Near-infrared spectral imaging of the female breast for quantitative oximetry in optical mammography

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We present a hybrid continuous-wave, frequency-domain instrument for near-infrared spectral imaging of the female breast based on a tandem, planar scanning of one illumination optical fiber and one collection optical fiber configured in a transmission geometry. The spatial sampling rate of 25 points/cm² is increased to 400 points/cm² by postprocessing the data with a 2D cubic spline interpolation. We then apply a previously developed spatial second-derivative algorithm to an edge-corrected intensity image (N-image) to enhance the visibility and resolution of optical inhomogeneities in breast tissue such as blood vessels and tumors. The spectral data at each image pixel consist of 515-point spectra over the 650–900 nm wavelength range, thus featuring a spectral density of two data points per nanometer. We process the measured spectra with a paired-wavelength spectral analysis method to quantify the oxygen saturation of detected optical inhomogeneities, under the assumption that they feature a locally higher hemoglobin concentration. Our initial measurements on two healthy human subjects have generated high-resolution optical mammograms displaying a network of blood vessels with values of hemoglobin saturation typically falling within the 60%–95% range, which is physiologically reasonable. This approach to spectral imaging and oximetry of the breast has the potential to efficiently exploit the high intrinsic contrast provided by hemoglobin in breast tissue and to contribute a useful tool in the detection, diagnosis, and monitoring of breast pathologies. © 2009 Optical Society of America

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1. Introduction
Breast cancer is the second leading cause of cancer death for American women, and roughly one in eight women will develop an invasive breast cancer during her lifetime [1]. White-light illumination was first introduced for breast cancer imaging in the late 1920s [2], and a significant method refinement occurred around the early 1980s [3,4], which led to the development of several commercially available light-scanning instruments. Some researchers were reporting encouraging results toward breast cancer detection [5–7] while others were showing inferior performance of light-scanning compared with x-ray mammography in terms of both sensitivity and specificity [8,9]. As a result, light scanning did not gain clinical applicability, even though it demonstrated the intrinsic high contrast provided by blood vessels and a variety of benign and cancerous lesions in the breast. In the 1980s and 1990s, a number of research groups developed analytical models for near-infrared light propagation inside tissues [10] and more advanced instrumentation and methods for the collection and analysis of optical data in tissues [11–16]. These efforts resulted in the development of the fields of near-infrared tissue spectroscopy (NIRS) and diffuse optical tomography, which led to a number of applications in tissue diagnostics and monitoring, including optical imaging and spectroscopy of the female breast [17–20].

NIRS and diffuse optical tomography are usually classified into continuous-wave (CW) [21] and time-resolved [22,23] domains, according to the temporal features of the source and detection systems used to probe the tissues. Near-infrared optical
mammography (typically performed by using wavelengths in the optical diagnostic window of 600–1000 nm) has spurred great interest as a diagnostic imaging modality complementary to x-ray imaging because of its advantages of low cost, bedside use, and noninvasiveness and, more importantly, because it provides functional information about the distributions of oxyhemoglobin and deoxyhemoglobin concentrations in tissues that are not accessible with x-ray mammography. The importance of developing alternative approaches to breast cancer detection is further justified by noting that x-ray mammography features a false-positive rate that can be as high as 50% among women screened for ten years [24,25] and introduces risks connected with the employment of ionizing radiation. While NIRS optical mammography features a lower spatial resolution (5–10 mm), it can provide physiological information that offers the potential to achieve a noninvasive discrimination of benign and malignant lesions and the metabolic characterization of breast tumors. Furthermore, the applicability of optical mammography to younger women with mammographically dense breasts that are not transparent to x-rays and its use as a repeatable modality for monitoring therapeutic effectiveness have opened new potential opportunities in breast cancer detection and clinical management that have not been available before [26].

The approaches used in NIRS optical mammography can be divided into two groups: (a) tomographic reconstructions [27–29] and (b) linear or planar scanning of a well-defined source–detector configuration (typically just one source–detector pair) [30–33]. In tomographic reconstructions, optical measurements are typically acquired by using a set of illumination and collection optical fibers (of the order of 16–32) arranged around the breast to deliver and detect near-infrared light at different sites of the breast. For data processing, analytical forward models of light propagation and inverse reconstruction procedures are applied to obtain spatial distributions of the optical properties within the breast. The optical properties that are usually reconstructed according to the diffusion forward model are the absorption coefficient (\(\mu_a\)) and the reduced scattering coefficient (\(\mu_s\)). Tomographic approaches are important because they can, in principle, provide detailed information on the spatial distribution of the optical properties and of the physiological or functional parameters associated with them. However, owing to the complexity of the models used and the large amount of information that they seek to retrieve, they are computationally time consuming, are more prone to artifacts, and sometimes require (or can substantially benefit from) independent a priori information. In contrast, a planar scanning of a single source–detector pair is simpler to implement, does not require the calibration needed to account for the different properties of multiple sources and detectors, and allows for higher spatial sampling rates. Depth discrimination is not directly available from a single projection, but the combination of multiple projections can provide depth information [34]. In this approach of dual scanning of source and detector, typical forward models of light propagation are derived from perturbation theory in the slab geometry, and the inversion procedures usually require some a priori knowledge of the location and the size of the optical perturbation (tumor) [35].

Our research group has recently developed a method, based on a planar tandem scan of one illumination fiber and one collection fiber in a transmission geometry, aimed at detecting optical inhomogeneities in the breast (such as blood vessels and tumors) with higher spatial resolution and at quantifying the oxygenation associated with such optical inhomogeneities by using a novel paired-wavelength spectroscopic approach [36,37]. In this paper we report the implementation of these methods in an instrument for spectral imaging of the female breast, and we show the images collected on two healthy human subjects. The linear spatial sampling rate of 0.5 mm\(^{-1}\) is increased to 2 mm\(^{-1}\) by data interpolation, and, in combination with a second-derivative image-processing algorithm, it allows the discrimination of structures that are separated by as little as ~4 mm. The spectral sampling rate of 2 mm\(^{-1}\) yields continuous optical spectra in the 650–900 nm wavelength range that are translated into quantitative values of hemoglobin oxygen saturation of the detected optical inhomogeneities in the breast. The oxygenation values retrieved with our method typically fall in the 60%–95% range, which is consistent with the range of values of hemoglobin saturation found in the venous, capillary, and arterial compartments. The enhanced spatial and spectral information of the diffuse optical images reported here open new opportunities for optical mammography in the detection, diagnosis, and monitoring of treatment of breast cancer.

2. Instrumentation and Methods of Data Collection

A. Approach to Optical Imaging of the Breast

The mechanical scanning platform for the optical breast imaging instrument was originally developed by Siemens Medical Engineering, Erlangen, Germany, as part of a prototype for frequency-domain (FD) optical mammography [38].

We perform a tandem, 2D raster scan of two collinear optical fibers (one for light delivery and one for light collection) placed on opposite sides of the slightly compressed breast. We collect optical data in the CW domain (for spectral imaging) and in the FD (for the determination of breast thickness), respectively, in two separate scans. We perform another data collection with a handheld probe (see Subsection 2.B) for the average optical properties of the breast at two discrete wavelengths. The acquisition time is of the order of 1–2 min per scan, depending on breast size. We imaged the right breasts of two healthy human subjects in a crano-
caudal (cc) projection. The protocol was approved by the Tufts University Institutional Review Board. The two subjects are labeled here as subject 1 and subject 2 and are 40 and 33 years old, respectively.

B. Frequency-Domain Measurement of Average Optical Properties of the Breast

Prior to the 2D scanning, the optical properties of the breast are measured at two wavelengths (690 and 830 nm) by means of a commercial oximeter (OxiplexTS, ISS Inc., Champaign, Illinois) and a handheld optical probe using a multidistance (2.0, 2.5, 3.0, 3.5 cm) method of data collection and analysis [39]. The optical probe is first calibrated on a tissue-like phantom with known optical properties and then applied to the breast to determine \( \mu_a \) and \( \mu_e \) at 690 and 830 nm. Three different breast sites for each breast are measured to obtain average optical properties of breast tissue at these two wavelengths. The full scattering spectrum is obtained from \( \mu_a \) at 690 and 830 nm by extrapolating the formula \( \mu_a(\lambda) = \mu_{a0}(\lambda/\lambda_0)^{2b} \), where \( \mu_{a0} \) is the reduced scattering coefficient at a reference wavelength \( \lambda_0 \) (650 nm in this case) and \( b \) is the scattering power [40,41]. This preliminary measurement of the scattering spectrum of breast tissue is required to apply our paired-wavelength spectral approach to oximetry (see Subsection 3.D).

C. Scanning for Spectral Imaging: Continuous-Wave Optical Data

We use a xenon arc lamp (Model 6258, Oriel Instrument, Stratford, Connecticut), bandpass filtered in the 400–1000 nm spectral range, with an average emission power on the breast of 325 mW. The illumination light is delivered through a 3 mm diameter optical fiber bundle, resulting in a power intensity of 4.6 W/cm\(^2\) onto the human breast, which is below the 12.6 W/cm\(^2\) skin exposure limit defined by the CIE Standard 009: E 2002 (photobiological safety of lamps and lamp systems). This source fiber is embedded into the source scanning slider, which is driven by a five-phase stepper motor (Model PK543NAWA-T10, Oriental Motor Corp., Torrance, California). The transmitted light is collected by a 5 mm diameter optical fiber bundle on the opposite side of the breast. This detection fiber is fixed to the detector scanning slider, which is controlled by another five-phase stepper motor that is synchronous with the source stepper motor to keep the illumination and collection fiber bundles collinear at all times. This detection fiber is split into two distinct fibers (Fig. 1), one ending with a rectangular cross section (7 mm × 2 mm), and the other ending with a circular cross section (3 mm in diameter). The rectangular end is placed at the entrance port of a spectrograph (Model SP-150, Acton Research Corp., Acton, Massachusetts) where the different wavelengths are dispersed spatially by a 300 G/mm grating blazed at 700 nm. The circular end is connected to a photomultiplier tube (PMT) detector for FD detection as described in Subsection 2.D. A 16 bit CCD camera (Model DU420A-BR-DD, Andor Technology, South Windsor, Connecticut) is located at the exit port of the spectrograph and acquires one spectrum every 57 ms (45 ms of exposure, and 12 ms of charge transfer) on a 1024 × 256 detector array chip. The CCD sensitivity is 12.8 electrons per A/D count at our 100 kHz A/D rate. The CCD pixel size of 26 \( \mu \)m × 26 \( \mu \)m results in a measured spectral range of 444–950 nm and a spectral sampling rate of 2 nm\(^{-1}\). The optical signal at wavelengths shorter than 650 nm is suppressed by the strong absorption of hemoglobin in breast tissue, and the signal at wavelengths longer than 900 nm features a significant absorption from water, which restricts the useful wavelength range to the 650–900 nm that we report in this work. The spectral resolution is worse than the spectral sampling of one data point per 0.5 nm because of the 1 mm entrance slit width of the spectrograph (the slit height is 7 mm), which also results in an effective light collection capability equivalent to a 3 mm diameter optical fiber bundle. However,
the broad spectral features of oxyhemoglobin, deoxyhemoglobin, lipids, and water (the main near-infrared chromophores in breast tissue) somewhat limit the requirements for high spectral resolution in breast tissue spectroscopy. A schematic diagram of the instrument for optical breast imaging is shown in Fig. 1.

The CCD and stepper motor control, as well as the spectral and time data recordings, are performed by LabVIEW. Mechanical scanning of the source and detector fibers starts from the right side of the chest wall in the –x direction (see Fig. 1). The end point of each scanning line along x is automatically determined by software when the spectrum (also shown in real time on a computer screen during the breast imaging measurement) is saturated because the source light reaches the detector fiber without passing through the breast tissue. At that time, the scanner stops and moves in the y direction for 2 mm and starts a new scanning line along +y. This determines a pixel size along y of 2 mm. The x scanning speed of 35 mm/s, combined with an optical detection time of 57 ms per spectrum, results in a pixel size of 2 mm along x. The pixel size is therefore 2 mm × 2 mm, and the total acquisition time per scan is 1–2 min, depending on breast size. When the length of one scanning line is smaller than a set threshold length (1 cm), the LabVIEW program stops the scanning and returns the optical probing fibers to the home position. The scanning speed along x, the interline steps along y, and the optical exposure time are all adjustable parameters that the users may modify to adapt to different breast sizes and signal-to-noise ratio (SNR) requirements.

D. Scanning for Breast Thickness Estimation: Frequency-Domain Optical Data

To preserve high optical contrast and limit the dynamic range over the whole imaged area of the breast, a so-called N-image method for edge effect corrections was proposed by our group [42]. The N-image method relies on the estimation of the breast thickness at each pixel r(x,y)], which is obtained by measuring the phase lag of intensity-modulated light with a FD system and by assuming the linearity of the phase versus breast thickness [42]. Some details on the N-image derivation are found in Subsection 3.B. We used intensity-modulated light at 690 nm, emitted by laser diodes housed in a commercial oximeter (OxiPlexTS, ISS Inc., Champaign, Illinois) that operates at a modulation frequency of 110 MHz. A 3 mm diameter optical fiber bundle, which is attached to the source scanning slider, delivers the 690 nm modulated light onto the human breast. The transmitted light is carried by the 5 mm diameter fiber bundle first (shared with the CW light path) and then goes into the 3 mm diameter collection optical fiber bundle before reaching a PMT detector, whose gain is modulated at 110.005 MHz. Heterodyne detection, low-pass filtering, and fast Fourier transformation are applied to measure the pixel-by-pixel phase delay in the breast image. The scanning procedure and parameters are identical to the CW domain acquisition except for the presence of a mechanical shutter installed in the scanning slider to block the light path to the collection optical fiber bundle at the end of each scanning line to prevent overexposure of the PMT detector.

3. Data Analysis

A. Two-Dimensional Spatial Interpolation to Achieve a Pixel Size of 0.5 mm × 0.5 mm

The spatial sampling rate of our data is 0.5 mm−1 along both x and y (2 mm × 2 mm pixel size). We increase the spatial density of data points to 2 mm−1 along both x and y (0.5 mm × 0.5 mm pixel size) by postprocessing the collected data, using a 2D cubic spline interpolation [43]. Because of the intrinsic low-pass filtering of spatial frequencies in diffuse optical imaging, not only does this interpolation yield smoother images, it also allows for further data processing (in particular using the second-derivative algorithm described in Subsection 3.C) on a finer spatial grid of interpolated data that represent a reasonable estimate of data that would have actually been collected with such a finer sampling rate. Both the CW spectral data and the breast thickness distribution measured with FD data are spatially interpolated to yield a 2D density of data points of 4 mm−2 (0.5 mm × 0.5 mm pixels).

B. Optical Density Images (N-Images)

When the scanning approaches the edge of the breast, because of the reduced thickness of breast tissue there is a significantly higher photon transmission (greater transmitted CW intensity) and a shorter mean path length of photons in tissue (a smaller phase delay in FD measurements). We have previously observed that the phase delay is affected mostly by the change in tissue thickness (this is the basis for using the FD phase to estimate the breast thickness), while the CW intensity can be significantly affected by changes in both thickness and local optical properties of tissue. Based on an empirical inverse relationship between the transmitted intensity (I) and the breast thickness (r), an edge-corrected intensity parameter is defined as follows: N(x,y;λ) = r′I′(λ)/r(x,y)I(x,y;λ), where r′ is the maximum breast thickness (equal to the distance between the two parallel glass plates that slightly compress the breast), and I′(λ) is the intensity at a reference pixel (x′,y′) where thickness is r′ [42]. This edge-corrected parameter defines the so-called N-images, which report N(x,y;λ) at any given pixel (x,y) and for a given wavelength λ. The N parameter can be seen as the inverse of a normalized edge-corrected intensity, so that it assumes larger values where the tissue optical density is higher and smaller values where the tissue optical density is lower. More precisely, the logarithm of N(x,y) is the difference between the optical densities that would be acquired on uniform-thickness breast tissue at pixels (x,y) and
\((x', y')\). For this reason, one can look at \(N\)-images as representing the spatial distribution of the optical density of breast tissue.

C. Spatial Second-Derivative Images (\(N\)-Images)

We have previously reported the enhanced display of blood vasculature, tumors, and other optical inhomogeneities in optical mammograms yielded by a spatial second-derivative operator [36]. We have applied this second-derivative algorithm to the \(N\)-images resulting from the spatial interpolation that forms a pixel size of 0.5 mm \(\times\) 0.5 mm (as explained in Subsection 3.A). To reduce the noise level, we first apply a 3 \(\times\) 3 moving average, low-pass filter twice, and then we find the minimum of the second derivatives along four directions \((x, y, x+y, x-y)\) [44] to maximize the contrast of directional structures. Such a minimum value of the second derivative is displayed in the second-derivative images. The pixels associated with negative second-derivative values, which indicate a localized increase in optical density, are displayed in gray-scale levels, whereas the pixels with nonnegative values are set to zero and displayed in white.

D. Oxygenation Images: Paired-Wavelength Spectral Analysis for Oximetry

We recently proposed a method of paired-wavelength spectral analysis to measure the oxygen saturation of tissue inhomogeneities that feature a locally higher hemoglobin concentration [37,45]. This method is based on spectra of \(\Delta I/I_0 = (I-I_0)/I_0\), where \(I\) is the transmitted intensity at the inhomogeneity and \(I_0\) is the averaged local background transmitted intensity. In our breast imaging approach, \(N\) is proportional to the inverse of the edge-corrected intensity, so that \(\Delta I/I_0 = I/I_0-1 = N_0/N - 1 = (N_0-N)/N = -\Delta N/N\), and the latter term (or equivalently \(+\Delta N/N\)) is the one that we use for the paired-wavelength spectral analysis. The pixels of interest for the computation of the oxygen saturation are those associated with a local absorption peak, or with a local minimum in the second-derivative matrix \(N^\prime\) in one of the four directions \((x, y, x+y, x-y)\). Therefore, we first identify those pixels \((x, y)\) that correspond to a local minimum in \(N^\prime\), and for each of those pixels we calculate the corresponding \(N_0(x, y)\) by considering an area of 3 cm \(\times\) 3 cm centered around the pixel of interest. The value of \(N_0(x, y)\) is taken as the average value of \(N\) in pixels \((\xi, \eta)\) in this area for which \(N(\xi, \eta) < N(x, y) - 0.8[N(x, y) - N_{\text{min}}]\), where \(N_{\text{min}}\) is the minimum \(N\) in the 3 \(\times\) 3 cm\(^2\) area. We then select the set of wavelength pairs \((\lambda_1, \lambda_2)\) that satisfy the condition \(\Delta N/N\max = \Delta N/N\max\), where the “max” notation represents the pixel \((x, y)\) that is associated with the local absorption maximum peak. The oxygenation \((SO_2)\) associated with the hemoglobin-based inhomogeneity at the pixel of interest for each wavelength pair \((\lambda_1, \lambda_2)\) is given by [37,45]

\[
SO_2 = \frac{c_{\text{Hb}}(\lambda_2) - c_{\text{HbO}_2}(\lambda_1)\Delta\varepsilon_{\text{Hb}}(\lambda_2)}{[c_{\text{Hb}}(\lambda_2) - c_{\text{HbO}_2}(\lambda_2)] + [c_{\text{HbO}_2}(\lambda_1) - c_{\text{Hb}}(\lambda_1)\Delta\varepsilon_{\text{Hb}}(\lambda_2)]}
\]

where \(c_{\text{Hb}}\) is the molar extinction coefficient of deoxyhemoglobin, \(c_{\text{HbO}_2}\) is the molar extinction coefficient of oxyhemoglobin, and \(\Delta\varepsilon_{\text{Hb}}\) is the background reduced scattering coefficient measured as described in Subsection 2.B. The value of oxygenation associated with a given pixel is the average of the values obtained from Eq. (1) for a set of wavelength pairs that have a minimum contrast-to-noise ratio of 8 and that yield an oxygenation value that is affected by an error below a given threshold [37,45], which for the data reported here was set to 6%.

4. Results

A. Signal-to-Noise Characterization on a Tissuelike Phantom

The approach to spectral imaging reported here, based on a mechanical scan of a source–detector pair over the imaged area, a broadband light source, and a parallel spectral acquisition by a spectrograph–CCD combination, needs to balance the requirements of fast scanning and high SNR. We measured the SNR of the transmitted intensity over the spectral band of interest of 650–900 nm by using a tissue-like phantom made of Delrin that mimics the optical properties of breast tissue (thickness 6.3 cm, \(\mu_s \sim 0.01\) cm\(^{-1}\), \(\mu_s' \sim 10\) cm\(^{-1}\)). In this measurement, we used a 40 mW illumination power and a 10 \(\mu\)m spectrograph entrance slit. The measured SNR\(^2\) as a function of CCD exposure time (from 0.01 s to 1 s) is reported in Fig. 2 for the two wavelengths for which the SNR was the lowest (650 nm) and highest (829 nm), respectively. The measured SNR\(^2\) is linearly dependent on the exposure time, with slopes of \(2.54 \times 10^4\) s\(^{-1}\) at 650 nm and \(11.8 \times 10^4\) s\(^{-1}\) at 829 nm, consistent with a dominant source of error from shot noise. In fact, the measured slopes are consistent with the number of photoelectrons detected per second at a given wavelength channel of the CCD (the illumination optical fiber delivers about \(10^{14}\) photons/s/nm, which are attenuated by a factor of about \(10^6\) by the phantom, by a factor of \(10^2\) by the slit width, and by 1–2 more orders of magnitude by the compression glass plates, optical fiber losses, spectrograph, etc.). Furthermore, the factor of \(\sim 4.6\) between the SNR\(^2\) slopes at 829 and 650 nm reflects the ratio of \(\sim 5\) between the arc lamp emission irradiance at 829 and 650 nm (the absorbance of the sample at the two wavelengths is comparable, as is the quantum efficiency of the CCD). At the exposure time of 45 ms used for imaging the human breast, the SNR for intensity measurements in the phantom ranged from 33 to 73, but in the actual measurements on human breasts was found to be of the order of 100 or
greater because of the wider slit width and larger illumination power employed.

B. Spectral Imaging: $N$-Images and $N'$-Images

Figure 3 (Media 1) shows the $N$-images [Fig. 3(a)] and the second-derivative, $N'$-images [Fig. 3(b)] measured on subject 1, right breast, cranio-caudal projection (Rcc). The glass plate separation was 3.7 cm in this case, and the scanning time per acquisition was 75 s. Our spectral imaging approach generates 515 spectrally resolved $N$-images and their corresponding $N'$-images, one approximately every 0.5 nm over the 650–900 nm wavelength range. While Fig. 3 (Media 1) shows only representative images at 700 nm (for the $N$-image) and 800 nm (for the $N'$-image), and a few images at immediately shorter and longer wavelengths, the whole set of spectral images can be viewed at external Web pages for the $N$-image at http://ase.tufts.edu/biomedical/research/Fantini/om/n.html and for the $N'$-image at http://ase.tufts.edu/biomedical/research/Fantini/om/2nd-derivative.html. In each Web site, we also present the spectrally-averaged $N'$-image and the oxygenation map. We also arranged the 515 spectrally resolved second-derivative images in a movie that displays them in a rapid sequence to allow the visualization of the variability or consistency of structures over images at different wavelengths (see Media 1). We note that, while the wider structures are present in all the sequence of images, a few of the finer structures appear and disappear randomly by changing the wavelength. Even though we implemented a low-pass filter to suppress the noise, this behavior is due to noise in pixels where the contrast-to-noise ratio is not large enough.

It may seem surprising that the second-derivative algorithm applied to the $N$-image of Fig. 3(a) results in the visualization of a large number of structures in the $N'$-image of Fig. 3(b) that are not visible in the blurred $N$-image. Figures 4(a) and 4(b), respectively,
clarify this point by showing a representative line scan (the one at $y = 2$ cm) for the spectrally averaged $N$ and $N^\prime$ images for subject 1. The line graphs are shown in Figs. 4(c) and 4(d) for $N$ and $N^\prime$, respectively. The narrow spatial peaks in $N^\prime(x)$ are determined by visible changes in curvature of the $N(x)$ function that are in turn determined by the superposition of the effects of closely spaced optical inhomogeneities. While such closely spaced inhomogeneities cannot be distinguished by the broad $N$ peaks, they can be resolved by the narrow $N^\prime$ peaks. Some specific cases that illustrate the enhanced resolving power of the second-derivative image are highlighted in Fig. 4 by the vertical lines at selected $N^\prime$ peaks.

C. Oxygenation Images

Figure 5 shows the oxygenation images for the two human subjects. The parallel glass plate distances for the two subjects are 3.7 and 6.0 cm, respectively. The scanning time for each subject is 75 and 96 s, respectively. As described in Subsection 3.D, we used the paired-wavelength spectral analysis to assign an oxygenation value to the detected inhomogeneities identified by local minima of $N^\prime$. For this reason, we overlap the color-coded oxygenation map onto the gray-scale second-derivative maps, Figs. 5(a) and 5(c). We analyze only the pixels associated with local absorption peaks identified by the $N^\prime$-image because the paired-wavelength method requires local absorption peaks. As a result, the white blotches in the oxygenation map, Fig. 5, simply represent all those pixels for which we did not perform the oxygenation calculation. The distribution of oxygenation values, which indicates hemoglobin saturation under the assumption that the detected optical inhomogeneities feature a locally higher hemoglobin concentration, are represented by the histograms of Figs. 5(b) and 5(d). For subject 1, 86% of the oxygenation values fall within the range 60%–95% [Fig. 5(b)], while for subject 2 they are more narrowly distributed in the range 85%–95%. These values are consistent with data reported for in vivo healthy breast oxygenation [33,46,47].

Figure 6 shows the spectra of $\Delta N/N$ measured at ten representative pixels of the image for subject 1. The oxygenation values in these ten pixels range from 25% to 98%, and the corresponding spectra shift from the spectral features of deoxyhemoglobin (a pronounced peak at 758 nm) to those of oxyhemoglobin (a positive derivative between 700 and 900 nm).

5. Discussion

We have reported an approach to optical mammography that emphasizes the spatial and spectral information content achievable with near-infrared diffuse imaging.

A relatively high 2D spatial sampling density of 25 points/cm$^2$ (for data collection) is further increased to 400 points/cm$^2$ by data interpolation. We have shown how such a fine pixel distribution has allowed the generation of second-derivative images that enhance the visualization and resolution of optical inhomogeneities in the breast and lead to a better identification of regions of interest associated with breast inhomogeneities. Of course, data interpolation cannot introduce additional information into the tissue optical property distribution that is not already present in the original data. However, it can help generate higher-quality images and enhance the display of spatial information in the original data. The structures that are visible in the optical mammograms of these healthy subjects, Figs. 5(a) and 5(c), are assigned to blood vessels. In fact, blood features a high intrinsic optical contrast in breast tissue, having a near-infrared absorption coefficient more than one hundred times greater than that of the surrounding breast tissue. Such a high intrinsic optical contrast provided by blood in breast tissue is one of the key aspects to be exploited by optical mammography, especially given the modifications to the blood distribution, oxygenation, and concentration induced by carcinogenesis and by changes in the local metabolic demand of tissues [46,48]. Currently, we are not able to assign the tissue depth of the structures that we detect in the second-derivative images,
Fig. 5. (a), (c) Hemoglobin saturation maps measured for subjects 1 and 2, respectively, with paired-wavelength spectral analysis. The false-color representation of oxygenation values is superimposed on the gray-level display of the second derivative of $N$. (b) and (d) are the histograms of the oxygenation values displayed in (a) and (c), respectively.

Fig. 6. Measured spectra of $(N - N_0)/N = \Delta N/N$ at ten representative locations of the optical mammogram for subject 1. The oxygen saturation values corresponding to each spectrum are indicated and range from 25% to 98%. 
but this can be done by introducing off-axis illumination and/or detection [49,50], which we plan to do in future developments of the instrument.

Acquiring optical data over a broad range of wavelengths is important to obtain information on the major near-infrared absorbers in tissue (deoxyhemoglobin, oxyhemoglobin, lipids, water) as well as the spectral features of tissue scattering [20]. In this work, we have focused on determining the relative concentrations of oxyhemoglobin and deoxyhemoglobin in absorbing inhomogeneities, for which we have applied a paired-wavelength spectral method that requires a high spectral sampling rate. We have previously demonstrated the reliability and accuracy of our paired-wavelength method in the measurement of the relative concentration of two localized chromophores within a liquid tissue-like phantom [45]. Here we have reported a spectral sampling of 2 wavelength/nm, which is adequate given the broad spectral features of oxyhemoglobin and deoxyhemoglobin. The importance of broadband spectroscopy in optical mammography has been pointed out both for more accurate chemometrics [26,51] and for potential improvements in image reconstruction algorithms [52]. The oximetry results reported here, Fig. 5, demonstrate the applicability of the paired wavelength method in vivo and represent physiologically reasonable values of oxygenation in the optical mammograms. Some of the oxygenation values in Fig. 5, for example, those in the 25%–60% range, seem lower than expected in a healthy breast. However, we observe that the associated spectra reported in Fig. 6 do show the spectral signatures of deoxyhemoglobin, and we therefore consider these low oxygenation values to actually indicate hypoxic areas. However, the lack of independent oxygenation measurements does not allow us to discuss the accuracy of the oxygenation measurements, which will need to be tested for their clinical potential in a pilot human study on patients with breast cancer.

6. Conclusion

The most notable features of the optical mammograms reported here are (1) a high spatial density of pixels at every wavelength, and (2) continuous spectral data over the wavelength range of 650–900 nm at every image pixel. More specifically, our spectral imaging approach generates optical mammograms with 400 pixels/cm² and 515-point spectra (one point approximately every 0.5 nm over the wavelength band 650–900 nm) at every pixel. These features have the potential to better exploit the high intrinsic contrast provided by hemoglobin in breast tissue and to perform more accurate spectral measurements to detect, diagnose, and monitor breast pathologies. In this work we have used the spectral information to determine the oxygenation of hemoglobin in detected breast inhomogeneities. The absolute value of hemoglobin saturation is an important parameter to determine tissue abnormality and hypoxic fractions in breast lesion sites [46]. It is highly possible that a robust method to measure the hemoglobin saturation associated with breast lesions will allow reliable study of the correlation between hemoglobin saturation and the nature (benign or malignant) of breast lesions. While it is not yet demonstrated that quantitative oximetry in optical mammography can reliably discriminate benign from cancerous breast lesions, it is nevertheless a powerful diagnostic tool that can advance the research in this field and find a role in the diagnosis and monitoring of breast cancer.

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