Broadband optical mammography instrument for depth-resolved imaging and local dynamic measurements

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(Received 29 October 2015; accepted 30 January 2016; published online 25 February 2016)

We present a continuous-wave instrument for non-invasive diffuse optical imaging of the breast in a parallel-plate transmission geometry. The instrument measures continuous spectra in the wavelength range 650–1000 nm, with an intensity noise level <1.5% and a spatial sampling rate of 5 points/cm in the x - and y -directions. We collect the optical transmission at four locations, one collinear and three offset with respect to the illumination optical fiber, to recover the depth of optical inhomogeneities in the tissue. We imaged a tissue-like, breast shaped, silicone phantom (6 cm thick) with two embedded absorbing structures: a black circle (1.7 cm in diameter) and a black stripe (3 mm wide), designed to mimic a tumor and a blood vessel, respectively. The use of a spatially multiplexed detection scheme allows for the generation of on-axis and off-axis projection images simultaneously, as opposed to requiring multiple scans, thus decreasing scan-time and motion artifacts. This technique localizes detected inhomogeneities in 3D and accurately assigns their depth to within 1 mm in the ideal conditions of otherwise homogeneous tissue-like phantoms. We also measured induced hemodynamic changes in the breast of a healthy human subject at a selected location (no scanning). We applied a cyclic, arterial blood pressure perturbation by alternating inflation (to a pressure of 200 mmHg) and deflation of a pneumatic cuff around the subject's thigh at a frequency of 0.05 Hz, and measured oscillations with amplitudes up to 1 μ M and 0.2 μ M in the tissue concentrations of oxyhemoglobin and deoxyhemoglobin, respectively. These hemodynamic oscillations provide information about the vascular structure and functional integrity in tissue, and may be used to assess healthy or abnormal perfusion in a clinical setting. © 2016 AIP Publishing LLC. [<http://dx.doi.org/10.1063/1.4941777>]

I. INTRODUCTION

A. X-ray and optical breast imaging

X-ray mammography is the primary screening method for breast cancer, and it provides structural information based on high resolution images of breast tissue density. However, the identification of breast cancer using X-ray mammography is difficult in the mammographically dense breasts of younger women, and X-rays are ionizing radiation which may have carcinogenic effects when used more frequently than every 1–2 years. Furthermore, false-positive rates in X-ray mammograms can be as high as 50% requiring additional testing at a financial cost to health care institutions and an emotional cost to the patients.^{1,2} These are some of the reasons that led to an interest in alternative methods for breast imaging.

White-light illumination was initially proposed as a diagnostic method for detecting breast lesions in the late 1920s,³ and by the 1980s, light-scanning instruments became commercially available for optical imaging of the breast. A number of researchers showed promising results towards the capability of light-scanning mammography to detect breast cancer using non-ionizing optical radiation.^{4–6} Although

they were able to identify regions of contrast due to the increased optical absorption of the cancerous tissue, light-scanning was deemed inferior to X-ray mammography in terms of its sensitivity to breast cancer.^{7,8} In the 1990s, analytical models to describe near-infrared light propagation in tissues,⁹ combined with sophisticated instrumentation for data collection and rigorous methods of data analysis, led to the development of more advanced near-infrared spectroscopy (NIRS) and diffuse optical tomography (DOT) systems aimed at detecting, diagnosing, and monitoring breast cancer.^{10–22}

Near-infrared spectroscopy and DOT of the breast take advantage of the intrinsic optical contrast provided by the tissue concentrations of oxyhemoglobin, deoxyhemoglobin, water, and lipids ([HbO], [Hb], [water], and [lipids], respectively). These are the four major tissue chromophores that absorb light in the near-infrared wavelength range 650–1000 nm. The ratio of oxyhemoglobin to total hemoglobin concentration ([HbT]) yields the oxygen saturation of hemoglobin, which is a measure of the balance of oxygen supply and oxygen utilization in tissue. Localized changes in the concentration and oxygen saturation of hemoglobin in breast tissue have been linked to angiogenic developments and perturbations to the tissue metabolic rate associated with the development of breast cancer.^{11,12,14,16,19,23–25} Additionally, localized changes in [water] and [lipids] can be indicative of structural modifications in the tissue associated with changes in cellularity and fibrous tissue content. These changes have also been shown to be indicative of tumor development.^{12,14,16,17,23–25}

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B. Relevance of spectral information

Near-infrared spectroscopy and DOT systems can be classified as continuous-wave (CW),^{10,11,16,17} time-domain (TD),^{12,23} and frequency domain (FD)^{10,14} based on the temporal characteristics of the light source and optical detection systems. Absolute measurements of [HbO], [Hb], [water], and [lipids] are dependent on the accurate recovery of the tissue absorption coefficient (μ_a), which in turn requires the determination of the tissue reduced scattering coefficient (μ_s'). Time-domain and FD systems have the capability to measure μ_a and μ_s' simultaneously, but usually feature limited spectral sampling (<10 wavelengths). Theoretically, to measure the concentrations of four chromophores ([HbO], [Hb], [water], and [lipids]), one only needs measurements at four wavelengths that feature sets of extinction coefficients of the four chromophores that are linearly independent. However, because of random and systematic experimental errors, accurate measurements of μ_a and μ_s' benefit from greater spectral sampling both in terms of the number of wavelengths and the wavelength range. In this regard, TD systems may take advantage of super-continuum lasers^{18,19} to achieve broadband spectral collection. However, TD systems often require longer acquisition times for usable signal-to-noise ratios, and each wavelength requires an individual photon counting system increasing the complexity of achieving high spectral sampling rates.

Systems based on CW light inherently have less information content than TD or FD systems and are unable to measure the absorption and scattering properties of tissue separately.²⁶ However, CW systems generally provide higher spectral sampling (>100 wavelengths over the full spectral range of interest) with a standard spectrograph and charge-coupled device (CCD) camera system. Spectral sampling at wavelengths longer than 900 nm can provide greater sensitivity to lipids and water as a result of their absorption peaks at 930 and 970 nm, respectively.¹⁹ The inability of CW systems to independently measure the reduced scattering coefficient requires some kind of assumption about the scattering properties of the breast tissue. For example, one may perform FD measurements at a set of wavelengths and obtain a reduced scattering spectrum by interpolation,¹⁶ or a scattering spectrum may be assumed on the basis of measurements reported in the literature for breast tissue.¹⁷

C. Spatial and temporal information

The spatial information of NIRS and DOT images yields the distribution of tissue chromophores within the breast tissue. Such spatial distributions relate the location of breast lesions to associated perturbations to the concentration and oxygen saturation of hemoglobin, or to perturbations in water and lipid content. Full 3D spatial information is desirable to accurately identify the location and extent of breast lesions. Approaches to 3D imaging can be grouped into tomographic reconstructions^{20,27} and depth discrimination methods in planar scanning systems.^{10,19,23} Diffuse optical tomography systems use a number of source-detector pairs (typically 32–64 pairs)^{11,21} placed around the breast or in a parallel-plate geometry^{20,28} to solve the inverse imaging problem in 3D.²⁹

While DOT systems offer full 3D spatial reconstructions, the task is complex, has high demands on data collection and computational routines, and suffers from the ill-posed nature of the inverse imaging problem in the diffusion regime. Planar scanning systems may use a single source-detector pair that is scanned to create a 2D projection image of the breast in a transmission geometry,^{17,19,23} or may employ parallel planar detection by a lens-coupled CCD camera.²⁰ Scanning systems typically provide a fine spatial sampling ($>1\text{ cm}^{-1}$) to identify detailed superficial structures, such as blood vessels, within the breast.^{10,23,30} Depth discrimination can be obtained from multiple projections realized by offset detectors that are not collinear with the optical illumination.^{31–33} Compared to a full tomographic reconstruction, this approach to depth discrimination is more straightforward and potentially robust. However, it is not capable of resolving multiple structures in depth, and it provides a coarser depth assessment than tomographic approaches based on a complete optical data set around the breast.

Temporal information, as collected with dynamic optical measurements of breast tissue, can broaden the scope of optical mammography to include intrinsic hemodynamic features and hemodynamic responses to external stimuli or perturbations. Recently, research groups have been exploring the possibility that induced changes of HbO and Hb within the breast may be used to discriminate healthy and cancerous tissue. Different methods of dynamic breast compression^{22,34} or regulated respiration^{21,35,36} have been shown to cause optically measurable hemodynamic fluctuations in breast tissue. Manual compression of the breast induces an increase in the interstitial fluid pressure which can cause a decrease in blood content of up to 20% in healthy subjects.³⁴ Similarly, regulating respiration by either paced breathing or breath-holding induces systemic changes in blood pressure that result in changes in blood content that can range from 5% to 10% in healthy subjects.^{21,36} It has been reported that differences in the measured magnitude of this change and in the associated washout rate are indicative of differences in the vasculature of healthy and cancerous tissues.^{21,34,36,37} To measure these changes, systems with acquisition rates on the order of 1 Hz are required since the relevant frequency band of tissue hemodynamics is generally limited to frequencies below the heart rate ($\sim 1\text{ Hz}$). Since the characteristic times of induced hemodynamics may be on the order of a few seconds, a sampling frequency of 0.5–1 Hz is sufficient in most cases. Hand-held probes^{38,39} generally provide the highest temporal resolution ($>10\text{ Hz}$) and have the flexibility to measure different locations on the breast. However, concurrent measurements, which are required to make comparative analyses of multiple locations on the breast, require multiple probes with unknown calibration factors related to individual probe-tissue coupling and pressure. Depending on the number of source-detector pairs, tomographic systems are limited in their temporal resolution ($<1\text{ Hz}$) but benefit in being able to provide detailed spatial maps of optical properties over time. However, recently, Flexman *et al.* have developed a tomographic system capable of 3D reconstructions at sampling frequencies up to 2 Hz depending on the number of wavelengths and source-detector pairs used.

D. Main features of the instrument presented here

We have previously reported an optical mammography instrument based on a CW, planar tandem scan of a collinear source-detector pair (wavelength range: 650–850 nm) to generate 2D snapshots of chromophore maps in breast cancer patients.¹⁷ We report here the development of a new CW breast scanning system (wavelength range 650–1000 nm) using one source and four detector optical fibers (one collinear and three off-axis with respect to the source fiber) with high spectral ($\sim 2.5 \text{ nm}^{-1}$), spatial (25 cm^{-2}), and temporal ($\sim 10\text{--}20 \text{ Hz}$) sampling rates. With respect to our previous instrument, the extended spectral range allows for a higher sensitivity to water and lipids, the off-axis detection scheme allows for depth sensitivity and 3D imaging, and the temporal resolution (at a stationary location) allows for hemodynamic measurements. Here, we demonstrate the 3D capabilities of this new instrument by imaging a tissue-like optical phantom containing absorbing structures, and the dynamic measurement capabilities by assessing physiological hemodynamics in healthy volunteers. We also discuss the relevance of implementing these advanced spectral, spatial, and temporal methods in a consolidated system, and how it can enhance the detection and monitoring of breast cancer in a clinical setting.

II. METHODS

A. System for scanning optical mammography

The emission of a QTH lamp (Model No. 66635, Newport Instruments, Irvine, CA) was band pass filtered by a spectral filter (Model YSC1100, Asahi Spectra USA, Torrance, CA) to a 340–1130 nm spectral bandwidth. The illumination light was delivered to the breast or optical phantom using a 2.5 mm diameter optical fiber bundle with a maximum illumination power exiting the fiber of 350 mW. The source-fiber was

mounted onto a 2D scanning slide controlled by stepper motors (Model XN10-0180-E02-71, Velmex, Bloomfield, NY). The end of the fiber was separated from the breast by a 6.5 mm thick, clear, polycarbonate plate. Although light-channeling is a concern with illumination through clear polycarbonate plates, in a transmission geometry, the effects of light channeling on our measurements are minimal due to the large source-detector distance and strong attenuation of light in tissue. The source fiber numerical aperture (NA) was 0.55, which resulted in a spot diameter on the breast of about 11 mm, and a maximum radiant exposure of $\sim 250 \text{ mJ/cm}^2$. With a 16 mm/s scan speed, the maximum illumination time at any given location on the breast (pixel resident time) was $\sim 690 \text{ ms}$, for which the maximum permissible exposure of skin to near-infrared light is $\sim 1 \text{ J/cm}^2$ while the fiber was scanned (ANSI Z136.1-2007). Typically, measurements were carried out using an illumination power of $\sim 100 \text{ mW}$ (yielding a radiant intensity at the tissue of $\sim 75 \text{ mJ/cm}^2$). However, for subjects with larger breasts (requiring $>6 \text{ cm}$ plate separation), the ability to increase the power level allowed us to maintain a suitable signal to noise ratio (SNR). For static measurements (no scanning), where the pixel resident time of the fiber could be as long as 10 min, the maximum permissible exposure was limited to a radiant intensity of 0.2 W/cm^2 (ANSI Z136.1-2007). For these measurements, we had to ensure that the power exiting the fiber was less than 190 mW. In cases where it was desirable to increase the SNR, we had the capability to increase the integration time since our temporal sampling rate ($\sim 10\text{--}20 \text{ Hz}$) was greater than the required temporal resolution of 1 Hz for physiological hemodynamics.

The breast or phantom was placed between the two polycarbonate plates attached to the scanning slide housing (Fig. 1(a)). The transmitted light was collected by four 2.5 mm diameter detector optical fibers with a NA of 0.22 which were arranged in a “T” pattern (Fig. 1(b)) and mounted onto another 2D scanning slide. The smaller acceptance angle of

FIG. 1. (a) Block diagram of the optical mammography instrument for spectral imaging of the human breast. The emission of a quartz-tungsten-halogen (QTH) lamp is spectrally filtered from 340 to 1130 nm and light is delivered to the breast through an optical fiber for illumination. (b) A four-legged detector fiber is arranged in a transmission geometry with the fibers oriented in the x - y plane. The offset between each fiber and D_0 was set at $\delta D = 20 \text{ mm}$. (c) The $2 \times 1 \text{ mm}^2$ rectangular arrangement of each detection fiber bundle into a single ferrule spanning 6.4 mm in height. The spectrograph spatially resolves light of different wavelengths from each fiber simultaneously onto a $26.8 \times 8 \text{ mm}^2$ charge-coupled device (CCD) chip which acquires four spectra (wavelength range: 650–1000 nm), corresponding to the four detection fibers, at each source position.

the detector fibers provided a smaller spot diameter at the breast (~ 3 mm) allowing for finer spatial sampling in our images. The “T” pattern for the detectors maximized the size of the imaged area for which depth information was collected. The source and detector fibers were scanned in tandem such that the central detector fiber (D_0) remains collinear with the source fiber during the scan. The three off-axis detector fibers were offset in the positive and negative x -directions (D_{+x} and D_{-x} , respectively) and in the positive y -direction (D_{+y}). The offset distances of D_{+x} and D_{-x} in the x -direction (δD_{+x} and δD_{-x} , respectively) and D_{+y} in the y -direction (δD_{+y}) were set to the same value $\delta D = 20$ mm with respect to the location of D_0 (the collinear detector). To obtain depth information for structures oriented in multiple directions, we have previously shown that two orthogonally offset detectors, are sufficient.³⁰ The arrangement of the detector fibers with two offset detector locations along the x -direction ensured that one of the off-axis fibers offset in the x -direction would remain within the x - y projection of the imaged tissue, thus providing depth information at the edges of the breast. Ideally, a fifth offset detector fiber in the negative y -direction would maximize the depth information in both spatial dimensions. However, to maximize the imaged area, we omitted this fiber to allow D_0 to scan as close to the chest wall as possible. The four detector fibers were joined into a single ferrule with four 2×1 mm² rectangular areas corresponding to each of the detector fibers (Fig. 1(c)). The four rectangular fiber ends were vertically distributed over 6.4 mm on the end of the ferrule with 0.8 mm spacing on either side of each fiber which resulted in a total vertical dimension of 8 mm. These 8 mm corresponded to the height of the CCD chip within the camera (Model Pixis400, Princeton Instruments, Princeton, NJ). The ferrule was placed at the entrance slit of the spectrograph (Model SP-150, Princeton Instruments, Princeton, NJ) which contains an internal shutter with an actuation time of 8 ms. The light from the four fibers was spectrally dispersed by a 300 G/mm grating blazed at 830 nm resulting in a dispersion of 18.7 nm/mm.

The cooled, 16-bit CCD camera was attached at the exit port of the spectrograph and acquired four spectra, corresponding to the four detection fibers, simultaneously. The sensitivity of the CCD was 1 electron per analog-to-digital (A/D) count with a 2 MHz A/D conversion rate. The CCD was made up of a 1340×400 pixel² array with 20×20 μm^2 pixels (resulting in a 26.2×8 mm² CCD chip). The lateral dimension of the CCD was used for spectral sampling whereas the longitudinal dimension was used for the spatial sampling of the four detection fibers. The resulting spectral sampling rate was ~ 2.5 nm⁻¹, and the measured spectral range was 580–1081 nm based on a center wavelength of 830 nm. The optical signal below 650 nm is strongly attenuated by the absorption of hemoglobin in breast tissue, and the signal at wavelengths longer than 1000 nm was restricted by the lower quantum efficiency ($< 30\%$) of the CCD. Consequently, only the wavelength range 650–1000 nm was used in this work. The spectral resolution of the system was limited by the slit width; to achieve the highest signal-to-noise ratio, we maximized the detected intensity by using a slit width of 2 mm, which resulted in a spectral resolution of ~ 50 nm.

The broad spectral features of hemoglobin, water, and lipids limit the requirement for high spectral resolution in NIRS of the breast. Considering the limited requirement for spectral resolution, the spectral sampling provided by this camera was more than necessary. However, it was chosen because it provided a quantum efficiency above 90% for light in the wavelength range 650–900 nm and above 30% up to 1000 nm which was necessary for robust measurements of water and lipids. Additionally, we found that laterally binning the CCD chip only marginally improved our acquisition rate and SNR at the cost of reducing our dynamic range. Consequently, we maintained the full spectral sampling rate of the CCD chip in our data collection scheme.

Control of the stepper motors and CCD, as well as data collection, was done with LabVIEW (National Instruments, Austin, TX). Scanning started close to the chest wall moving continuously in the $+x$ -direction at a speed of 16 mm/s. The scanner was stopped automatically by the software at the edge of the breast when the tissue thickness was reduced by approximately 20 mm and the transmitted intensity exceeded the dynamic range of our CCD and saturated it. At that point, the scanner moved 2 mm in the $+y$ -direction and started scanning in the $-x$ -direction. With an exposure time (including the 8 ms shutter actuation) of 100 ms and a readout rate of 28 ms, spectra were acquired every 128 ms resulting in a pixel size of ~ 2 mm along the x -direction. When the length of a scanned line was less than 10 mm (5 pixels) the data acquisition was stopped and the fibers returned to their starting position. The typical scanning time for a 100 mm $\times$ 100 mm area was about 6 min. Our method of raster-scanning optical fiber bundles to create spectral images of the transmitted intensity requires a balance of scan speed, spatial sampling rate, and SNR. Increasing the scan speed, while maintaining the spatial sampling rate along the scanning direction, requires a reduction of the exposure time per pixel. This would result in an inherently lower signal and SNR. A spatial map of intensity at 830 nm was updated during the scan for real-time display in the graphical user interface. Data collection on phantoms took place in a dark room to prevent leakage of room light into the optical detector, whereas in the case of breast measurements, we found that the subject's body provided some shielding of room light to allow for dim illumination in the room.

B. Achieving depth sensitivity

1. Approach to depth discrimination

It was previously shown that an off-axis detection scheme provides information regarding the depth, z_{obj} , of an inhomogeneity embedded within a turbid slab of thickness d_0 .^{30,31} To obtain this information, one combines data from the on-axis detector, D_0 , and a detector offset, by a distance δD , in the x - y plane. The location of a given inhomogeneity will appear shifted (along the direction of the detector offset) between the transmission intensity images of the on-axis and off-axis detectors. The magnitude of this shift, Δ , can be normalized to δD yielding $\alpha = \Delta/\delta D$,³⁰ where α is a dimensionless parameter that is related to the normalized depth, z_{obj}/d_0 . Furthermore, to achieve depth sensitivity for

linear structures along x , we included an off-axis detector D_{+y} (offset along the positive y -direction). By measuring the parameter α and the thickness of the medium, d_0 , the depth of an absorbing structure can be found for any pair of detectors using a calibration curve relating α to z_{obj}/d_0 . For example, for the detector D_{+x} , $\alpha_{+x} = \Delta_{+x}/\delta D_{+x}$, where Δ_{+x} is the measured spatial shift along the positive x -direction between the structure in the image generated by D_{+x} and the one generated by D_0 , and δD_{+x} is the detector offset in the $+x$ -direction. Similarly, α_{-x} , and α_{+y} can be recovered using the spatial shifts measured from detectors D_{-x} and D_{+y} , respectively. It was previously shown that this calibration curve is weakly dependent on the background optical properties of the medium and on the source-detector separation.^{30,31} Also, depth reconstruction using this method is not affected by the contrast between the structure and the background medium because it is based on the spatial offset of intensity minima. As a result, for our experiments, we use simple black inclusions of varying shapes to show the capabilities of our instrument and this technique.

2. Comparison of theoretical and experimental depth calibration curve

A first-order perturbation approach within diffusion theory in an infinite slab geometry was used to derive the depth calibration curve, henceforth called the “depth curve,” which translates measured values of α into the corresponding values of z_{obj}/d_0 . In the diffusion based calculations, a single highly absorbing (effectively black) perturbation with a volume of 8 mm³ is embedded in a turbid infinite slab with optical properties $\mu_a \sim 0.04 \text{ cm}^{-1}$ and $\mu_s' \sim 9 \text{ cm}^{-1}$ and thickness $d_0 = 60 \text{ mm}$. Perturbation theory was used to generate 1D line scans with two detection optical fibers separated by $\delta D = 20 \text{ mm}$. The calculations were carried out for depths of the inhomogeneity, z_{obj} , in the range 4–56 mm.

We also acquired an experimental depth curve in order to assess the discrepancy between an experimental measurement of the depth curve in a slab geometry with finite boundaries and the theoretical depth curve derived from diffusion theory in an infinite slab geometry. For this, we used two wedge-shaped silicone phantoms (labeled “1” and “2” in Fig. 2(a))

FIG. 2. Description of the tissue-like phantom to experimentally derive the relationship between α and z_{obj}/d_0 . (a) Lateral view of the combined phantom. A black strip 7.5 mm wide is printed on a transparency sheet and is used as an optical inclusion between the two halves (labeled 1 and 2) of the phantom. The inclusion is imaged from depths ranging from 8 to 50 mm. (b) Front view of the same phantom depicting the location of the inclusion and the source (S), collinear detector (D_0), and off-axis detector (D_{+x}), which is offset by $\delta D_{+x} = 20 \text{ mm}$. The optical properties of the phantom at 690 nm are $\mu_a \sim 0.04 \text{ cm}^{-1}$ and $\mu_s' \sim 9 \text{ cm}^{-1}$.

with optical properties $\mu_a \sim 0.04 \text{ cm}^{-1}$ and $\mu_s' \sim 9 \text{ cm}^{-1}$ at 690 nm, which are combined into a rectangular block that allows for a thin inclusion to be inserted between the two phantoms. A transparency sheet with a single 7.5 mm wide black line was printed and used as an inclusion between these two pieces. For this experiment, two separate scans were performed: the first scan with a single detector collinear to the source (D_0), and the second scan with a single detector offset by $\delta D = 20 \text{ mm}$ (D_{+x}) (Fig. 2(b)). The detector offset was chosen as a compromise between the depth sensitivity and the size of the imaged area. Increasing δD would increase our depth sensitivity, however, the area imaged by both detectors would decrease. We found that, given our spatial sampling rate, a 20 mm detector offset provided good depth sensitivity. Each scanned line along the x -direction sampled the depth-varying inclusion at a unique depth. The spatial sampling rate of our data (0.5 mm^{-1}) was increased to 2 mm^{-1} by 2D spline interpolation after low-pass filtering with a 2D moving average to reduce the intensity noise-level. The inherently low spatial resolution afforded in diffuse optics allows interpolation to provide a finer spatial grid that mimics collecting data with a higher spatial sampling rate. Using this data interpolation, the shift in location of an inclusion between the images collected with the collinear and offset detectors (D_0 and D_{+x}) can be measured with greater precision. That is, if the true intensity minimum associated with a structure were to occur between two sampled points in the intensity image, interpolation provides a better measure of the shift in location. If the true minimum is sampled exactly by a pixel in the intensity image, interpolation does not affect the measured depth. The depth of the inclusion at each x -line was associated to a corresponding value α_{+x} by dividing the measured shift (Δ_{+x}) by the detector offset $\delta D_{+x} = 20 \text{ mm}$. By relating the measured value of α_{+x} to the known value of z_{obj}/d_0 , a depth curve was created.

3. Performance test on a breast-shaped phantom

We have previously presented the depth recovery method in an infinite-medium geometry, where boundary effects are absent,³⁰ and we have presented the case of a semi-infinite slab geometry in Sec. II B 2. To address the capability of our instrument to perform depth measurements in a geometry that is more representative of a real breast, we designed a breast-shaped optical phantom made of silicone ($\mu_a \sim 0.04 \text{ cm}^{-1}$, $\mu_s' \sim 9 \text{ cm}^{-1}$ at 690 nm) depicted in Fig. 3.

The phantom has a semi-circular projection with a diameter of 140 mm. A 70 mm diameter portion of the phantom (outlined by a dashed semi-circle in Fig. 3(a)) is of uniform thickness to resemble the portion of the breast in contact with the polycarbonate plates. A 17 mm diameter circle of black tape was placed between the two halves of the phantom ($z_1 = 29 \text{ mm}$) to mimic a deeply embedded tumor. A 3 mm wide strip of black tape was placed along the surface of the top half of the phantom ($z_2 = 0 \text{ mm}$) to mimic a superficial blood vessel. The phantom was imaged using a detector offset of $\delta D = 20 \text{ mm}$ for all three directions ($+x$, $-x$, and $+y$), recovering intensity images from D_{+x} , D_{-x} , and D_{+y} simultaneously over the wavelength range 650–1000 nm. The same low-pass filter and interpolation used in Section II B 2

FIG. 3. Drawing of breast shaped optical phantom. (a) Top view of two inclusions placed on the phantom. The grey circle is black tape placed between the two solid halves of the phantom ($z_1 = 29$ mm) and the solid black line is a 3 mm wide strip of black tape placed over the top of the phantom ($z_2 = 0$ mm). (b) A lateral view of the phantom depicting the two halves. The total thickness of the phantom is $d_0 = 60$ mm. The optical properties of the phantom at 690 nm are $\mu_a \sim 0.04 \text{ cm}^{-1}$, $\mu_s' \sim 9 \text{ cm}^{-1}$.

were applied to the intensity images recovered simultaneously from the four detectors. Since this method is largely insensitive to the absorption and scattering properties of the background medium,^{30,31} and since we used a black absorber, we maximized the contrast-to-noise ratio by using the integrated spectrum of the transmitted intensity at each pixel.

To account for the shape-based edge effects associated with the variable slab thickness, a normalization was applied using information from the intensity image at 912 nm. At 912 nm, the absorption of silicone has a peak and subsequently the background absorption from the phantom is the highest. At this wavelength, we minimize the contrast of the two black structures, and spatial variations in the intensity are dominated by fluctuations associated with the shape of the phantom. We divided the integrated intensity image by the one recovered at 912 nm, as a method of removing changes in intensity resulting from changes in thickness. The resulting image was then normalized to its maximum value creating a normalized intensity image at each detector. We represent these images taken from D_0 , D_{+x} , D_{-x} , and D_{+y} with the matrices of normalized, interpolated intensity data $\mathbf{N}_0$, $\mathbf{N}_{+x}$, $\mathbf{N}_{-x}$, and $\mathbf{N}_{+y}$, respectively, where $\mathbf{N}_0(x_0, y_0)$ represents the normalized intensity value taken from D_0 at source location (x_0, y_0) . We observe that we have previously presented an alternative method for tissue thickness correction for breast measurements *in vivo*, which is based on data collected at an optical wavelength of 925 nm.¹⁷

The location of the circle and stripe structures in the on- and off-axis images need to be identified to measure their corresponding spatial shifts between images taken from different detectors. To do this, regions of interest (ROIs) (or submatrices) within $\mathbf{N}_0$ were first defined for the circle ($\mathbf{n}_{\text{circ},0}$) and stripe ($\mathbf{n}_{\text{stripe},0}$) as templates to identify similar regions in off-axis images. Here, the location of $\mathbf{n}_{\text{circ},0}$ was defined by the location of its first element (at the top-left of the ROI). Meaning, if $\mathbf{n}_{\text{circ},0}(1, 1) = \mathbf{N}_0(x_1, y_1)$, then the location of $\mathbf{n}_{\text{circ},0}$ is (x_1, y_1) . To define $\mathbf{n}_{\text{circ},0}$, the center of the circle inhomogeneity was identified by a local minimum in $\mathbf{N}_0$, and it was used to define the center of a 17 mm (34 pixel) square region. The ROI for the stripe, $\mathbf{n}_{\text{stripe},0}$, was defined by finding minima along $\mathbf{N}_0(x, 36)$ and $\mathbf{N}_0(x, 0)$, where 36 and 0 were the y location in mm of the top and bottom of the stripe, respectively. These two points identified the center locations of the stripe along the x -direction and were used as the top-

left and bottom-right corners for the rectangular $22 \times 36 \text{ mm}^2$ ($44 \times 72 \text{ pixel}^2$) ROI, $\mathbf{n}_{\text{stripe},0}$.

The 2D cross-correlations were calculated between $\mathbf{n}_{\text{circ},0}$ or $\mathbf{n}_{\text{stripe},0}$ and ROIs of the same size taken from $\mathbf{N}_{+x}$, $\mathbf{N}_{-x}$, and $\mathbf{N}_{+y}$ to identify the location of the inhomogeneities in each off-axis image. For $\mathbf{N}_0$ and $\mathbf{N}_{+x}$, a registered ROI for the circle, $\mathbf{n}_{\text{circ},+x}$, was identified by shifting $\mathbf{n}_{\text{circ},0}$ pixel by pixel over the offset image in the $-x$ -direction up to a distance of δD_{+x} to maximize the value of the 2D cross-correlation between $\mathbf{n}_{\text{circ},0}$ and the offset ROIs of equal size in $\mathbf{N}_{+x}$. Then the magnitude of the shift for the circle between $\mathbf{N}_0$ and $\mathbf{N}_{+x}$, $\Delta_{+x,1}$, is defined as the difference in location between $\mathbf{n}_{\text{circ},+x}$ and $\mathbf{n}_{\text{circ},0}$. Since the shift should only occur along the offset direction, only the magnitude of the difference in x locations between $\mathbf{n}_{\text{circ},+x}$ and $\mathbf{n}_{\text{circ},0}$ is considered for $\Delta_{+x,1}$. Similarly, regions in the other off-axis images for the circle $\mathbf{n}_{\text{circ},-x}$ and $\mathbf{n}_{\text{circ},+y}$, and the regions for the stripe, $\mathbf{n}_{\text{stripe},+x}$, $\mathbf{n}_{\text{stripe},-x}$, and $\mathbf{n}_{\text{stripe},+y}$ can be identified to calculate their respective shifts, $\Delta_{-x,1}$ and $\Delta_{+y,1}$ for the circle, and $\Delta_{+x,2}$, $\Delta_{-x,2}$, and $\Delta_{+y,2}$ for the stripe. The maximum of the 2D cross-correlations between template ROIs and ROIs within the off-axis images were found using the *normxcorr2* function in MATLAB (R2012a, Mathworks, Natick, MA). The recovered shifts were then normalized by δD to obtain $\alpha_{+x,1}$, $\alpha_{-x,1}$, $\alpha_{+y,1}$ as the normalized shifts for the circle in each direction ($+x$, $-x$, and $+y$, respectively), and $\alpha_{+x,2}$, $\alpha_{-x,2}$, $\alpha_{+y,2}$ for the stripe in each direction. The theoretically derived depth curve described in Section II B 2 was used as a look-up table to assign a depth to each α value. The measured depth from all three directions for the circle, $z_{1,\text{meas}}$, and the stripe, $z_{2,\text{meas}}$, are reported in Section III B 2.

C. Dynamic spectroscopy on the finger

To determine the capability of our imaging system to measure physiological hemodynamics, we measured the arterial pulsation at the distal phalanx of the index finger of a 24-yr old, male, healthy volunteer. The Tufts University Institutional Review Board approved the experimental protocol, and the subject provided written informed consent prior to the experiment. Transmitted intensity spectra were collected at 20 Hz for 60 s resulting in 1200 spectra. Only a single source-detector pair was used to collect data through the finger with the polycarbonate plates removed. The source-detector separation was 1.5 cm. From the raw intensity data, $I(\lambda, t)$, we calculated the relative intensity changes, $\Delta I(\lambda, t)/I_0(\lambda)$, where $I_0(\lambda)$ is the average intensity at each wavelength over time, and $\Delta I(\lambda, t) = I(\lambda, t) - I_0(\lambda)$.

To test the ability of our instrument to perform physiological hemodynamic measurements over the entire spectral range, we estimated the spectral shape of the change in the tissue absorption coefficient, $\Delta\mu_a(\lambda)$, using the modified Beer-Lambert law,⁴⁰

$$\frac{\Delta I}{I_0}(\lambda, t) = -\Delta\mu_a(\lambda, t) \cdot \text{DPF}(\lambda) \cdot d, \quad (1)$$

where $\text{DPF}(\lambda)$ is the differential pathlength factor and d is the geometrical source-detector distance. The DPF, which is defined as the ratio between the mean path length of detected photons in the scattering media and d , was assumed to be

time-independent. The average heart rate was measured by taking the Fourier transform of $\Delta I/I_0(\lambda, t)$ and identifying the peak in the Fourier spectrum around ~ 1 Hz. We measured the amplitude of the oscillations in $\Delta I/I_0(\lambda)$ at the heart rate, $|\Delta I/I_0|_{\text{HR}}(\lambda)$, by integrating the Fourier coefficients around the peak pulsation frequency (1.16-1.33 Hz) at each wavelength. Assuming that the changes in attenuation are dominated by changes in the volume of arterial blood in tissue, we can omit the contributions of changes in blood flow, lipids, and water to relate $|\Delta I/I_0|_{\text{HR}}(\lambda)$ directly to the change in tissue absorption due to arterial pulsation, $\Delta\mu_{a|\text{HR}}(\lambda)$. Since we were only interested in recovering the arterial saturation, we only needed the spectral shape of $\Delta\mu_{a|\text{HR}}(\lambda)$ to be within a factor. Accordingly, we used a DPF spectrum normalized to its value at 790 nm measured by Kohl *et al.*⁴¹ We solved for $\Delta\mu_{a|\text{HR}}(\lambda)$ from Eq. (1) using this normalized spectrum of the DPF, the measured values of $|\Delta I/I_0|_{\text{HR}}(\lambda)$, and d . We then normalized $\Delta\mu_{a|\text{HR}}(\lambda)$ to its value at 790 nm and fit it with the known absorption spectra of hemoglobin also normalized to 790 nm (which depend only upon the saturation of hemoglobin) to recover the arterial saturation.

D. Dynamic spectroscopy on the breast

We tested the sensitivity of our system to physiological dynamics induced by systemic changes in blood pressure, and acquired time-resolved spectra in the breast. One healthy female subject (24-yr old) participated in the study. The Tufts University Institutional Review Board approved the experimental protocol, and the subject provided written informed consent prior to the experiment. The subject's breast was placed between the scanning plates of the optical mammography system and gently compressed to keep the breast stationary. Pneumatic thigh cuffs were wrapped around both of the subject's legs and inflated using an automatic cuff inflation system (E-20 rapid Cuff Inflation System, D. E. Hokanson, Inc., Bellevue, WA) to induce systemic changes in arterial blood pressure. Intensity spectra from 650 to 1000 nm were recovered at 5 Hz with the fibers positioned in the center of the breast close to the chest wall to mitigate boundary effects.

Baseline measurements, during which the subject is at rest and the thigh cuffs are deflated, were recorded for 2 min. To induce cyclic changes in blood pressure at a given frequency, the thigh cuffs were then periodically inflated (to a pressure of 200 mmHg) and deflated at a frequency of 0.05 Hz for a total time of 2 min. Slow temporal drifts in the data were removed by detrending the intensity data with a third-order polynomial. The plate separation was $d = 52$ mm. The modified Beer-Lambert law (Eq. (1))⁴⁰ was used to obtain time traces of the absorption changes, the changes in tissue concentrations of hemoglobin, $\Delta[\text{HbO}]$, $\Delta[\text{Hb}]$, and $\Delta[\text{HbT}]$ by the following relationship:

$$\Delta\mu_a(\lambda) = \Delta[\text{HbO}] \cdot \epsilon_{\text{HbO}}(\lambda) + \Delta[\text{Hb}] \cdot \epsilon_{\text{Hb}}(\lambda), \quad (2)$$

where, $\epsilon_{\text{HbO}}(\lambda)$ and $\epsilon_{\text{Hb}}(\lambda)$ are the molar extinction coefficients of oxy- and deoxyhemoglobin, respectively. The coefficients $\epsilon_{\text{HbO}}(\lambda)$ and $\epsilon_{\text{Hb}}(\lambda)$ ⁴² and the wavelength dependence of the DPF⁴¹ are known from the literature, and an absolute value of the DPF was chosen at 700 nm to be 7.⁴³ Using

relative intensity data from 700 to 970 nm (where the wavelength dependence of the DPF was defined⁴¹), $\Delta\mu_a(\lambda)$ was calculated from Eq. (1). The magnitude of $\Delta[\text{HbO}]$, $\Delta[\text{Hb}]$, and subsequently $\Delta[\text{HbT}]$ were obtained from Eq. (2) in the least squares sense using the *linsolve* function in MATLAB.

III. RESULTS

A. Signal-to-noise characterization on a tissue-like phantom

We measured the SNR of the transmitted intensity over the spectral band of interest (650-1000 nm) by using a rectangular optical phantom made of silicone that mimics the optical properties of breast tissue ($d_0 = 60$ mm, $\mu_a \sim 0.04$ cm⁻¹, $\mu_s' \sim 9$ cm⁻¹ at 690 nm). Using a 90 mW illumination power we measured the SNR² as a function of the resulting signal by adjusting the CCD exposure time (Fig. 4). The SNR² as a function of optical signal detected by the CCD at 830 nm for the on-axis fiber is reported in Fig. 4. The measured SNR² is linearly dependent on the optical signal detected by the CCD for all four fibers which is indicative of noise dominated by the statistical variation of photons arriving at the detector, also termed shot noise (which follows a Poisson distribution). At the exposure time of 100 ms the SNR for the intensity measurements on the optical phantom ranged from 67 to 130 (equivalent to less than 1.5% intensity noise) over the wavelength range 650-1000 nm.

B. Depth discrimination

1. Experimental validation of the depth curve

We experimentally measured a depth curve and compared it to a theoretical depth curve obtained using diffusion theory

FIG. 4. Results of SNR measurement on an optical phantom. The SNR² shows a linear dependence on the number of detected CCD counts. With a 2 MHz A/D rate and a gain setting of 3, the CCD sensitivity is about 1 e⁻/count. The symbols refer to data taken at 830 nm over exposure times ranging from 0 to 500 ms. The thickness of the phantom was 60 mm while its optical properties at 690 nm were $\mu_a \sim 0.04$ cm⁻¹ and $\mu_s' \sim 9$ cm⁻¹.

FIG. 5. Comparison between measured (crosses) (see the experimental setup in Fig. 2) and theoretically calculated (solid line) depth curve. The theoretical depth curve is obtained by using a first order perturbation within diffusion theory using an infinite slab with experimental parameters similar to the experimental setup— $d_0 = 60$ mm and background optical properties at 690 nm of $\mu_a = 0.04 \text{ cm}^{-1}$ and $\mu_s' = 9.0 \text{ cm}^{-1}$.

(Fig. 5). We measured the depth of an inclusion for depths ranging from $z_{\text{obj}} = 8\text{--}50$ mm ($z_{\text{obj}}/d_0 = 0.13\text{--}0.83$) resulting in $\Delta_{+x} = 1.5\text{--}18$ mm ($\alpha_{+x} = 0.08\text{--}0.89$). The measured depth curve closely approximates the theoretical curve with a discrepancy in Δ_{+x} to within 0.5 mm, which is equivalent to the size of one interpolated pixel. The average discrepancy in Δ_{+x} was 0.28 ± 0.13 mm ($\alpha_{+x} = 0.014 \pm 0.006$). The only exceptions, where the discrepancy in Δ_{+x} is greater than 0.5 mm, occur when the fibers are scanned to within 2.5 cm of the phantom's edge, where the maximum discrepancy in Δ_{+x} is 0.8 mm. We attribute this larger error to shifts in the intensity minima due to edge effects resulting from light leakage through the side of the phantom. Accordingly, we use the theoretical depth curve for the rest of this work.

The accuracy with which a depth can be assigned to a given inhomogeneity using this method is dependent on the accurate estimation of α . The measured values of α depend on the pixel width, k , and the detector offset δD , and are consequently discretized into N “bins” where N is the nearest integer value of $\delta D/k$. For example, in the case of this experiment, $k = 0.5$ mm and $\delta D = 20$ mm, so α is discretized into 40 bins. Since the relationship between α and z_{obj}/d_0 is non-linear, the estimation of depth will inherently have an error that depends non-linearly on the depth, and each “bin” will correspond to a different accuracy based on the z -location of the inhomogeneity. However, by dividing d_0 by N , we obtain an estimate of the average bin width in z , $d_0/N = 1.5$ mm. This value is an approximate measure of the accuracy with which a depth can be assigned to a given inhomogeneity within these experimental parameters. Note that by changing d_0 , k , or δD , the accuracy of the depth measurement is altered. In a clinical setting, we do not have control over d_0 , however k and δD

are adjustable system parameters. Ideally, a larger value of δD would improve our measurement accuracy, but we chose $\delta D = 20$ mm to maximize the area around the edges of the breast from which depth information can be recovered. The value of k can be decreased through further interpolation, but this provides minimal improvements in accuracy due to the physical limitation of the source and detector fiber diameters and the spatial resolution afforded in diffuse imaging.

2. Recovery of depth on a breast shaped phantom

We tested the depth discrimination capabilities of our system using a silicone optical phantom designed to simulate the shape and optical properties of the human breast. The depth of two optical inclusions, a circle ($z_1 = 29$ mm) resembling a tumor and a black stripe ($z_2 = 0$ mm) resembling a superficial blood vessel (Fig. 3) were recovered using information from D_0 , and the three offset detectors, D_{+x} , D_{-x} , and D_{+y} (Fig. 6).

The registered ROI, $\mathbf{n}_{\text{circ},+x}$, and the template ROI, $\mathbf{n}_{\text{circ},0}$, had a 2D cross correlation value of 0.95. The shift between the two ROIs was $\Delta_{+x,1} = 10 \pm 0.5$ mm translating to a depth of $z_{1,\text{meas}} = 30 \pm 1$ mm where the actual depth was $z_1 = 29$ mm. The registered ROIs for both the circle and the stripe in all images were always representative of unique maxima. Meaning, in $\mathbf{N}_{+x}$ (as well as the other images), no other regions exist that had 2D cross-correlations with $\mathbf{n}_{\text{circ},0}$ equal to or greater than 0.95. For $\mathbf{N}_{-x}$ and $\mathbf{N}_{+y}$, the recovered shifts $\Delta_{-x,1}$, and $\Delta_{+y,1}$ were also 10 ± 0.5 mm. Similarly, the shift measured between $\mathbf{n}_{\text{stripe},+x}$ and $\mathbf{n}_{\text{stripe},0}$ was $\Delta_{+x,2} = 0 \pm 0.5$ mm. The same shift was measured for all three directions associated with a recovered depth of $z_{2,\text{meas}} = 0 \pm 5.6$ mm, where the actual depth was $z_2 = 0$ mm. The errors are defined by assuming a one-pixel inaccuracy in the measurement of the shifts and translating that error to the depth based on the depth curve. The 2D cross-correlations between template and registered ROIs were always at least 0.95. The discrepancy between the actual depth of each object and the measured depth of each object is within the previously defined measurement error of ~ 1.5 mm.

C. Dynamic spectroscopy on the finger

We tested the temporal sensitivity of our imaging system by measuring the arterial pulsation in the subject's index finger over the course of 60 s. In Fig. 7(a) the relative intensity changes $\Delta I/I_0$ over time at 820 nm are observed for a time period of 10 s. At a 20 Hz acquisition rate, each individual pulse over the entire wavelength range (650–1000 nm) is visualized.

The Fourier spectrum has a clear peak at 1.27 Hz corresponding to a heart rate of 76 bpm (Fig. 7(b)). The full width at half maximum (FWHM) of the peak is ~ 0.1 Hz, corresponding to a width of 6 bpm, which is comparable to the normal fluctuations in the heart rate over a period of 60 s.

We use the magnitude of the peak in the Fourier spectra surrounding the heart rate at each wavelength, $|\Delta I/I_0|_{\text{HR}}(\lambda)$, to calculate the change in the tissue absorption coefficient due to arterial pulsation, $\Delta\mu_a|_{\text{HR}}(\lambda)$ (Fig. 7(c)). We recovered a 97% oxygen saturation of hemoglobin by fitting $\Delta\mu_a|_{\text{HR}}(\lambda)$ with

FIG. 6. Experimental result of depth recovery for the circle and single black stripe in a breast shaped phantom. Interpolated normalized intensity images for the four detectors are shown. The solid boxes represent the template ROI ($n_{\text{circ},0}$ for the circle and $n_{\text{stripe},0}$ for the black stripe) selected from the D_0 image, and the dashed boxes represent the registered ROIs in the off-axis images where the circle and stripe structures were found. The shift in the ROI of the circle for N_{+x} was $\Delta_{+x,1} = 10 \text{ mm} \pm 0.5 \text{ mm}$ associated with a recovered depth of $z_{1,\text{meas}} = 30 \pm 1 \text{ mm}$ where the exact depth was 29 mm. The shift in the ROI of the stripe was $\Delta_{+x,2} = 0 \pm 0.5 \text{ mm}$ associated with a recovered depth of $z_{2,\text{meas}} = 0 \pm 5.6 \text{ mm}$, where the exact depth is 0 mm.

the molar extinction spectrum of hemoglobin with oxygen saturation as the fitting parameter. The average discrepancy between the curves (Fig. 7(c)) is 0.9% with the largest errors ($\sim 7\%$) observed at the extremes of the measured spectral range where the error in the measured wavelength dependence of the DPF is the largest.⁴¹

FIG. 7. Measurement of arterial pulsation and normalized hemoglobin absorption coefficient in the index finger of a human subject. (a) Relative intensity changes over 10 s at 820 nm. (b) Magnitude of Fourier coefficients of $\Delta I/I_0$ over the 60 s acquisition at 820 nm. (c) Change in the absorption coefficient normalized to 790 nm recovered from the magnitude of the Fourier components. The dashed line represents the normalized absorption of total hemoglobin with an oxygen saturation of 97%.

D. Dynamic spectroscopy in the breast

Figure 8 shows measurements of $\Delta[\text{HbO}]$, $\Delta[\text{Hb}]$, and $\Delta[\text{HbT}]$ in the breast of a healthy subject induced by the cyclic inflation of a pneumatic thigh cuff as described in Section II D. Periods of cuff inflation are shaded in Fig. 8. Inflation of the cuffs induced an increase by $\sim 0.5\text{--}1 \mu\text{M}$ in $\Delta[\text{HbO}]$ and an increase by $\sim 0.1\text{--}0.2 \mu\text{M}$ in $\Delta[\text{Hb}]$. Based on

FIG. 8. Measurement of changes in $\Delta[\text{HbO}]$, $\Delta[\text{Hb}]$, and $\Delta[\text{HbT}]$ in a single location of the breast as a result of induced oscillations from pneumatic thigh cuffs. Shaded areas indicate the periods when the cuffs are fully inflated to 200 mmHg, and white areas indicate the periods when the cuffs are deflated. Each inflation cycle lasts 20 s, with the cuffs inflated for $\sim 10 \text{ s}$ and deflated for $\sim 10 \text{ s}$.

typical values of hemoglobin concentration in breast tissue (15–30 μM), this is indicative of approximately a 2%–8% fluctuation in total hemoglobin content. The oscillations of $\Delta[\text{HbO}]$, $\Delta[\text{Hb}]$, and $\Delta[\text{HbT}]$ are all in phase with each other and with the cuff inflation, suggesting that the observed changes are associated with a blood volume oscillation induced by the change in blood pressure.⁴⁴

IV. DISCUSSION

We have reported an instrument for optical mammography that can measure broadband spectral data (wavelength range: 650–1000 nm) with high spatial sampling (25 cm^{-2}) and recover the depth of embedded absorbing structures to within 1 mm. We also showed that this system has the capability to resolve temporal dynamics with an acquisition rate as high as 20 Hz, as demonstrated by measuring induced hemodynamics in the breast. The spectral information provided by this method allows for the generation of 2D concentration maps of the major absorbing chromophores in breast tissue (deoxyhemoglobin, oxyhemoglobin, lipids, and water).¹⁷ Furthermore, we apply this spectral information to recover the arterial saturation of a healthy volunteer from dynamic measurements taken on his finger. We observe that while the absolute value of the DPF is strongly dependent on the value of the reduced scattering coefficient (as determined by the tissue architecture, cellular density, etc.) and the absorption coefficient (as determined by vascularization, blood volume, etc.), the spectral shape of the DPF is mostly determined by the wavelength dependence of the scattering and absorption coefficients. Such wavelength dependence, being related to the cellular size distribution and hemoglobin saturation, respectively, is comparable in various soft tissues under rest conditions, and this justifies the application of the cerebral DPF wavelength dependence to finger tissue. In the remainder of this work, we focused on the depth information realized through an off-axis detection scheme, and the dynamic information obtained through measuring intensity spectra at a single location.

This instrument is capable of identifying the depth of specific inhomogeneities observed in a 2D projection with a single scan of four detection fibers simultaneously. By spatially multiplexing detection fibers into a large CCD chip, this instrument can assess depth without requiring additional scans for each offset direction, and, more importantly, this method avoids patient movement between scans and does not require calibration measurements. The accuracy of the depth assessment is mostly affected by the detector offset δD . A larger detector offset can improve the accuracy of the depth assessment, but it would also increase the source-detector distance and reduce the SNR accordingly. Furthermore, a large offset may introduce artifacts close to the edges of the breast where inhomogeneities may not appear in all detector images.

We reported here a method to apply this technique to a breast-shaped phantom using cross-correlation to validate its efficacy in a curved, slab geometry (Fig. 6). In the simplest case, the location and size of absorbing structures are known *a priori*, and the structures of interest are separated by a distance greater than δD . Here, the cross-correlation serves as

an accurate measure of the spatial offset of different structures. The average reported error of 1.5 mm in depth assessment is associated with the discretization of the depth look-up table due to the physical dimensions of our source-detector setup and the experimental parameters. In the simple case of a single structure or multiple structures separated by a distance greater than δD , the accurate pairing of spatial locations is a simple task, and the error associated with it is ignored. However, in a real breast, the complex network of blood vessels and the intrinsic tissue heterogeneity complicates the depth discrimination method.

The main challenge in extending this method to the real breast is eliminating the requirement for *a priori* information. Kainerstorfer *et al.*³⁰ have detailed a robust algorithm where a window-based registration is done by pairing ROIs that are smaller than δD surrounding vessel-like structures. Additionally, this method uses a spatial second derivative to improve the contrast of absorbing structures in the case where intensity minima are ambiguous. This method does not rely on *a priori* information. However, the challenge in applying this window method to spherical objects is that if the optical footprint of the object is larger than δD , identifying an accurate minimum could still be ambiguous. Although increasing the window size may avoid this issue, a larger window may encompass more than one structure at multiple depths where assigning Δ to the appropriate structures becomes difficult. This method can still be a valuable tool, since presumably even if only a portion of the object is encompassed by the window, the same portion would exist in the offset image. However, the error when using this registration method on a dense array of unknown structures (as is the case in the human breast) needs to be further investigated. It is likely that although we can achieve an error in our depth assessment of ~ 1.5 mm in ideal conditions, the additional error associated with measuring Δ in a more complex case will reduce the accuracy of these measurements. Different approaches to generate images with selective depth sensitivity from additional off-axis information are based on phased-array³³ or tomosynthesis³² schemes. These methods generate 2D images with selective sensitivity at different depths without *a priori* knowledge of the location or shape of the detected inhomogeneities. In this way, the location of detected lesions can be assessed in 3D.

The coarse depth resolution provided by our method and alternative methods based on limited off-axis information^{32,33} makes them unsuitable to generate detailed 3D spatial maps comparable to full 3D reconstructions. However, the depth information they provide is still valuable and effectively complements the 2D spatial information of projection images. The depth information can improve the instrument's sensitivity to spatial changes in chromophore concentrations by allowing for a more accurate implementation of a perturbation based diffusion model.¹³ Additionally, depth information regarding detected blood vessels can determine whether they are superficially located, or are larger feeding vessels that present high contrast near a breast tumor.

Dynamic measurements done on the breast show the sensitivity of our instrument to induced changes in hemodynamics. A challenge with inducing hemodynamic changes is to separate direct mechanical effects of the stimulus from

the hemodynamic response of interest. In cases where breast compression is applied using the measurement hardware (e.g., scanning plates or a hand-held probe), careful consideration is required to ensure changes in detector contact from the applied pressure to the tissue do not affect the measurement. Additionally, when the patient is required to regulate their own respiration, such as with the Valsalva maneuver, patient compliance can become an issue. To visualize similar controllable hemodynamics in the breast, we induced systemic blood pressure changes with cuffs around the thighs. In this way, we provide a hemodynamic stimulus that requires no effort from the patient, and is completely independent of the measurement hardware. It can induce changes in total hemoglobin content of a similar magnitude to those obtained using manual compression or regulated respiration while minimizing artifacts. Furthermore, our multiplexed detection system can be easily adapted to measure multiple regions of interest (such as healthy and cancerous tissue) simultaneously. Defining an ROI for dynamic measurements is another challenge when studying breast cancer patients. For the purpose of detecting cancer signatures in the temporal data, an initial 2D spatial map can be obtained to identify suspicious locations for further hemodynamic study. This combination of measurement schemes can provide additional specificity when static optical imaging is insufficient to differentiate breast cancer. In the case of monitoring neoadjuvant chemotherapy, the cancer location is known *a priori* and can be used to define ROIs for dynamic measurements.

V. CONCLUSION

The instrument presented here highlights the advantage of diffuse optics to non-invasively acquire both functional and structural information simultaneously to help diagnose and monitor breast cancer while limiting burden to the patient. We have demonstrated both the flexibility of this system to measure a number of relevant physiological parameters and the simplicity with which the measurements are done. From an investigative standpoint, this allows us to maximize the information we collect, and use it to identify key physiological parameters that can translate to clinical diagnoses. While it has not yet been demonstrated that observed changes in hemodynamics are a direct measure of angiogenesis in the breast, the information may provide new ways to discriminate healthy and cancerous tissues. Furthermore, the additional spatial information we collect can help improve our sensitivity to suspicious lesions. Although we have demonstrated the feasibility and simplicity of these measurements, the relevance of the diagnostic information provided will need to be further tested in future studies on patients with breast cancer.

ACKNOWLEDGMENTS

This research is supported by the National Institutes of Health (Grant No. R01 CA154774).

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