Near infrared transillumination imaging of breast cancer with vasoactive inhalation contrast

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Abstract: Inhalation of vasoactive gases such as carbon dioxide and oxygen can provide strong changes in tissue hemodynamics. In this report, we present a preliminary clinical study aimed at assessing the feasibility of inhalation-based contrast with near infrared continuous wave transillumination for breast imaging. We describe a method for fitting the transient absorbance that provides the wavelength dependence of the optical pathlength as parametrized by tissue oxygenation and scatter power as well as the differential changes in oxy- and deoxy-hemoglobin. We also present a principal component analysis data reduction technique to assess the dynamic response from the tissue that uses coercion to provide single temporal eigenvalues associated with both oxy- and deoxy-hemoglobin changes.

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1. Introduction

The development of complementary imaging modalities for imaging breast cancer has been an important priority for the imaging community. Specifically, the ability of near infrared (NIR) light, 600–1000 nm, to image breast tissue has led to significant advances, including the development of optical tomography [1–5] and diffuse optical imaging and spectroscopy [6–8] modalities. NIR imaging lends itself easily to the use of endogenous contrast due to the strong absorption of oxy- (HbO₂) and deoxy- (Hb) hemoglobin in blood. In this wavelength window, the absorption due to water is low, enabling instruments to capture contrast due to these blood chromophores. In breast tissue, where the vasculature is not dense, the presence of tumors results in increased vasculature to feed the growing tumor tissue [9]. This vasculature is often chaotic in structure and can be leaky in nature. It has been postulated that exogenous vasoactive contrast agents such as carbogen gas (95% O₂ and 5% CO₂) could provide contrast when coupled with a NIR imaging system to image breast tumors.

Our laboratory has been investigating the use carbogen gas as a contrast agent to image cancer using a differential NIR imaging method. In the differential imaging approach, baseline images are acquired during inhalation of room air. Logarithms of these images are then subtracted from logarithms of the images acquired during the inhalation of the vasoactive gas to produce the differential images used in all of our studies. Animal model studies from our group using this approach established the viability of this hypothesis [10]. The development of a continuous wave (CW) NIR imaging instrument designed to image breast tissue has also been reported [11]. In that report we detail various aspects of the instrument design and gas delivery protocols with images of the palm. This study revealed several important trends. We noted that the Bain's circuit provided more efficient delivery of carbogen gas, the use of air + 5% CO₂ and 95% O₂ + 5% N₂ was well tolerated, and the effect of carbogen appeared to indicate a vasoconstriction effect.

Here we report data from our studies on imaging breast tissue in healthy volunteers as a function of carbogen inhalation and compression. We also show data from imaging diseased breast tissue using carbogen inhalation to induce contrast. While we have reported early data from these studies [12], this account is a detailed compilation of the work. There have been a limited number of reports in the literature regarding the effect of vasoactive gas inhalation on contrast from breast tissue. An x-ray mammography study reported results on a single subject [13] where a signal increase of roughly 15% was observed between the first air and oxygen period. Recent reports using magnetic resonance guided near-infrared imaging of healthy breast tissue revealed that carbogen acts as a vasodilator [14]. A follow-up study by the same authors on a pilot clinical investigation involving two cancer subjects was indicative of a greater correlation of the total hemoglobin to the carbogen gas stimulus in healthy versus tumor tissue [15]. Inhalation based vasoactive imaging has also been reported in a clinical setting using bold MRI [16] on various carcinomas.

2. Experimental details

Instrument design

The design of the transillumination-based NIR instrument has been detailed in a previous publication from our laboratory [11]. Spot paddles for x-ray mammography (AR Custom Medical Products, Commack, New York) were used to support the breast for imaging in combination with an NIR light source and a CCD camera. Five wavelengths were available for the transillumination measurements with nominal values of 690, 735, 810, 850, and 940 nm. The LED illuminators (Epitex, Kyoto, Japan) had the following center wavelengths as measured by a spectrometer (Ocean Optics, Dunedin, Florida): 680, 734, 813, 830, and 933 nm. These wavelengths were stable to within 2 nm for measurements lasting up to 25 min. The measured wavelengths were used in calculations as described in Section 3. The instrument was assembled on an optical rail and was placed on the C-arm of a stereo-tactic breast biopsy table as shown in Fig. 1. The arm of the table could be rotated to allow different imaging positions such as craniocaudal (CC), mediolateral oblique (MLO), and mediolateral (ML). Masks were prepared using black paper to prevent light leakage through the spot paddles from regions that did not touch the breast tissue as shown in Fig. 1(c). Phantom measurements indicated no light transmission through these masks. The camera (Apogee Instruments) was tilted during image acquisition to allow imaging close to the chest wall; in this configuration, the imaging system could be raised until the spot paddles touched the bottom of the opening in the table. Mild compression, on the order of 1 pound force, was applied to the breast to minimize motion during the imaging experiments. The load cell, which measured the compression force, was connected to the paddle closer to the camera. Labview software was used to automate data collection. Phantom measurements were routinely performed in the clinic prior to imaging runs to verify software performance. In some cases, imaging was also performed in the sitting position when the stereo-tactic table was unavailable. In these cases the imaging system was supported by a tripod. We have set the smallest exposure time to default at 0.05 ms in the imaging software. We have been able to get signal in our images with exposure times as low as 0.06 ms for 810 nm and 850 nm when tissue transmission is good.

Breathing circuit

Two types of breathing circuits were used in this study: a non-rebreathing circuit and a Bain's circuit. Details for each type of circuit have been reported [11]. Prior to data collection a capnometer and pulse oximeter (Tidal Wave® Hand-held Capnograph) recorded baseline values of end tidal CO₂, inspired CO₂, blood saturation (SpO₂) and pulse rate values. Samples of inhaled and exhaled air were recorded by the capnometer via an airway adapter on the mouthpiece. This mouthpiece was used to deliver room air or carbogen to the volunteer during

an imaging cycle. The maximum value of the inspired CO₂% that the capnometer can measure is 3% although the capnometer can read higher values for end tidal CO₂%. Room air was inhaled through the mouthpiece for a time interval of two minutes after which the breathing circuit tube was manually connected to the mouthpiece adapter for a total carbogen inhalation time of two minutes. The carbogen gas flow rate was maintained at or close to 15 liters per minute using a regulator during the entire two minutes in the data reported here. After the end of the two minute gas delivery, the breathing circuit tube was removed from the mouthpiece adapter and room air was inhaled for a final two minutes. The carbogen gas flow was turned on before the tube was attached to the mouthpiece adapter and turned off after the tube was disconnected from the mouthpiece adapter. This ensured smooth delivery of the gas to the volunteer. A typical inhalation cycle lasted a total of six minutes; 2 min (air)/2 min (carbogen)/2 min (air), during which imaging is performed continuously. A nose clip was used during the entire imaging run to ensure that the volunteer inhaled only through the mouthpiece. A typical imaging run involved collection of background images for each wavelength followed by data collection during a gas inhalation cycle. During the gas inhalation cycle, typically 15 frames per wavelength were collected for each gas, although this number may increase to about 20 frames if we get lower exposure times for a subject because the transmission through the breast tissue is strong. Medical grade carbogen gas was used in the study and was supplied by Airgas.

Clinical aspects

All imaging was performed in the radiology department of the Moores Cancer Center at the UCSD School of Medicine under appropriate consent. The image protocol was approved by both the UCSD Institutional Review Board (IRB) and the IRB of SRI International. The instrument was transported by plane from Menlo Park to San Diego for the imaging sessions. Table 1 summarizes the total number of subjects imaged in this study at UCSD.

Table 1. Number of Subjects Imaged in this Study.

Tissue Type	Number of Subjects	Type of Run (Test/Compression/Inhalation)
Healthy	17	7 Runs: Preliminary Tests / Imaging Only 7 Runs: Inhalation 3 Runs: Only Compression
Fibroadenoma	4	Inhalation
Cancer	6	Inhalation

Fig. 1. (a) The NIR imaging instrument placed on the C-arm of a stereo-tactic breast biopsy table. The red arrows indicate the position of the breast during imaging. (b) Top view as seen from the table platform. The space between the two spot paddles localizes the breast tissue. (c) A schematic of the front face of the spot paddles. The white region is the only transparent zone on the paddle. This zone interfaces with the breast tissue.

3. Data analysis

We convert our multi-wavelength images to images of deoxyhemoglobin and oxyhemoglobin based on a differential pathlength analysis [17] similar to our previous studies [10,11]. In this case the transient intensity I_T is related to the baseline intensity I_B according to

$$I_T^{\lambda_i} = I_B^{\lambda_i} \exp(-\Delta\mu_a^{\lambda_i} L^{\lambda_i}) \quad (1)$$

where $\Delta\mu_a^{\lambda_i}$ and L^{λ_i} are the change in the absorption coefficient and the optical pathlength, which is equal to the product of the source-detector separation distance and the differential pathlength factor. The superscripts for all variables denote that the parameters are for wavelength λ_i . We assume that the changing absorbance is due primarily to changes in the concentrations of deoxyhemoglobin and oxyhemoglobin, $\Delta[\text{Hb}]$ and $\Delta[\text{HbO}_2]$, respectively. This is a good assumption because the inhalation protocol primarily influences blood vessels and blood oxygenation. In this case

$$\Delta\mu_a^{\lambda_i} = \ln(10) \{ \epsilon_{\text{Hb}}^{\lambda_i} \Delta[\text{Hb}] + \epsilon_{\text{HbO}_2}^{\lambda_i} \Delta[\text{HbO}_2] \} \quad (2)$$

where $\epsilon_{\text{Hb}}^{\lambda_i}$ and $\epsilon_{\text{HbO}_2}^{\lambda_i}$ are the (base 10) absorption coefficients at wavelength λ_i . We will ratio all pathlength factors to the pathlength factor at a single wavelength, 813 nm. Although our instrumentation supports measurements at 5 wavelengths, we have omitted the 933 nm images from our analysis because first, the 933 nm wavelength did not always provide good images and second, the absorbance portion of the wavelength dependence of the optical pathlengths for the other wavelengths are primarily influenced by deoxyhemoglobin and oxyhemoglobin, while 933 nm is also influenced by water and fat, which complicates the derivation of optical pathlengths. For a typical data set involving 50 images at each of 5 wavelengths, we have 200 measurements for each pixel. For this typical data set, there are 103 unknown values for each pixel: the 3 optical pathlength ratios at that pixel and 50 values each for $\Delta[\text{Hb}]$ and $\Delta[\text{HbO}_2]$. Thus we can potentially determine the 103 unknowns from the 200 measurements. For any given set of values for the optical pathlengths, we can calculate the $\Delta[\text{Hb}]$ and $\Delta[\text{HbO}_2]$ 6

ways corresponding to each pair of the 4 wavelengths. We solve for the unknowns by varying the optical pathlength ratios until the root mean square variation of the estimates of $\Delta[\text{Hb}]$ and $\Delta[\text{HbO}_2]$ are minimized; i.e., until all possible estimates are very close together. To improve this convergence we make two modifications. First, we omit the 813 nm / 830 nm wavelength pair because they respond quite similarly to changes in $\Delta[\text{Hb}]$ and $\Delta[\text{HbO}_2]$. Second, instead of fitting three parameters (the three optical pathlength ratios), we fit two parameters, the tissue oxygenation and scatter power. We note that the optical pathlength at a single wavelength can be written as [18]

$$L^{\lambda_i} \approx \frac{\sqrt{3}}{2} \sqrt{\frac{\mu_s^{\lambda_i}}{\Delta\mu_a^{\lambda_i}}} \rho \quad (3)$$

where μ_a is the absorption coefficient, μ_s' is the reduced scattering coefficient, and ρ is the pathlength without scattering. If we also assume that the only significant chromophores contributing to the wavelength dependence of the optical pathlength are deoxyhemoglobin and oxyhemoglobin, we can write μ_a as

$$\mu_a^{\lambda_i} = \ln(10) \{ \varepsilon_{\text{Hb}}^{\lambda_i} [\text{Hb}] + \varepsilon_{\text{HbO}_2}^{\lambda_i} [\text{HbO}_2] \} \quad (4)$$

We can rewrite Eq. (4) as

$$\mu_a^{\lambda_i} = \ln(10) \{ (1-x) \varepsilon_{\text{Hb}}^{\lambda_i} + x \varepsilon_{\text{HbO}_2}^{\lambda_i} \} [\text{Hb}_{\text{tot}}] \quad (5)$$

where x is the oxygenation ($x = [\text{HbO}_2]/[\text{Hb}_{\text{tot}}]$) and $[\text{Hb}_{\text{tot}}]$ is the total hemoglobin concentration.

The wavelength dependence of μ_s' can be modeled using a scatter power model [19]

$$\mu_s' = A \lambda^{-SP} \quad (6)$$

where A is a constant and SP the scatter power. Using Eqs. (3), (5), and (6), we can write the optical pathlengths as

$$\frac{L^{\lambda_i}}{L^{\lambda_0}} \approx \sqrt{\left(\frac{\lambda_i}{\lambda_0} \right)^{-SP} \frac{(1-x) \varepsilon_{\text{Hb}}^{\lambda_0} + x \varepsilon_{\text{HbO}_2}^{\lambda_0}}{(1-x) \varepsilon_{\text{Hb}}^{\lambda_i} + x \varepsilon_{\text{HbO}_2}^{\lambda_i}}}. \quad (7)$$

where $\lambda_0 = 813$ nm. Thus we can fit the optical pathlength ratios based on two unknowns, oxygenation (x) and scatter power (SP). Thus the differential imaging analysis can provide information not only on dynamic properties $\Delta[\text{Hb}]$ and $\Delta[\text{HbO}_2]$, but also on the static tissue properties (oxygenation and scatter power). As is typical for differential pathlength analysis, we assume that the changes $\Delta[\text{Hb}]$ and $\Delta[\text{HbO}_2]$ are not large enough to change the optical pathlength values.

Finally, to better understand the temporal variations in the data as a result of the carbogen inhalation, we process the $\Delta[\text{Hb}]$ and $\Delta[\text{HbO}_2]$ images using a principal component analysis algorithm [20–22]. Briefly, the method involves the subtraction of a mean image from an image stack. Thus, if there are t images with i rows and j columns then,

$$I^*(i, j, t) = I(i, j, t) - \langle I(t) \rangle \quad (8)$$

Each image in the resulting stack of mean-subtracted images is transformed into a one-dimensional vector with $i \times j$ elements. The covariance of this new matrix $I^*(i \times j, t)$ is computed, followed by the computation of the eigenvalues, temporal eigenvectors and spatial eigenimages. Each temporal eigenvector is a linear combination of the individual pixel time series and each spatial eigenimage is a linear combination of the reconstructed images. The

resulting principal components are basically a rearrangement of the original time series. The original data can be recovered by summing the eigenvalue-temporal eigenvector-eigenimage products. No information is lost in this transformation [23]. The temporal eigenvectors are uncorrelated because they are orthogonal. This is because the covariance matrix is symmetric. Finally, note that the temporal and spatial eigenvectors corresponding to the largest eigenvalues correspond to the largest variations in the image set. Also, to understand the information presented in a given eigenvalue set, it should be noted that the temporal eigenvector indicates the temporal evolution of each pixel in the corresponding spatial eigenimage.

We have used three software packages for data analysis. In the first step, ImageJ is used to crop the image, calculate images of $\Delta\mu_a L$ at 680, 734, 813, and 830 nm using Eq. (1), and bin the processed images to reduce the image size from 512 by 512 to 128 by 128. When any raw pixels are saturated, the processed pixels are set to not-a-number to exclude them from the subsequent pathlength ratio calculation. In the second step, we used the Igor Pro graphing and data analysis package to convert the images of $\Delta\mu_a L$ at four wavelengths to images of $\Delta[\text{HbO}_2] \cdot L^{813}$ and $\Delta[\text{Hb}] \cdot L^{813}$ in units of mM*cm as well as images for oxygenation and scatter power using Eqs. (2) and (7). The tissue oxygenation and scatter power at each pixel are varied until the root mean square estimates of $\Delta[\text{Hb}]$ and $\Delta[\text{HbO}_2]$ are minimized. Although the optical pathlength ratios or oxygenation and scatter power values should not change significantly over a spatial distance of a single pixel, we performed fits at every pixel in order to gain information of the variability in this analysis. An example of the variability may be found in the histograms for the optical pathlength ratios and the oxygenation and scatter power images shown in Fig. 2(c), 2(d), and 2(e). In the final step, MATLAB was used to compute the eigenvalues, temporal eigenvectors and the spatial eigenimages. In the present analysis, a mean image from the temporal image stack, one each for the $\Delta[\text{Hb}]$ and $\Delta[\text{HbO}_2]$ image sets, is subtracted from each image in that stack following Eq. (8). Next, the mean subtracted stacks are concatenated into a single image stack for both $\Delta[\text{HbO}_2]$ and $\Delta[\text{Hb}]$. This image matrix is then subjected to the PCA analysis as mentioned above. The coercion of the mean subtracted $\Delta[\text{Hb}]$ and $\Delta[\text{HbO}_2]$ images simultaneously through the PCA algorithm ensures that the temporal eigenvectors show simultaneous changes for both the $\Delta[\text{Hb}]$ and $\Delta[\text{HbO}_2]$ images, that is, there is a single temporal eigenvector for both $\Delta[\text{Hb}]$ and $\Delta[\text{HbO}_2]$. When PCA is performed on $\Delta[\text{Hb}]$ and $\Delta[\text{HbO}_2]$ separately, the temporal eigenvectors can have similar shapes, but aren't necessarily associated with each other because the eigenvalues can differ. The coercion results in single temporal eigenvector and eigenvalue associated with the physiological changes for each eigenimage, whether they be due to the individual components, $\Delta[\text{Hb}]$ and $\Delta[\text{HbO}_2]$, or their combinations, a change in total hemoglobin, $\Delta[\text{HbO}_{\text{tot}}] = \Delta[\text{HbO}_2] + \Delta[\text{Hb}]$, or a pure oxygenation change, i.e., $\Delta[\text{HbO}_2] - \Delta[\text{Hb}]$.

4. Results

Experiments were performed on healthy subjects to investigate the response of the breast tissue to carbogen inhalation. Mild compression to stabilize the breast against motion was a part of this study, but compression alone also produces tissue changes. To characterize the effect of the compression, data were also collected for the same subjects under steady compression. Comparison between the two sets of data revealed a different response from the breast tissue for a given subject under steady compression and compression accompanied by carbogen gas inhalation.

As an example, in Figs. 2 and 3, we show the response of healthy breast tissue to carbogen inhalation. The tissue was held at a compression load of 0.46 pounds. Data acquisition lasted for a total of 6 minutes where each two minute interval consisted of room air, carbogen, and room air gases respectively, in that order. Carbogen administration is indicated by the blue shaded region in all of the temporal data presented in the figures.

Fig. 2. Data during carbogen inhalation for a healthy volunteer. For all time dependent data, the region shaded blue shows the period of carbogen inhalation. (a) End tidal and inspired percent CO_2 from capnometer (capnometer readings are delayed relative to gas changes). (b) Time dependence of natural absorbance data, $-\ln(I_t/I_b)$, at four wavelengths (solid lines) at a single pixel. (c) Histograms of pathlengths calculated from absorbance data. (d) and (e) Scatter power and oxygenation values for each pixel from fitting. The resulting static images are for the entire imaged regions after processing each pixel through the analysis methodology presented in Section 3. (f) and (g) Time dependence of $\Delta[\text{HbO}_2] \cdot L_{810}$ and $\Delta[\text{Hb}] \cdot L_{810}$ in units of $\text{mM} \cdot \text{cm}$ for the same pixel as in (b). There are 5 overlapping traces calculated using combinations of the multi-wavelength data in (b) and the pathlengths summarized in (c-e). The mean values of the $\Delta[\text{HbO}_2]$ and $\Delta[\text{Hb}]$ data from (d) and (e) were used with the pathlengths in (c-e) to calculate absorbance at each wavelength, which are also plotted as the dashed lines in (b); these are only partially visible due to the high overlap with the measurements (solid lines). (h) Eigenvalues from coerced PCA. Temporal eigenvectors and eigenimages for this data set are shown in Fig. 3.

A robust response is noted in Fig. 2(a) for the end tidal $\text{CO}_2\%$ values and the inspired $\text{CO}_2\%$ values during carbogen inhalation. There is a lag between the capnometer response and the gas changes (blue shaded region). We often find the capnometer data to be delayed relative to the gas changes. Part of this delay is due to the fact that the capnometer only provides updated readings every 8 seconds. For this data set, the rise in the inspired $\text{CO}_2\%$ values had an additional lag beyond the rise in the end tidal $\text{CO}_2\%$ values. The reason for this additional lag is not known. The timing of the gas administration (blue shaded regions) is reliable in each case reported here. Regardless of the timing shift, the end tidal CO_2 values show that the carbogen gas is being delivered properly. The change in absorbance for all 4 wavelengths at a single pixel is shown in Fig. 2(b). From such single pixel data we calculate values for the pathlength ratios, scatter power, and oxygenation using the fitting procedure detailed in the Experimental Methods section above. The resulting pathlength histogram, scatter power image, and oxygenation image for all the pixels are shown in Figs. 2(c), 2(d) and 2(e), respectively. The pathlengths are all ratioed to the pathlength at 813 nm. Therefore

the 813 pathlength ratios in the histogram all have a value of one. Figures 2(f) and 2(g) show the trends in the $\Delta[\text{HbO}_2]$ and $\Delta[\text{Hb}]$ values over the 6 minute time interval calculated for the same pixel as Fig. 2(b) using the pathlength factors for that pixel. The PCA eigenvalues are shown in Fig. 2(h).

Fig. 3. Temporal eigenvectors and eigenimages from coerced PCA for the data in Fig. 2. The first two eigenvalues indicate that the corresponding eigenimages show maximum deviations from the mean and also carry signals in response to the gas stimulus. The first set of eigenimages indicate a marked increase the $\Delta[\text{HbO}_2]$ values with a corresponding decrease in the $\Delta[\text{Hb}]$ values. The magnitudes of the corresponding eigenvalues are also indicated at the bottom of the figure.

The principal component analysis for this data set revealed strong variations from the mean for the first two temporal eigenvectors as evidenced by the large eigenvalues. From the plot of the eigenvalues shown in Fig. 2(h), it is clear that the first few eigenvalues carry the maximum weight. We typically obtain over 45 eigenvectors for each image set. The eigenvectors are ordered according to the size of the eigenvalues. Of these, only the smallest 10 or so show a white noise structure in the eigenimages as is commonly associated with noise. A large number of the temporal eigenvectors appear noisy, but the fluctuations in many of them are probably actually changes that are occurring faster than our imaging rate such as the cardiac cycle, respiration, movement, and other physiological fluctuations. Multiplication of the eigenvalue times the temporal eigenvectors times the $\Delta[\text{HbO}_2]$ or $\Delta[\text{Hb}]$ eigenimage

gives a component of either $\Delta[\text{HbO}_2] \cdot L^{813}$ or $\Delta[\text{Hb}] \cdot L^{813}$ in units of mM*cm. Summation of all of the components will reproduce the original data set for $\Delta[\text{HbO}_2] \cdot L^{813}$ or $\Delta[\text{Hb}] \cdot L^{813}$. Typically however, only the first few eigenvalues are associated with the temporal eigenvectors that carry a response to the gas inhalation stimulus for a given subject. For the data shown in Fig. 2, the first four temporal eigenvectors are shown in the first column of Fig. 3; the first four eigenimages for $\Delta[\text{HbO}_2]$ and $\Delta[\text{Hb}]$ are shown adjacent to the corresponding temporal eigenvector. Each temporal eigenvector describes the temporal variation of its corresponding spatial eigenimage. It is clear that the first eigenset indicates an increase in the $\Delta[\text{HbO}_2]$ values upon the onset of the gas and a corresponding decrease in the $\Delta[\text{Hb}]$ values. This is presumably due to the increased oxygen inhaled in the carbogen. The second eigenset points to a mild decrease in the values of both $\Delta[\text{HbO}_2]$ and $\Delta[\text{Hb}]$ during the carbogen administration phase. This is expected to be vasoconstriction, possibly also from the increased oxygen inhalation. Each eigenimage shows the spatial variation of $\Delta[\text{HbO}_2]$ and $\Delta[\text{Hb}]$ corresponding to this associated temporal eigenvector.

In order to compare the effect of steady compression on the transillumination images obtained with this instrument, compression data were collected for the same volunteer for Fig. 2 over a period of 7 minutes and 30 seconds at a load of 0.65 pounds. These data are shown in Fig. 4. The pathlength histograms, scatter power image, and oxygenation image are shown in Fig. 4(a)–4(c). The first temporal eigenvector and the corresponding spatial eigenimages of $\Delta[\text{HbO}_2]$ and $\Delta[\text{Hb}]$ are shown in Fig. 4(d). In this set, the dominant response was noted only from the first eigenvalue. From the plot, it is apparent that after a delay of about 150 seconds there is a general trend of increasing $\Delta[\text{Hb}]$ values and an overall decrease in the $\Delta[\text{HbO}_2]$ values. This response is opposite to that seen for inhalation and much smaller in size as seen from the first temporal eigenvectors and the corresponding spatial eigenimages in Fig. 3. Thus, pure compression without the carbogen inhalation results in a reduction of blood oxygenation over time. This is expected to result from reduced circulation under compression, leading to less oxygen delivery. We find differences in the pathlength factors between carbogen inhalation and compression alone, as seen by comparing Fig. 2(c), 2(d) and 2(e) with Fig. 4(a), 4(b), and 4(c). The scatter power has extremely low values, essentially zero, and the oxygenation is rather low, in the range of 30%. The source of these differences is not known. It may be due to the fact that carbogen and compression impact different compartments of the circulation vasculature, or the pure compression response may differ more strongly from the differential pathlength model than the inhalation response.

For our data on healthy subjects, we find common trends. First, carbogen inhalation results in a stepwise (persistent) increase in oxygenation and a transient decrease in total hemoglobin. Second, mild compression without carbogen inhalation leads to a stepwise decrease in oxygenation, in essence the opposite response of the dominant response to carbogen and much smaller in magnitude. We have two data sets comparing carbogen inhalation with mild compression without carbogen inhalation in healthy subjects. The subject with a stronger response to carbogen also had a stronger response to compression only. There is intersubject variability in the magnitude of the response and relative changes in HbO_2 and Hb for compression alone.

Fig. 4. Data acquired for mild compression without gas inhalation for the same subject as Figs. 2 and 3. (a), (b), and (c) summarize pathlength ratios as a histogram, scatter power image, and oxygenation image, respectively. (d) First four temporal eigenvectors and eigenimages from coerced PCA with the corresponding magnitudes for the eigenvalues for this set.

We also measured the response from breast tissue of subjects with confirmed cases of fibroadenomas as per pathology reports provided to the radiologist in this study (Dr. Comstock). Data for one such subject are shown in Fig. 5. The capnometer registered efficient gas breathing parameters, however, the inspired $\text{CO}_2\%$ and end tidal $\text{CO}_2\%$ values lagged the onset of gas delivery. The location of the lesion was not available at the time of data collection. Figure 5(a), 5(b), and 5(c) show the pathlength ratio histogram, scatter power image and oxygenation image from the fitting analysis performed on the difference absorbance data for this set. The scatter power is close to zero over much of the image. Higher oxygenation values near the dense vascular bed are observed. In Fig. 5(d), we show the plots for the first four temporal eigenvectors with the corresponding $\Delta[\text{HbO}_2]$ and $\Delta[\text{Hb}]$ eigenimage maps. In this set, the first and third temporal eigenvectors carried signatures in the carbogen inhalation phase and the first three eigenvalues were the most significant. Again, the

dominant response seen in the first temporal eigenvector is that of an increase in the $\Delta[\text{HbO}_2]$ values in the entire imaged tissue and a corresponding decrease in the $\Delta[\text{Hb}]$ values.

Fig. 5. (Media 1) Data from a subject with fibroadenoma. (a), (b), and (c) Pathlength ratio histogram, scatter power image, and oxygenation image, respectively. The images in (b) and (c) are obtained after analyzing each pixel in the original image according to the methodology presented in Section 3. (d) First four temporal eigenvectors and eigenimages from coerced PCA with the corresponding magnitudes for the eigenvalues for this set.

In the $\Delta[\text{HbO}_2]$ and $\Delta[\text{Hb}]$ image maps for Fig. 5, the vasculature is seen clearly with the maximum response seen from this zone. The third temporal eigenvector shown in the third row of Fig. 5(d) along with the corresponding $\Delta[\text{HbO}_2]$ and $\Delta[\text{Hb}]$ image maps shows a transient carbogen specific response that has a dominant contribution from the tissue vasculature in a section of the tissue imaged. This heterogeneous response from specific zones in the tissue may be indicative of varied effects of the gas on different vascular beds in the tissue, with regions showing both vasoconstriction and vasodilation. The second and fourth temporal eigenvectors do not appear to be associated with the inhalation. Altogether, 4

subjects with confirmed fibroadenomas were imaged in this study with data from one subject reporting efficient gas inhalation. The data presented in Fig. 5 are for this subject.

Lastly, we present data from subjects with confirmed cases of cancer. In Fig. 6 we show data from a subject where the tumor was located as a large mass in the 12 o'clock position. A compression of 0.3 pounds was applied to the tissue. The tumor zone registered in the raw transillumination image as a bright zone. Raw transillumination images for this subject are shown in Fig. 6(a). Also shown in Fig. 6(b), 6(c), and 6(d) are the pathlength ratio histogram and maps for the scatter power and the oxygenation for the absorbance data obtained from the fitting analysis method described earlier in this report. The tumor region shows up as a reduced oxygenation zone. The scatter power for the tumor zone is also reduced. In this set, the capnometer failed to register values when the gas delivery tube was inserted into the mouth piece adapter. While the values of blood saturation ($\text{SpO}_2\%$) and the pulse rate were measured, it was difficult to ascertain how efficient the carbogen delivery was to this subject. Nevertheless, the consistent saturation of the $\text{SpO}_2\%$ value during the carbogen inhalation phase, coupled with the marked increase in the transmitted intensity from the breast during carbogen inhalation, points to sufficient carbogen inhalation of the carbogen gas by the subject. Finally, we show the first six temporal eigenvectors along with their corresponding $\Delta[\text{HbO}_2]$ and $\Delta[\text{Hb}]$ image maps in Fig. 6(e). Of these, the first, fourth and sixth vectors showed a response associated with the carbogen inhalation phase. Again, the first temporal eigenvector indicates an increase in the $\Delta[\text{HbO}_2]$ values and a decrease in the $\Delta[\text{Hb}]$ values with a marked decrease in the tumor region. The fourth temporal eigenvector along with the heme maps, shows yet again a response from the vasculature where a decrease is observed in the $\Delta[\text{HbO}_2]$ values in the area surrounding a large blood vessel and an increase in the tumor zone during carbogen inhalation. Finally, the sixth temporal eigenimage shows an increase in fluctuation associated with the carbogen inhalation, with a dominant response from the tumor zone. The nature of these fluctuations is not understood.

Lastly, we show another data set where the subject has a diagnosed infiltrating ductal carcinoma. The capnometer registered efficient carbogen delivery and inhalation by the subject. There was some light leakage in this data set so the images have been cropped to process zones that were not affected by the light leakage significantly. Only the scatter power and oxygenation images, together with the pathlength histogram, are shown for this set in Fig. 7. The zone at the base of the image shows a markedly reduced oxygenation. The location of this zone corresponds to a tumor location from the pathology report that was very close to the chest wall.

Altogether, six cancer subjects were imaged in this study. Two imaging runs resulted in localized zones clearly visible in the inhalation data, which corresponded to the location of the lesions detailed in the pathology report. The other four data sets had problems that made analysis difficult, including a patient coughing throughout the measurements, capnometer failure and no dominant temporal eigenvector, strong light leakage, and LED misfiring. We note that our instrument was our first generation clinical device, and an improved instrument should have much better clinical results. Problems with light leakage and LED misfiring have already been corrected. Automated gas administration will further improve reliability. Excessive coughing may require rescheduling the imaging.

Fig. 6. Data from subject with invasive carcinoma with mixed ductal and lobular features. (a) Raw transillumination images at 680, 734 and 813 nm acquired during air inhalation without any image processing are shown. (b), (c), and (d) pathlength histogram, scatter power imaging and oxygenation image, respectively obtained by analyzing the signal at each pixel. (e) The first six temporal eigenvectors and eigenimages from coerced PCA. The corresponding eigenvalues are also indicated. A video file showing the temporal dependence of $\Delta[\text{HbO}_2] \cdot L^{813}$ and $\Delta[\text{Hb}] \cdot L^{813}$ in units of $\text{mM} \cdot \text{cm}$ (media 1) shows the onset of carbogen inhalation stimulating a sharp rise in oxygenation and fluctuations in the tumor region and the blood vessel at the top of the image.

Fig. 7. Pathlength histogram, scatter power image, and oxygenation image for subject with infiltrating ductal carcinoma.

5. Conclusions

The measurements reported in this study provide a description of the response of the vasculature in various types of breast tissue under the effect of compression and inhalation of carbogen gas. Compression alone results in a persistent change in oxygenation similar in shape to compression plus inhalation, but much smaller in magnitude and opposite in sign. We conclude that NIR CW transillumination based measurements can provide unique information on the vascular response of breast tissue in response to vasoactive gas inhalation. We present a method for analysis of optical parameters based on the transient response to the gas inhalation, producing static images of oxygenation and scatter power from the resulting pathlength factors as well as dynamic images for $\Delta[\text{HbO}_2]$ and $\Delta[\text{Hb}]$. We use coerced principal component analysis for the combined $\Delta[\text{HbO}_2]$ and $\Delta[\text{Hb}]$ images to determine eigenvectors from the data sets corresponding to different types of vascular response. The dominant gas-dependent response includes a stepwise (persistent) increase in oxygenation and a transient response that shows variation between individuals and tissue type. We do not currently understand the physiological source of the persistent or transient responses and more study will be required to better understand this behavior. The method generally shows large contrast for cancer, but the number of subjects is relatively small and the interpersonal variation is not well understood. More study is required to better understand the behavior of this imaging method and its value for cancer imaging.

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