oxygenation, tissue hemoglobin oxygenation, and mean hemoglobin oxygenation are used in the literature\textsuperscript{7,24,26} to indicate this concept. Although tissue hemoglobin oxygenation is not rigorous because hemoglobin molecules are located in only blood, the term is used specifically to differentiate between the hemoglobin saturation in the tissue vascular bed, as measured by the NIR method, and the arterial hemoglobin saturation S\textsubscript{a}O\textsubscript{2}, as measured by a pulse oximeter.

The goal of this paper is to demonstrate the NIR technique as a real-time, noninvasive means of monitoring hemoglobin oxygenation dynamics, i.e., changes in the concentrations of total hemoglobin (Hb\textsubscript{t}) and oxygenated hemoglobin (HbO\textsubscript{2}), in the vascular bed of breast and prostate rat tumors in response to respiratory challenge. Compared with previous NIR studies of tumors \textit{in vivo}, our approach has the following features: (1) The transmission mode, as opposed to the reflectance mode used by Hull \textit{et al.},\textsuperscript{22} interrogates deeper regions (central parts) of the tumor. (2) Only two wavelengths, as opposed to the spectrum of 300–1100 nm used by Steen \textit{et al.},\textsuperscript{21} are employed and provide a fast and low-cost instrument. (3) A source–detector separation of 1–2 cm interrogates a large tumor noninvasively, as opposed to the needlelike probe used by Steinberg \textit{et al.}.\textsuperscript{20} More innovatively, on the basis of the experimental observation of tumor hemoglobin oxygenation dynamics, we developed a tumor hemoperfusion model that provides important insight into tumor blood perfusion.

This paper is organized as follows: In Section 2, we describe our animal model, the NIR instrument, and the algorithm for calculations of tumor blood oxygenation. In Section 3, we show experimental results measured from both breast and prostate tumors under respiratory interventions and calculate time constants for the hemoglobin oxygenation dynamics of the tumors. In Section 4, we develop a tumor hemoperfusion model to interpret the experimental data obtained in the tumor-intervention studies and to relate the time constants to tumor blood perfusion. Finally, in Section 5, we discuss the results, the future extensions, and the potential uses of the NIR technique as a novel diagnostic–prognostic tool for tumor therapy and cancer research.

2. Materials and Methods

A. Animal Model and Measurement Geometry

NF13762 breast tumor was implanted in adult female Fisher rats, and Dunning prostate adenocarcinoma R3327-AT1 was implanted in adult male Copenhagen rats. The tumors were grown in pedicles\textsuperscript{27} on the forebacks of the rats until the tumors were approximately 1–2 cm in diameter. Rats were anesthetized with 200-\textmu l ketamine hydrochloride (100 mg/ml) and maintained under general gaseous anesthesia with 33\% inhaled O\textsubscript{2} (0.3 dm\textsuperscript{3}/min O\textsubscript{2}, 0.6 dm\textsuperscript{3}/min N\textsubscript{2}O, and 0.5\% methoxyflurane) through a mask placed over the mouth and nose. Tumors were shaved to improve the optical contact for transmitting light. Body temperature was maintained with a warm-water blanket. In some cases, a fiber-optic pulse oximeter (Nonin, Inc., Model 8600V) that was manufacturer calibrated was placed on the hind foot to monitor arterial oxygenation S\textsubscript{a}O\textsubscript{2}, and a fiber-optic probe was inserted rectally to measure temperature. The tumor volume V (in centimeters cubed) was estimated as \[ V = \left(\frac{4\pi}{3}\right) \left[ L + \frac{W + H}{2}\right]^\frac{3}{2}, \] where \( L, W, \) and \( H \) are the three respective orthogonal dimensions.

Most measurements were performed with 33\% oxygen as inhaled gas to achieve a stable baseline for a period of 5 to 15 min. The inhaled gas was then switched to carbogen (95\% oxygen, 5\% carbon dioxide) for at least 20 min and then switched back to 33\% O\textsubscript{2} for approximately 15 min. The complete cycle lasted 1 hour. Sometimes repeated carbogen interventions were performed sequentially to evaluate the reproducibility of the time profiles of the tumors. In certain cases alternative gases were used, as defined in the results and figures, and some rats were sacrificed by KCl-induced cardiac arrest.

Figure 1 shows the measurement geometry: Horizontally, the delivering and the detecting fiber bundles were face to face in the transmittance mode, and both were in contact with the tumor surface without hard compression. The separation of the two bundle surfaces was between 1.0 and 2.5 cm, depending on the tumor size. Vertically, the two bundle tips (with diameters of 0.5 cm) were placed around the middle of the tumor. Thus the current setup of the probes provides an optimal geometry for the NIR light to interrogate deep tumor tissue with minimal interference from the forehead of the rat.

B. Near-Infrared Instrument and Data Analysis

As shown in Fig. 1, we used a homodyne frequency-domain photon-migration system\textsuperscript{28,29} that was capable of determining the amplitude and the phase changes of amplitude-modulated light passing through tumors. In this setup a rf source modulates
the light from two laser diodes (wavelengths of 758 and 782 nm) at 140 MHz. The laser light passes through a combined fiber-optic bundle, is transmitted through the tumor tissue, and is collected by a second fiber bundle. The light is then detected by a photomultiplier tube and demodulated with a commercially available in-phase and quadrature (IQ) demodulator chip into its I and Q components. After these components are put through a low-pass filter they can be used to calculate the amplitude and the phase changes caused by the tumor. These steps are expressed mathematically by

\[
I(t) = 2A \sin(\omega t + \theta) \sin(\omega t)
\]

\[
= A \cos(\theta) - A \cos(\omega t + \theta) \frac{\text{low pass}}{I_{dc}}
\]

\[
= A \cos(\theta),
\]

\[
Q(t) = 2A \sin(\omega t + \theta) \cos(\omega t)
\]

\[
= A \sin(\theta) + A \sin(\omega t + \theta) \frac{\text{low pass}}{Q_{dc}}
\]

\[
= A \sin(\theta),
\]

\[
\theta = \tan^{-1}(Q_{dc}/I_{dc}),
\]

\[
A = (I_{dc}^2 + Q_{dc})^{1/2},
\]

where \( A \) and \( \theta \) are the amplitude and the phase of the detected light, respectively, and \( \omega \) is the angular modulation frequency (\( = 2\pi \times 140 \) MHz).

The two laser lights were time shared, and the controlling process and the data acquisition both interfaced through a 12-bit analog-to-digital board (Real Time Devices, Inc., Model AD2100) with a maximum sampling rate of 4 Hz. However, slower sampling rates were used in measurements to compensate for experimental noise. Simple time averaging among a few adjacent data points was performed during data analysis to further decrease the noise. However, data smoothing was not applied for (1) calculating the experimental uncertainty (error bars) or (2) fitting the time constants to prevent the fast-changing component from being oversmoothed and overlooked. The pulse-oximeter data were not averaged because they were recorded manually and appear discrete compared with the NIR data. The experimental uncertainties for arterial saturation and changes in hemoglobin concentrations were calculated by use of the baseline data taken over 5–10 min without respiratory perturbation to the rat. Nonlinear curve fitting based on the Marquardt algorithm was performed by use of KaleidaGraph. The software also provided the errors (or uncertainties) for each fitted parameter, the optimized \( \chi^2 \) values, and the fitting correlation coefficient \( R \), together with the goodness of the fit \( R^2 \). The significance of changes was assessed on the basis of Fisher protected-least-significant-difference analysis of variance by use of Statview software.

C. Calculation for Changes in the Hemoglobin Concentration

It is well known that the NIRS of tissue can be used to determine the total hemoglobin concentration \( H_b \), and the hemoglobin oxygen saturation \( S_O_2 \) of an organ in vivo. When two NIR wavelengths are used (758 and 782 nm, in this case) it is assumed that tissue background absorbance is negligible and that the major chromophores in organs are oxygenated and deoxygenated hemoglobin molecules. In principle, because the IQ system can give both phase and amplitude values, we should be able to obtain absolute calculations of HbO2, Hb, and \( S_O_2 \). However, given the tumor’s small size and large spatial heterogeneity, it is very difficult to obtain such absolute quantification accurately with conventional algorithms that are based on the diffusion approximation. Instead, on the basis of the modified Beer-Lambert law, we can use the amplitude of the light transmitted through the tumor to calculate concentration changes in HbO2, Hb, and Hb (expressed as \( \Delta H_b \), \( \Delta H_b \), \( \Delta H_b \), respectively) of the tumor that are caused by respiratory intervention. These changes can be derived and expressed as (see Appendix A for derivations and justifications)

\[
\Delta H_b = H_b(\text{transient}) - H_b(\text{baseline})
\]

\[
= \frac{\varepsilon_{HbO_2}^{k_1} \log A_{k_1} - \varepsilon_{HbO_2}^{k_2} \log A_{k_2}}{L(\varepsilon_{Hb}^{k_1} \varepsilon_{HbO_2} - \varepsilon_{Hb}^{k_2} \varepsilon_{HbO_2})},
\]

where \( L \) is the optical path length of the tumor, \( \varepsilon_{HbO_2} \) and \( \varepsilon_{Hb} \) are the molar absorptivity of HbO2 and Hb, respectively, and \( A \) is the absorbance of the tissue at the wavelength of the laser light.
\[
\Delta \text{HbO}_2 = \text{HbO}_2(\text{transient}) - \text{HbO}_2(\text{baseline})
\]
\[
= \frac{\epsilon_{\text{Hb}} \log \left( \frac{A_b}{A_t} \right)_{758} - \epsilon_{\text{HbO}_2} \log \left( \frac{A_b}{A_t} \right)_{782}}{L (\epsilon_{\text{HbO}_2} - \epsilon_{\text{Hb}})}
\]
where \(\epsilon_{\text{Hb}}\) and \(\epsilon_{\text{HbO}_2}\) are extinction coefficients\(^{35}\) of deoxygenated and oxygenated hemoglobin, respectively, at wavelength \(\lambda\); the variable \(A_b\) is a constant amplitude of baseline; \(A_t\) is the transient amplitude under measurement; and \(L\) is the optical path length between the source and the detector.

Using the approach suggested by Cope and Delpy,\(^{10}\) we can express \(L\) as \(L = \text{DPF} \times d\), where \(d\) is the direct source–detector separation in centimeters and DPF is the ratio between the optical path length and the physical separation and is tissue dependent. The DPF for tumors has not been well studied; for simplicity, we assume the DPF to be 1 in our calculations. The justification for this simplification is given in Section 5. After substituting the extinction coefficients\(^{35}\) at 758 and 782 nm in Eqs. (5) and (6) with values of \(\epsilon_{\text{Hb}} = 0.359\), \(\epsilon_{\text{HbO}_2}^{758} = 0.1946\), \(\epsilon_{\text{HbO}_2}^{782} = 0.265\) and \(\epsilon_{\text{HbO}_2} = 0.178\), respectively, in units of inverse millimoles times inverse centimeters, we arrive at
\[
\Delta \text{Hb} = \frac{[7.34 \log(A_b/A_t)_{758} - 6.17 \log(A_b/A_t)_{782}]}{L},
\]
(7)
\[
\Delta \text{HbO}_2 = \frac{[-10.92 \log(A_b/A_t)_{758} + 14.80 \log(A_b/A_t)_{782}]}{L},
\]
(8)
\[
\Delta \text{Hb}_t = \Delta(\text{HbO}_2 + \text{Hb})
\]
\[
= \frac{[-3.58 \log(A_b/A_t)_{758} + 8.63 \log(A_b/A_t)_{782}]}{L},
\]
(9)
where the units are in millimoles. Equations (7) and (8) permit the calculation of changes in Hb and HbO\(_2\) that are due to respiratory challenge, respectively, whereas Eq. (9) quantifies a relative increase in the total hemoglobin concentration that is caused by the intervention. The last quantity also reflects a change in blood volume because it is proportional to the total Hb concentration.

3. Results

A. Instrument Drift Tests

The stability of the NIR instrument was tested in terms of baseline drift after a warm-up period of 30 min by use of a tissue phantom\(^{25,36}\) with stable optical properties. Figure 2 shows an example of a phantom measurement that displays the variation of relative changes in apparent HbO\(_2\) and Hb concentrations, as calculated from Eqs. (8) and (9). In this example, the standard deviations over the entire period of 100 min were less than 0.007 and 0.004 mM, respectively, for \(\Delta \text{HbO}_2\) and \(\Delta \text{Hb}\). Furthermore, we calculated uncertainties for both of these quantities on the basis of the propagation of errors, and the results are consistent with those shown in Fig. 2.

B. Breast Tumors

Figure 3(a) shows the results taken from a breast tumor (4.5 cm\(^3\)) with a source–detector separation of 1.8 cm. The data were smoothed, and the measurement uncertainties are shown at only discrete locations. The figure shows the relative changes in total hemoglobin concentration \(\Delta \text{Hb}\) and oxygenated hemoglobin concentration \(\Delta \text{HbO}_2\). The arterial Hb saturation was also obtained to show a relatively rapid change in arterial signals when the inhaled gas was switched from 33% O\(_2\) to carbogen. Respiratory challenge caused a sharp rise in \(\Delta \text{HbO}_2\) (\(p < 0.01\) after 1 min, \(p < 0.0001\) by 1.5 min) that was followed by a further slow, gradual, but significant, increase over the next 25 min (\(p < 0.001\)). \(\Delta \text{Hb}\) also changed significantly (\(p < 0.001\)) within the first minute, but the total change was only approximately 10% of that of \(\Delta \text{HbO}_2\). Given the exponential appearance of the rising part of \(\Delta \text{HbO}_2\), we used single-exponential and double-exponential expressions to fit the data in the rising portion to better understand and quantify the dynamic features of \(\Delta \text{HbO}_2\). The unsmoothed data and the fitted curves are shown in Figure 3(b). The double exponential appears to give a much better fit, as is confirmed by the respective \(R\) values (0.98 versus 0.81). Time constants of 0.18 ± 0.02 min and 27.8 ± 3.9 min were obtained for fast and slow dynamic changes, respectively, in the tumor HbO\(_2\) concentration.

Figure 4(a) was obtained from a second breast tumor (5.9 cm\(^3\)) with a source–detector separation of 1.6 cm. Here \(\Delta \text{HbO}_2\) increased rapidly after the ini-
tial gas switch but did not exhibit the continued slow rise afterward. $\Delta$Hb was found to increase with carboxen inhalation, although the magnitude was smaller than that of $\Delta$HbO$_2$ during the period of the intervention. Again, changes in $\Delta$HbO$_2$ were modeled by a single-exponential term that yielded a time constant of 2.00 ± 0.04 min ($R = 0.97$) and by a double-exponential formula with two time constants of 0.8 ± 0.2 min and 3.0 ± 0.3 min ($R = 0.98$). In this case both expressions fit the data well, as shown in Fig. 4(b).

To demonstrate the reproducibility of the dynamic changes in response to respiratory challenge, we subjected one animal to repeat carbogen inhalation. Figure 5(a) shows measurements taken from a breast tumor (6.7 cm$^3$) with a source–detector separation of 2 cm. In this case air with 1.2% isoflurane (anesthetic) was used as the baseline instead of 33% O$_2$. This figure shows a very consistent pattern in two repeated time responses with a fast and a slow increase in $\Delta$HbO$_2$. Here $\Delta$Hb$_t$ shows a similar dynamic pattern, i.e., a rapid rise followed by a slow continuation. Figures 5(b) and 5(c) show the unsmoothed data together with the fitted curves for the rising portions of the two repeated increases in $\Delta$HbO$_2$. Again, the double-exponential expression with two time constants produced much better fits than did the single-exponential term in both processes with two averaged time constants of $\tau_1$ (mean) = 0.26 ± 0.11 min and $\tau_2$ (mean) = 8.2 ± 1.8 min. Individual, respective time constants and coefficients are summarized in Table 1. Furthermore, single-exponential and double-exponential expressions were fitted to obtain time constants for the decay processes after the inhaled gas was switched repeatedly back to the baseline conditions. Similarly, the double-exponential expression fits the data better with two mean time constants of $\tau_1^{\text{decay}}$ (mean) = 0.17 ± 0.07 min and $\tau_2^{\text{decay}}$ (mean) = 12.2 ± 0.7 min for the two decay processes.

To further validate our experimental observations, we subjected some rats to cardiac arrest (with KCl) to observe the changes in HbO$_2$ and Hb$_t$ on death. Figure 6 shows an example of cardiac arrest on a rat with
Relative changes in the HbO2 detected with the NIR instrument from a rat breast tumor (6.7 cm³) while the breathing gas was alternated between air (21% O2) and carbogen. The best fits to the HbO2 data by use of both the double-exponential and the single-exponential expressions for (b) the first and (c) the second respiratory challenges are shown. The fitted equations that were obtained from (b) are 0.232 \( \exp\left(-t/21.9\right) + 0.18 \exp\left(-t/0.368\right) \), with \( R = 0.98 \), and 0.368 \( \exp\left(-t/21.9\right) + 0.232 \exp\left(-t/0.368\right) \), with \( R = 0.89 \), respectively. The fitted equations that were obtained from (c) are 0.321 \( \exp\left(-t/67\right) + 0.332 \exp\left(-t/9.47\right) \), with \( R = 0.99 \), and 0.485 \( \exp\left(-t/67\right) + 0.321 \exp\left(-t/9.47\right) \), with \( R = 0.81 \), respectively.

![Graph showing relative HbO2 changes](image)

Table 1. Summary of the Vascular Oxygen Dynamics

<table>
<thead>
<tr>
<th>Tumor Type</th>
<th>Volume (cm³)</th>
<th>( A_1 ) (mM)</th>
<th>( A_2 ) (mM)</th>
<th>( \tau_1 ) (min)</th>
<th>( \tau_2 ) (min)</th>
<th>( R^2 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breast (Fig. 3)</td>
<td>4.5</td>
<td>0.18±0.02</td>
<td>0.27±0.03</td>
<td>27.8±3.9</td>
<td>1.43±0.03</td>
<td>0.36±0.03</td>
</tr>
<tr>
<td>Breast (Fig. 4)</td>
<td>5.9</td>
<td>0.18±0.02</td>
<td>0.27±0.07</td>
<td>3.0±0.3</td>
<td>0.09±0.02</td>
<td>0.16±0.04</td>
</tr>
<tr>
<td>Breast (Fig. 5)</td>
<td>6.7</td>
<td>0.18±0.02</td>
<td>0.36±0.12</td>
<td>6.93±0.12</td>
<td>0.09</td>
<td>0.16±0.04</td>
</tr>
<tr>
<td>Prostate (Fig. 7)</td>
<td>8.2</td>
<td>0.18±0.02</td>
<td>0.36±0.12</td>
<td>6.93±0.12</td>
<td>0.09</td>
<td>0.16±0.04</td>
</tr>
<tr>
<td>Prostate (Fig. 8)</td>
<td>10.8</td>
<td>0.18±0.02</td>
<td>0.36±0.12</td>
<td>6.93±0.12</td>
<td>0.09</td>
<td>0.16±0.04</td>
</tr>
</tbody>
</table>

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a breast tumor (5.3 cm$^3$). Both $\Delta$Hb and $\Delta$HbO$_2$ dropped significantly, immediately after KCL was admitted intravenously. Within 1 min $\Delta$Hb$_t$ reached a plateau, whereas $\Delta$HbO$_2$ decreased rapidly within the first 30 s and then was followed by a slow prolongation.

C. Prostate Tumors

Figure 7(a) was obtained from a large prostate tumor (8.2 cm$^3$). In common with the breast tumors, $\Delta$HbO$_2$ showed a rapid initial increase that was followed by a slower continuation. $\Delta$Hb increased rapidly and then reached a plateau. Figure 7(b) shows that the double-exponential equation fits the unsmoothed data better ($R = 0.96$) than does the single-exponential term ($R = 0.82$). Here the fast and the slow time constants are 0.265 ± 0.007 min and 6.02 ± 0.15 min, respectively.

Figure 8(a) was obtained from another large prostate tumor (10.8 cm$^3$) with a source-detector separation of 2.5 cm. Here $\Delta$HbO$_2$ displayed a gradual increase throughout the entire period of carbogen inhalation, whereas the increase in $\Delta$Hb was considerably delayed. Variations in arterial hemoglobin saturation $S_a$O$_2$ are also shown and were very rapid in comparison with $\Delta$HbO$_2$, in common with Fig. 3. $\Delta$HbO$_2$ dropped rapidly when the inhaled gas was switched back from carbogen to 33% O$_2$. Both the single-exponential and the double-exponential expressions were used to obtain time constants for the rising portion of $\Delta$HbO$_2$ that was due to carbogen intervention. In this case both expressions gave equally good fits, as shown in Fig. 8(b) and Table 1. For the decay process, we obtained $t_1$ decay = 0.6 ± 0.2 min and $t_2$ decay = 6.6 ± 1.7 min with $R = 0.94$ for the double-exponential fitting, whereas the single-exponential fitting resulted in $\tau = 2.8 ± 0.4$ min with $R = 0.88$. For comparison the rat was also challenged with 100% O$_2$.

In summary, we observed dynamic changes in HbO$_2$ that were due to carbogen intervention for both breast and prostate tumors. In most cases these changes were modeled better by a double-exponential expression with a fast and a slow time constant than they were by a single-exponential fitting. Dynamic changes in arterial saturation preceded those in HbO$_2$. The detailed parameters regarding tumor size, fitted time constants, corresponding magnitudes, and $R^2$ are listed in Table 1.

4. Model for the Blood Oxygenation Dynamics of Tumors

As was shown in Section 3, the temporal changes in HbO$_2$ caused by respiratory challenge can be fitted with an exponential equation that has either one or two time constants (fast and slow). In this section, we further derive and simplify a hemoperfusion model to interpret these time constants and to correlate the experimental findings with the physiology of the tumors.

To develop the model, we follow an approach used to measure regional cerebral blood flow (rCBF) with diffusible radiotracers, as originally developed by Kety$^{37}$ in the 1950’s. The basic model was modified in a variety of ways to adapt it to positron emission tomography studies.$^{38,39}$ By analogy, we can evaluate tumor hemodynamics such as tumor blood flow (perfusion) by using the respiratory-intervention gas as a tracer.

Fig. 6. Influence of KCl-induced cardiac arrest on the values of HbO$_2$ and Hb, of a breast tumor (5.3 cm$^3$), while the rat was breathing air.

Fig. 7. (a) Influence of respiratory challenges (switching from air to carbogen) on the values of HbO$_2$ and Hb, of a large rat prostate tumor (8.2 cm$^3$). (b) The best-fitted equations are 0.090[1 − exp(−(t − 12)/0.265)] + 0.064[1 − exp(−(t − 12)/6.02)], with $R = 0.96$, and 0.140[1 − exp(−(t − 12)/1.13)], with $R = 0.82$, for the double-exponential and the single-exponential expressions, respectively.
In Eq. (10b), \( f \) and \( \lambda \) are constants, whereas \( C_i \) is a time-dependent variable that is written as \( C_i(t) \). In principle, the arterial-tracer concentration \( C_{a}(t) \) is a time-varying quantity. If a certain concentration of the arterial tracer is administered continuously starting at time 0, \( C_{a} \) can be expressed mathematically as a constant value of \( C_{a}(0) \) after time 0. Then Eq. (10b) can be solved as

\[
C_i(t) = \lambda C_{a}(0)[1 - \exp(-ft/\lambda)].
\]

Equation (11) indicates that, at time \( t \) after the onset of tracer administration, the local tissue (traditionally brain) \( C_i(t) \) concentration depends on the blood flow \( f \), the arterial time–activity curve \( C_{a}(0) \), and the partition coefficient \( \lambda \).

In response to respiratory intervention, a sudden small change is introduced into the arterial O\(_2\) saturation \( S_{a}O_2 \), and the resulting increase in arterial HbO\(_2\) concentration \( \Delta HbO_2^{\text{artery}} \) can be considered as an intravascular tracer. Following Kety’s method and assuming that changes in dissolved O\(_2\) are negligible, we have

\[
\frac{d}{dt}(\Delta HbO_2^{\text{vasculature}}) = \frac{f(\Delta HbO_2^{\text{artery}} - \Delta HbO_2^{\text{vasculature}})}{\gamma}.
\]

where \( f \) still represents blood flow (or perfusion rate) and \( \gamma \) is defined as a vasculature coefficient of the tumor. The coefficient \( \gamma \) is the ratio of the HbO\(_2\) concentration change in the vascular bed to that in veins and equals \( (\Delta HbO_2^{\text{vasculature}})/(\Delta HbO_2^{\text{vein}}) \). This definition implies that a change in the venous blood oxygenation \( \Delta HbO_2^{\text{vein}} \) is proportional to a change in the Hb oxygenation in the vascular bed, \( \Delta HbO_2^{\text{vasculature}} \).

In Eq. (12), \( f \) and \( \gamma \) are constants, whereas \( \Delta HbO_2^{\text{vasculature}} \) is a time-dependent variable. By analogy to Eq. (11), \( \Delta HbO_2^{\text{vasculature}} \) can be solved rigorously given a constant input \( H_0 \) for \( \Delta HbO_2^{\text{artery}} \) after time 0. Our data (Figs. 3 and 8) demonstrate that changes in the arterial HbO\(_2\) \( (S_{a}O_2) \) are much faster than in the vascular bed. Then solving Eq. (12) leads to

\[
\Delta HbO_2^{\text{vasculature}}(t) = \gamma H_0[1 - \exp(-ft/\gamma)].
\]

Equation (13) indicates that, at time \( t \) after the onset of respiratory intervention, the change in oxygenated hemoglobin concentration in the tumor vasculature \( \Delta HbO_2^{\text{vasculature}}(t) \) depends on the blood perfusion rate \( f \), the arterial oxygenation input \( H_0 \), and the vasculature coefficient of the tumor \( \gamma \).

As indicated by Eq. (8), our NIR instrument is able to measure an increase in the vascular HbO\(_2\) concentration \( \Delta HbO_2^{\text{vasculature}} \). Equation (13) gives an exponential of the same form as that used to fit our experimental data, indicating that the measured
time constant is associated with the blood perfusion rate \( f \) and the vasculature coefficient \( \gamma \) of the tumor in the measured area. If the measured volume involves two distinct regions, then we involve with two different blood-perfusion rates \( f_1 \) and \( f_2 \), two different vasculature coefficients \( \gamma_1 \) and \( \gamma_2 \), or all four. Here it is reasonable to assume that the measured signal results from both regions, as illustrated in Fig. 9. Consequently, Eq. (13) can be modified with a double-exponential expression and two time constants as

\[
\Delta \text{HbO}_2 \text{vasculature}(t) = \gamma_1 H_0 [1 - \exp(-f_1 t/\gamma_1)] + \gamma_2 H_0 [1 - \exp(-f_2 t/\gamma_2)] \\
= A_1 [1 - \exp(-f_1 t/\gamma_1)] + A_2 [1 - \exp(-f_2 t/\gamma_2)],
\]

(14)

where \( f_1 \) and \( \gamma_1 \) are the blood-perfusion rate and the vasculature coefficient, respectively, in region 1, \( f_2 \) and \( \gamma_2 \) are the same for region 2, \( A_1 = \gamma_1 H_0 \), and \( A_2 = \gamma_2 H_0 \). The two time constants are equal to \( \tau_1 = \gamma_1/ f_1 \) and \( \tau_2 = \gamma_2/ f_2 \). Then, if \( A_1, A_2, \) and the two time constants are determined from our measurements, we arrive at the ratios for the two vasculature coefficients and the two blood-perfusion rates:

\[
\frac{\gamma_1}{\gamma_2} = \frac{A_1}{A_2}, \quad \frac{f_1}{f_2} = \frac{A_1/A_2}{\tau_1/\tau_2}.
\]

(15)

With these two ratios, we can obtain insight into the tumor vasculature and blood perfusion. For example, a ratio of \( \gamma_1/\gamma_2 \) near 1 from a measurement implies that the vascular structure of the measured tumor volume is rather uniform. Then the coexistence of two time constants reveals two mechanisms of regional blood perfusion in the tumor. A large time constant implies slow perfusion through a poorly perfused area, whereas a small time constant indicates fast perfusion through a well-perfused area. In the meantime, the ratio of the perfusion rates in these two areas can also be obtained quantitatively. Furthermore, a ratio of \( \gamma_1/\gamma_2 > 1 \) (i.e., \( A_1/A_2 > 1 \)) means that the measured signal results more from region 1 than from region 2 within the measured tumor volume. Therefore, by studying tumor blood oxygenation dynamics and obtaining time constants together with their amplitudes, we can gain important information on regional blood perfusion and vascular structures of the tumor within the measured volume.

Our experimental data (Table 1) reveal that all the measurements can be fitted with the double-exponential model equivalently to or better than the single-exponential fitting. Ratios of \( \tau_1/\tau_2 \), \( \gamma_1/\gamma_2 \), and \( f_1/f_2 \) are also shown in Table 1 for respective cases.

5. Discussion

Using NIRS, we have measured relative changes in Hb and HbO$_2$ in breast and prostate rat tumors in response to respiratory intervention. We have observed that respiratory challenge caused the HbO$_2$ concentration to rise promptly and significantly in both breast and prostate tumors but that the total concentration of hemoglobin sometimes increased and sometimes remained unchanged. The dynamic changes of tumor oxygenation can be modeled by either one exponential term with a slow time constant or two exponential terms with fast and slow time constants. This relation suggests that there may be two vascular mechanisms in the tumor that are detected by the NIRS measurement. As indicated by Eqs. (13) and (14), these time constants are inversely proportional to the blood-perfusion rates of the measured volumes of the tumors. Based on the double-exponential model, determination of the two time constants and their corresponding amplitudes allows us to determine the relations between the two perfusion rates and between the vascular structures, as expressed in Eq. (15). Further investigation with more measured quantities may lead to quantification of each parameter individually by use of the NIRS technique.

To develop a model for interpreting the NIR data taken during carbogen inhalation, we have defined a vasculature coefficient \( \gamma \). It is a proportionality factor between \( \Delta \text{HbO}_2 \text{vein} \) and \( \Delta \text{HbO}_2 \text{vasculature} \), i.e., \( \Delta \text{HbO}_2 \text{vein} = \Delta \text{HbO}_2 \text{vasculature}/\gamma \). We expect that \( \gamma \) depends on (1) the oxygen consumption and (2) the capillary density of the tumor. If the oxygen consumption, the capillary density, or both of the tumor are large, changes in the venous HbO$_2$ concentration will be small; if the oxygen consumption, the capillary density, or both of the tumor are small, changes in the venous HbO$_2$ concentration will be large. Further studies are necessary to learn more about this coefficient and to confirm our speculation.

Our current NIR system allows us to quantify the
ratio of $\gamma/f$ by using the single-exponential model or to
count the ratios of $\gamma_1/\gamma_2$ and $f_1/f_2$ by using the
double-exponential model. We can obtain important
information on the blood perfusion of the tumor:
a large time constant usually represents slow blood
perfusion, whereas a small time constant indicates
fast blood perfusion. The coexistence of two time
constants implies a combination of well-perfused and
poorly perfused mechanisms of blood perfusion.
Indeed, some tumor lines have only 20% to 85% of ves-
sels perfused.41 Furthermore, tumor structures and
oxygen distributions8,42 are highly heterogeneous.
Therefore it is likely that our measurement detects a
well-perfused region, or a poorly perfused region, or a
mixture of both in the tumor, depending on the posi-
tion or the location of the source and the detector of
the NIR instrument.

Hull et al.22 reported carbogen-induced changes in
rat mammary tumor oxygenation by using spatially
resolved NIRS. To compare our results to theirs, we
applied our curve-fitting procedure to their published
hemoglobin saturation curve and obtained a time
constant of 0.27 min (or 16 s) for the rising edge,
which is consistent with our fast component. Their
data do not show a slow component, suggesting that
their measurement was dominated by active tumor
vasculature. This difference may be explained as
follows:

(1) The tumor volume mentioned in the paper by
Hull et al.,22 was approximately 1.5 cm$^3$, much
smaller than the volumes of the tumors that we mea-
sured (in Figs. 3 to 8, the largest tumor was 10.8 cm$^3$,
whereas the smallest tumor was 4.5 cm$^3$).

(2) Their measurement was in reflectance geo-
metry with multiple detectors located at distances 1 to
20 mm away from the source, and in the calculation
the tumor was assumed to be homogenous in order to
use diffusion theory. Thus their measurement was
more sensitive to the superficial area of the tumor,
emphasizing the tumor periphery, which is often bet-
ter vascularized than the central part of the tu-

The fast component observed in our tumors is con-
sistent with the rapid changes detected in $S_0O_2$ in the
leg as measured by use of the pulse oximeter, provid-
ing further evidence that this relates to the well-
vascularized, highly perfused region of the tumor.

The data shown in Fig. 8 in this paper are repre-
sentative of the measurement of a poorly perfused
region in which the measured tumor was large and
the portion for the fast oxygenation response was
small. The data given in Figs. 3–5 and 7 resulted
from a mixture of well-perfused and poorly perfused
areas in the tumors and exhibited a mixture of fast
and slow oxygenation responses to hyperoxgen con-
ditions. It is reasonable to expect that larger tumors
have more poorly perfused regions than do smaller
tumors. The time constant of the slow component
observed here approaches that observed previously
for changes in tissue pO$_2$ in an AT1 prostate tumor
measured by use of $^{19}$F nuclear magnetic resonance
spectroscopy to interrogate interstitial oxygenation.44
In general, well-oxygenated tumor regions had a
large and rapid response to respiratory challenge,
whereas poorly oxygenated regions were much more
sluggish.45 One would indeed expect changes in
vascular oxygenation to precede changes in the tis-
"ue, and combined investigations by NIRS and nu-
clear magnetic resonance spectroscopy in the future
will provide further insight into the delivery of oxy-
gen to tumors.

Dynamic changes in vascular oxygenation have
been assessed previously by several other techniques.
Following the infusion of Green 2W dye intrave-
nously into EMT-6 tumor-bearing mice, Vinogradov et
al.4 were able to image changes in the surface
vascular pO$_2$. On switching from air to carbogen
inhalation, they observed a very rapid increase in pO$_2$
with a rate similar to the fast component, which we
have seen here. Although the phosphorescence
method provides vascular pO$_2$, NIR methods gen-
ernally provide HbO$_2$ or SO$_2$ because there is some un-
certainty in the local affinity of hemoglobin for tumor
oxygen: the pO$_2$–SO$_2$ dissociation curve is subject to
pH, temperature, and other allosteric effectors, such
as 2,3-diphosphoglycerate in the immediate milieu.
A promising new approach is the blood-oxygen-level–
dependent (BOLD) contrast $^1$H MRI, which is sensi-
tive to vascular perturbations. Robinson et al.46
explored the response to respiratory challenge in var-
ious tumors and showed reversible regional changes
on switching from air to carbogen inhalation. In
common with our NIR data, their changes were often
biphasic with a large change occurring within the
first 2 min and followed by slower increases.46 How-
ever, interpreting the BOLD MRI results is compli-
cated by variations in vascular volume and flow, and
there is no direct measure of HbO$_2$ in tumors.

The time constants are not source–detector sepa-
ration sensitive. Equations (8) and (9) have demon-
strated that $\Delta$HbO$_2$ and $\Delta$Hb$_b$ are proportional to 1/d,
where d is the source–detector separation. This re-
lation indicates that a different d value will only
stretch or compress the entire temporal profile of
$\Delta$HbO$_2$, but it does not change the transient behavior
of the time response. The same argument can apply
to the DPF. In this study, we have assumed a DPF
ratio of 1 for simplicity. If the DPF value is larger
than 1, the values of $\Delta$HbO$_2$ and $\Delta$Hb$_b$ will decrease
by a factor of DPF. But this modification does not
affect the time constants $\tau_1$ and $\tau_2$, which constitute
the dynamic responses of $\Delta$HbO$_2$ of the tumors to
respiratory intervention.

Given the evidence for intratumoral heterogeneity
from MRI6,46 and histology,47 we believe it will be
important to advance our NIR system to have mul-
tiple sources, multiple detectors, or both to study not
only dynamic but also spatial aspects of blood oxy-
genation in tumor vasculature. Nonetheless, we be-
lieve the preliminary results described here are a
proof of principle for the technique, laying a founda-
tion for more extensive tests to correlate tumor size
with the rates of change of HbO$_2$ and Hb, with respect to respiratory challenge. Although Hull et al.\textsuperscript{22} and we have focused on respiratory challenge, we note that previous NIR studies of tumors also examined the influence of chemotherapy,\textsuperscript{21} pentobarbital overdose,\textsuperscript{21} ischemic clamping,\textsuperscript{20} and infusion of perfluorocarbon blood substitute.\textsuperscript{19} These studies demonstrate the potential versatility of the NIR approach and its application for diverse future studies.

In summary, we have demonstrated that the NIR technology can provide an efficient, real-time, noninvasive means for monitoring vascular oxygenation dynamics in tumors during hyperoxygen respiratory challenge. HbO$_2$ concentrations measured from both breast and prostate tumors often exhibit a prompt rise that is followed by a gradual persistence throughout the intervention. By developing a hemoperfusion model with two exponential terms and fitting the model to the increased HbO$_2$ data, we are able to recognize two mechanisms for blood perfusion in the tumor and to quantify the ratios of the two perfusion rates and those of the two vasculature coefficients. Thus the technique can enhance our understanding of the dynamics of tumor oxygenation and the mechanisms of tumor physiology under baseline and perturbed conditions. Moreover, it appears that the NIRS may have a great potential for monitoring tumor angiogenesis because the method can provide information on blood perfusion and oxygen consumption of the measured tumor.

Appendix A

A. Derivation

It has been shown that, in the NIR range, the major light absorbers in tissue are oxygenated and deoxygenated hemoglobin molecules.\textsuperscript{24,25} With this knowledge, the absorption coefficients (in inverse centimeters) at two wavelengths can be associated with the concentrations of HbO$_2$ and Hb by

\[
\mu a^\lambda = \epsilon_{\text{Hb}}^\lambda Hb + \epsilon_{\text{HbO}_2}^\lambda HbO_2, \quad (A1)
\]

\[
\mu a^\lambda = \epsilon_{\text{Hb}}^\lambda Hb + \epsilon_{\text{HbO}_2}^\lambda HbO_2, \quad (A2)
\]

where \(\epsilon_{\text{Hb}}^\lambda\) and \(\epsilon_{\text{HbO}_2}^\lambda\) are the extinction coefficients (in inverse centimeters times inverse millimoles) of deoxygenated and oxygenated hemoglobin, respectively, at wavelength \(\lambda\), and HbO$_2$ and Hb are the oxyhemoglobin and the deoxyhemoglobin concentrations. Because \(\epsilon_{\text{Hb}}^\lambda\) and \(\epsilon_{\text{HbO}_2}^\lambda\) are constants, changes in HbO$_2$ and Hb in tissue vasculature result in changes in \(\mu a^\lambda\). In turn, changes in HbO$_2$ and Hb can be determined by measuring changes in \(\mu a^\lambda\) at two wavelengths and can be expressed as

\[
\Delta Hb = Hb(\text{transient}) - Hb(\text{baseline}) = \frac{\epsilon_{\text{HbO}_2}^\lambda \Delta \mu a^\lambda - \epsilon_{\text{HbO}_2}^\lambda \Delta \mu a^\lambda}{\epsilon_{\text{Hb}}^\lambda \epsilon_{\text{HbO}_2}^\lambda - \epsilon_{\text{Hb}}^\lambda \epsilon_{\text{HbO}_2}^\lambda}, \quad (A3)
\]

\[
\Delta HbO_2 = HbO_2(\text{transient}) - HbO_2(\text{baseline}) = \frac{\epsilon_{\text{Hb}}^\lambda \Delta \mu a^\lambda}{\epsilon_{\text{Hb}}^\lambda \epsilon_{\text{HbO}_2}^\lambda - \epsilon_{\text{Hb}}^\lambda \epsilon_{\text{HbO}_2}^\lambda}, \quad (A4)
\]

where \(\Delta Hb\) and \(\Delta HbO_2\) refer to the change in the deoxyhemoglobin and the oxyhemoglobin concentrations between the baseline condition and the transient, or perturbed, condition, respectively, and \(\Delta \mu a^\lambda\) represents the change in the absorption coefficient at \(\lambda\) relative to the baseline condition. However, our current experimental setup with one source and one detector does not provide adequate information for quantifying \(\mu a^\lambda\) values for a solid rat tumor. Thus we take an approximate approach by using the modified Beer–Lambert relation to calculate \(\Delta Hb\) and \(\Delta HbO_2\).

According to the modified Beer–Lambert law,\textsuperscript{10} an optical density (OD) can be defined as

\[
OD = \log(I_0/I) = \mu a L, \quad (A5)
\]

where \(I_0\) and \(I\) are the incident and the detected optical intensities, respectively, and \(L\) is the optical path length traveled by light inside the tissue. When an organ or a tumor undergoes a change from its baseline condition to a transient condition under physiological perturbations, a change in the OD at wavelength \(\lambda\) will occur and can be expressed as

\[
\Delta OD^\lambda = OD^\lambda (\text{transient}) - OD^\lambda (\text{baseline}) = \log(I_b/I_b^\lambda) - \log(I_0/I_0^\lambda) = \Delta \mu a^\lambda L^\lambda, \quad (A6)
\]

With our current NIR instrument, we can obtain the ratios of \((I_b/I_b)^{\lambda 1}\) and \((I_b/I_b)^{\lambda 2}\) from the tumor measurement. By assuming a constant path length, i.e., \(L^{\lambda 1}\) (baseline) \(\approx\) \(L^{\lambda 2}\) (transient) \(\approx\) \(L\), we next substitute Eq. (A6) into Eqs. (A3) and (A4) and arrive at Eqs. (5) and (6) for calculations of tumor hemoglobin oxygenation dynamics. Note that \(I_b, I\) have been replaced with \(A_b, A\), in Eqs. (5) and (6).

B. Justification

The assumption of a constant path length as given above makes it possible to use relatively simple equations, for example, Eqs. (5) and (6), to quantify the \(\Delta Hb\) and the \(\Delta HbO_2\) of tumors under respiratory intervention. However, in principle, the optical path length \(L\) through tissue is wavelength dependent and could be variable under physiological perturbations. Therefore it is useful to know whether the relative error for calculated \(\Delta Hb\) and \(\Delta HbO_2\) caused by this assumption is within a reasonable range.

According to the diffusion approximation, the optical path length \(L\) of the NIR light traveling in tissue can be expressed approximately as

\[
L = \frac{\sqrt{3}}{2} d \left(\frac{\mu a}{\mu u}\right)^{1/2}, \quad (A7)
\]
where $d$ is the source–detector separation and $\mu_a$ and $\mu'_a$ are the absorption and the reduced scattering coefficients, respectively. Because in the NIR region the $\mu_a$ of tissue is not sensitive to either wavelength or perturbation, we assume that a change in $L$ results from only a change in $\mu_a$, which is both wavelength and perturbation dependent. With this assumption, Eq. (A7) leads to

$$\frac{\Delta L}{L} = -\frac{1}{2\mu_a} \Delta \mu_a.$$

Equation (A8) allows us to determine the relative errors of $\Delta L/L$ that are caused by (a) the wavelength dependence of $\mu_a$ and (b) the perturbation dependence of $\mu_a$ in the tumor. For case (a), we calculated this error by using

$$\frac{\mu_{a,758}^{758} - \mu_{a,782}^{782}}{2\mu_{a,782}^{782}}$$

under the baseline and the perturbed conditions; for case (b), we employed

$$\frac{\mu_a^{(\text{transient})} - \mu_a^{(\text{baseline})}}{2\mu_a^{(\text{baseline})}}$$

at both $\lambda = 758$ nm and $\lambda = 782$ nm for the error calculation. The $\mu_a$ values used here were taken from Hull et al.\textsuperscript{22} Although the rat tumor used in their study was different from ours, the absorption coefficients of the tumors should be in a similar order and follow a similar dynamic trend. The calculation shows that, with 758 and 782 nm under carbogen perturbation, the maximum value of $\Delta L/L$ is 12%. This result implies that the assumption of a constant path length that was used for Eqs. (5) and (6) gives rise to a maximal relative error of 12% in $L$.

On the basis of Eqs. (5) and (6) [or Eqs. (7) and (8)], we arrive at $\Delta X/X = -\Delta L/L$, where $X$ can be $\Delta$HbO$_2$, $\Delta$Hb, or $\Delta$Hb$_r$. Thus the assumption of a constant path length leads to a maximal relative error of 12% for the magnitude of the changes that we detected with regard to respiratory challenge. Although 12% is not completely negligible, the measurement and the calculation with the assumption of a constant path length are still worthwhile. Such an approach makes it possible, as a first-order approximation, to quantify the $\Delta$Hb and the $\Delta$Hb$_r$ of tumors under respiratory intervention, providing deep insight into tumor vascular phenomena and mechanisms of modulating tumor physiology for therapeutic enhancement.

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