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Localization of absorbers in scattering media by use of frequency-domain measurements of time-dependent photon migration

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Abstract

Frequency-domain studies of time-dependent light propagation in tissuelike phantoms that contain optical heterogeneities are described. Specifically the phase shift and amplitude modulation of reemergent light were measured when illuminated by an amplitude-modulated light source. Changes in the phase angle and the extent of modulation revealed the presence of a light-absorbing object. Furthermore the magnitude and direction of these changes were sensitive to the absorber depth and the light modulation frequency in a manner that could be used to infer the location of the heterogeneity. These data suggest the feasibility of optical imaging by frequency-domain methods.

Keywords

Biomedical optical imaging; photon-migration imaging; frequency-domain imaging

Introduction

Recent advances in laser and detector technologies and high-speed computers have resulted in several efforts to develop near-infrared (NIR) biomedical optical imaging. However, optical imaging is complicated, since the direct geometric correlation between the incident and detected light is destroyed by tissue scattering. Image reconstruction has been demonstrated to some extent in tissues or tissuelike phantoms by time-gated measurements of ballistic or minimally scattered light photons.^{1–10} Such measurements retain the direct geometric correlation and permit the use of traditional reconstruction techniques. However, these approaches have the limitations of sensitivity and depth of penetration as a result of the absorption and scattering properties of tissues.

In this paper we describe measurements of time-dependent photon migration in which the limitations of sensitivity and penetration depth may not be as severe, yet the correlation between incident and detected light is not known. In particular we chose to use frequency-domain or phase and modulation measurements of time-dependent light migration because of the technological simplicity, high time resolution, and clinical practicality of the

measurement. Such measurements may facilitate the development of biomedical optical imaging but present challenges for image reconstruction.

Recently investigators have shown that time-dependent measurements of photon migration in both time and frequency domains can be used to detect optical heterogeneities that are effectively located at tissue depths from 1 to 5 cm (for a review see Ref. 11). The solution to the forward imaging problem, i.e., the prediction of photon-migration measurements from information regarding the presence, position, and properties of an optical heterogeneity, is not completely understood. As a result, a direct approach to solving the inverse-imaging problem, i.e., predicting the presence, position, and properties of optical heterogeneities from time-dependent, photon-migration measurements, remains to be defined.¹¹ Successful biomedical optical imaging requires a clinically feasible solution to the forward problem (i.e., the actual measurement of photon migration) and at least a partial solution to the inverse problem (i.e., deriving information regarding detection, localization, and possibly characterization from photon-migration measurements).

Investigators have demonstrated that a solution to the inverse-imaging problem exists.^{12,13} From time-domain measurements of well-defined phantoms, these investigators employed iterative reconstruction algorithms to detect, locate, and characterize the optical properties of simple heterogeneities. In a way that is similar to the computation approaches used by Singer and co-workers,^{14,15} these algorithms converge on a spatial map of optical properties that gives the smallest difference between actual time-dependent measurements and those predicted by the solution to the forward-imaging problem. Convergence on the correct inverse solution or image is not guaranteed. In addition, such algorithms may overspecify the goals of biomedical optical imaging, especially when the imaging modality is to be used as a screening tool for detection and localization rather than a complete diagnostic characterization of diseased tissue. Here we examine features of the forward-imaging problem to determine whether information to detect and locate heterogeneities can be directly obtained from measurements of phase and modulation.

Recently we directly obtained actual images from multipixel measurements of phase shift and amplitude modulation at varying modulation frequencies by using newly developed techniques. These photon-migration measurements revealed the presence and three-dimensional location of a perfect absorber.¹⁶ Furthermore they were consistent with a previously proposed qualitative model of frequency-domain, photon-migration imaging.^{11,16} Specifically both the model and the array of phase-shift and amplitude-modulation data suggest that one may probe various depths by simply changing the modulation frequency. This result suggests that photon-migration measurements made at varying modulation frequencies may be employed to develop a more efficient inverse algorithm or at best provide direct information for detection and localization.

In the next section the statistical arguments for detecting and locating the three-dimensional position of an optical heterogeneity from multifrequency phase-shift and modulation measurements are presented. Changes in photon migration caused by the presence of an absorber are investigated from the following experimental measurements of phase shift and

modulation. In light of the experimental results, implications for solving the detection and localization aspects of the inverse-imaging problem are discussed.

Theory

Because of multiple scattering events, photons emitted at the source and ultimately received at the detector have traveled a distribution of optical path lengths L , of time of flights t , or of the number of scattering events n_{tot} (where $L = n_{\text{tot}} l^*$, l^* is the mean isotropic scattering length, and c is the speed of light divided by the refractive index of the media). In the limit of a large number of scattering events n^* , one can show that $P_S(\mathcal{X}, \zeta, n^*)$ or the probability that a photon emitted at the source $(-\rho^*/2, 0)$ can be found at dimensionless position $(\mathcal{X}, \zeta)$ in n^* scattering steps is given by (Fig. 1)

$$P_S(\mathcal{X}, \zeta, n^*) \simeq \left(\frac{4}{3}\pi l^{*2} n^*\right)^{-3/2} \exp(-\mu_a n^* l^*) \times \left(\exp\left\{-\frac{3}{4n^*} \left[(\zeta - 1)^2 + \left(\mathcal{X} - \frac{\rho^*}{2}\right)^2 \right]\right\} - \exp\left\{-\frac{3}{4n^*} \left[(\zeta + 1)^2 + \left(\mathcal{X} - \frac{\rho^*}{2}\right)^2 \right]\right\} \right), \quad (1)$$

where μ_a is the absorption coefficient and $\mathcal{X}, \zeta, \rho^*$ are the x and z directions and the source-detector separation normalized by l^* . Approximation (1) is simply the expression derived from the method of images for the time-dependent fluence in a semi-infinite medium resulting from a delta pulse of light at the surface.^{17,18} We employ reflectance geometry in our analysis since transillumination of thick tissues is less likely.

Similarly the probability $P_D(\mathcal{X}, \zeta, n')$ that a photon emitted at the detector $(+\rho^*/2, 0)$ can be found at dimensionless position $(\mathcal{X}, \zeta)$ in n' scattering steps is given by

$$P_D(\mathcal{X}, \zeta, n') \simeq \left(\frac{4}{3}\pi l^{*2} n'\right)^{-3/2} \exp(-\mu_a n' l^*) \times \left(\exp\left\{-\frac{3}{4n'} \left[(\zeta - 1)^2 + \left(\mathcal{X} + \frac{\rho^*}{2}\right)^2 \right]\right\} - \exp\left\{-\frac{3}{4n'} \left[(\zeta + 1)^2 + \left(\mathcal{X} + \frac{\rho^*}{2}\right)^2 \right]\right\} \right), \quad (2)$$

When one considers the entire photon migration from the source at $(\rho^*/2, 0)$ to the detector at $(\rho^*/2, 0)$, the probability $P(\mathcal{X}, \zeta, n_{\text{tot}})$ that a photon has sampled point $(\mathcal{X}, \zeta)$ within $n_{\text{tot}} = n^* + n'$ scattering steps is simply the product of P_D and P_S summed over all combinations of steps:

$$P(\mathcal{X}, \zeta, n_{\text{tot}}) = \sum_{n^*=1}^{n_{\text{tot}}-1} P_S(\mathcal{X}, \zeta, n^*) P_D(\mathcal{X}, \zeta, n_{\text{tot}} - n^*). \quad (3)$$

When the diffusion approximation applies, P_S and P_D can be determined from approximations (1) and (2) and $P(\mathcal{X}, \zeta, n_{\text{tot}})$ can be computed. Otherwise Monte Carlo simulations must be conducted to determine P_S and P_D . Figure 2 illustrates that for diffusing photons traveling small to moderate numbers n_{tot} of scattering steps, a most probable path intersecting $(0, \zeta)$ exists as indicated by the maximum in the probability $P(0, \zeta, n_{\text{tot}})$ for photon visitation. At greater ζ depths below the source and detector and at a greater number

of scattering steps n_{tot} , the most probable path is less defined. This observation is indicated by the broadened probability curve and is expected from previous statistical analyses.¹⁸ For a homogeneous medium $P(\mathcal{X}, \zeta, n_{\text{tot}})$ defines (1) the volume of probable photon sampling and (2) the depth of the most probable path through which photons, traveling n_{tot} scattering steps, migrate. The depth of the most probable path is deflected from the surface because of the probability for photon escape at the surface of the semi-infinite medium.

If a light-absorbing object were located at a depth greater than the most probable path, photons traveling to the greatest penetration depths would be eliminated from the photon-migration process. The volume available for photon migration would diminish. Since photons that travel to the greatest penetration depth also travel the greatest optical length,¹⁸ the presence of the absorber would cause shortening of the distribution of optical path lengths as well as of the mean of the distribution $\langle L \rangle$. Photon transport would be primarily between the source-detector plane and the absorber. On the other hand, if an absorber position intersected the most probable path, photon migration might occur with equal numbers and probabilities behind as well as in front of the absorber, which may result in no change in $\langle L \rangle$. If the absorber were located between the source-detector plane and the most probable path, the photon migration would occur primarily around the backside of the absorber, resulting in a lengthening in $\langle L \rangle$. Obviously the most probable path depends on the number of scattering events considered n_{tot} , the source-detector separation ρ , and the optical properties of the semi-infinite medium. As the absorption properties increase, the most probable path would become situated closer to the source and detector. As the source-detector separation increases, the most probable path would be located farther from the surface. The prediction of $P(\mathcal{X}, \zeta, n_{\text{tot}})$ resulting from the presence of an optical heterogeneity is difficult to obtain routinely through an analytical solution. Numerical methods such as Monte Carlo simulation,^{19–21} finite element,^{22,23} and other numerical techniques²⁴ are employed to predict photon-migration characteristics in the presence of optical heterogeneities. Nonetheless experimental measurements of the optical path length distribution from time-domain studies demonstrate that mean optical path lengths may increase or decrease depending on the absorber position.¹¹

To relate the probability for photon sampling at position $(\mathcal{X}, \zeta)$ to actual measurements conducted in the frequency domain, one can compute the $P(\mathcal{X}, \zeta, f^*)$ or the probability for detected photon visitation at $(\mathcal{X}, \zeta)$ resulting from light at modulated frequency f^* :

$$P(\mathcal{X}, \zeta, f^*) = \int_{-\infty}^{\infty} P(\mathcal{X}, \zeta, n_{\text{tot}}) \exp(2\pi i f^* n_{\text{tot}}) dn_{\text{tot}}, \quad (4)$$

where the dimensionless frequency f^* is equal to $1/n_{\text{tot}}$. The dimensional frequency is simply equal to $f^* c / l^*$. As the modulation frequency approaches zero, $P(\mathcal{X}, \zeta, f^*)$ represents the probability for detecting a photon that has visited $(\mathcal{X}, \zeta)$ when the photon is emitted from a continuous, steady point source. The ratio of absolute probabilities, $P(\mathcal{X}, \zeta, f^*) / P(\mathcal{X}, \zeta, 0)$, therefore represents the effect of modulation frequency on the probability for photon visitation at $(\mathcal{X}, \zeta)$.

Figure 3 illustrates that at low modulation frequencies, $P(0, \zeta, f^*)/P(0, \zeta, 0)$ is unity at penetration depths of 50 scattering lengths or less when $f^* = 1.22^{-4}$ [or $f = 26$ MHz when $f^* = 1$ mm and $c = 2.14^{10}$ cm/s (Ref. 25)]. Thus at low modulation frequencies the migration characteristics of photons traveling the entire volume and entire distribution of possible optical paths contribute to frequency-domain measurements of phase shift and modulation. It is not surprising that the direct relationship between θ and $\langle L \rangle$ has been proved mathematically at low modulation frequencies, $f \ll \mu_a c / 2\pi$ (Refs. 26–28):

$$\theta(\rho, f) = \tan^{-1} \frac{2\pi f \langle L \rangle}{c} \sim \frac{2\pi f \langle L \rangle}{c}. \quad (5)$$

However, at higher modulation frequencies $P(\mathcal{X}, \zeta, f^*)/P(\mathcal{X}, \zeta, 0)$ decreases rapidly with increased depth ζ below the source and detector, which indicates a smaller probability for photon visitation. Using the photon-density wave description, Tromberg *et al.*²⁹ show that photon-density waves modulated at high frequencies are increasingly damped as they propagate further in random media. This result implies that the migration characteristics of photons traveling greater depths and longer optical path lengths make a smaller contribution to measurements of phase shift and modulation when the modulation frequency is increased. Since long optical paths are not contributing, the most probable path for photon migration between a source and detector shifts closer to the surface with increased modulation frequency.

As a result of the reduced photon visitation, the phase shift can be considered to reflect time-dependent photon migration that is weighted toward smaller times of flight at higher modulation frequencies; $\theta(\rho, f)$ therefore predicts a mean optical path length that is weighted increasingly to smaller optical paths. When an absorber is present, its position relative to the most probable path changes with the modulation frequency. Thus, while the presence of an absorber may cause a positive phase-shift difference, $\theta (= \theta_{\text{presence}} - \theta_{\text{absence}})$, at low modulation frequencies (indicative of optical path lengthening in the above time-domain arguments), it may cause negative phase-shift differences at higher frequencies (again indicative of optical path shortening). The decrease in volume available for photon transport results in less attenuation of the traveling photon density wave. The increase in amplitude modulation caused by the presence of an absorber should therefore be reflected in values of the modulation ratio, $M_r (= M_{\text{presence}} / M_{\text{absence}})$, greater than 1.

In the analysis above the lossless case was considered since the current single-pixel measurements and the previous multipixel images were obtained through use of relatively nonabsorbing lipid emulsions. In tissues one may expect the absorption coefficients to be 10–100 times greater than experimental lipid solutions. In the lossy case the volume probed by a photon density wave propagating at increasing modulation frequencies would be considerably smaller than in the lossless case.²⁹ The distribution of the optical path lengths traveled would be significantly shortened, which might significantly limit the depths probed at even moderate frequencies. Increased source-detector separations, however, may compensate for the small penetration of the most probable paths, which would occur in these physiological conditions.¹⁸

From these arguments several predictions regarding the relationships between the phase-shift difference (θ), the modulation ratio M_r , the modulation frequency, and the absorber position can be made:

- (1) At a constant modulation frequency the approach of a distal perfect absorber midplane between a single source-detector in reflectance geometry causes a decrease and subsequent increase in phase shift θ .
- (2) At a constant modulation frequency the approach of a perfect absorber causes an increase in the modulation ratio M_r as the volume for photon transport decreases and a subsequent decrease in M_r as the volume again increases.
- (3) At increased modulation frequencies the presence of the absorber is detected from changes in the phase shift and amplitude modulation at distances closer to the source and detector.
- (4) For a given absorber position the phase-shift difference θ that results may be positive at low modulation frequencies but negative at higher modulation frequencies, depending on the absorber position. At an intermediate frequency, termed the zero crossing frequency (ZCF), there is no change in phase shift because of the presence of the absorber.
- (5) The ZCF decreases with the absorber positions located at increasing depths in the medium.

Such results would point to the clinical potential of variable frequency measurements in that the modulation frequency can be easily scanned under electronic control and that one can detect and localize the diseased tissue by noting the ZCF and other measurable values of phase shift and modulation.

Materials and Methods

Experimental frequency-domain measurements of photon migration were made as a function of modulation frequency and absorber position for the phantom system described in Fig. 4. The phantom consisted of a 0.5% emulsified fat solution, Intralipid (Kabi-Vitrum, Inc., Clayton, N.C.). The mean isotropic scattering length at 633 nm has been reported to be between 1 and 1.9 mm.³⁰ The laser source was a 3.81-MHz train of 5-ps pulses from a cavity-dumped dye laser (Coherent Model 701) operating at 720 nm with Pyridine-1 (Exciton, Dayton, Ohio) which was pumped by a mode-locked Nd:YAG laser (Antares, Coherent, Palo Alto, Calif.). The frequency-domain instrumentation at the Center for Fluorescence Spectroscopy has been described in detail.^{31,32} The laser source was focused onto the end of a 600- μ m-diameter quartz fiber, which was passed through a hole bored in a clear section of Plexiglas at a height of 11.4 cm. The tip of the fiber protruded 1 mm into the tank and was in contact with the scattering medium. The detectors consisted of three 600- μ m fibers with proximal ends similarly positioned inside the tank at distances of 2, 4, and 5 cm from the source. The distal end of each detector fiber was directed into the housing of an R928 photomultiplier tube (Hamamatsu), which was gain modulated at a cross-correlation frequency offset by 40 Hz from the laser modulation frequency. Four percent of the incident

beam was directed onto a second R928 photomultiplier tube (PMT), which acted as the reference detector, and the data were acquired by standard ISS software (ISS, Urbana, Ill.).

Two sets of experiments were conducted. In the first set of experiments measurements of phase shift θ_{presence} and modulation M_{presence} were made at 30.48, 41.91, 49.53, and 99.06 MHz as the absorber was moved in 0.2–1-cm increments from 0.25 to 15 cm away in the z direction from the x - y plane containing the source and detector. Measurements were also made of θ_{absence} and M_{absence} in the absence of the absorber. All measurements of θ and M were made with respect to the signal measured by the reference detector. Changes in the frequency-domain parameters caused by the absorber are reported as the phase-shift difference θ ($=\theta_{\text{presence}} - \theta_{\text{absence}}$) and as the modulation ratio $M_r = (M_{\text{presence}}/M_{\text{absence}})$. Phase-shift and modulation measurements were made at each detector (located 2, 3, 4, and 5 cm from the source fiber), and the absorber was always located on the y - z plane that intersected the midpoint between the source and detector and on the x - z plane containing the source and detector. For all experiments the absorber was a cylindrical black rod with a 2.5-mm radius. In the second set of experiments the absorber position associated with the ZCF's between 30.48 and 148.59 MHz was found by slowly moving the absorber away from the source-detector plane until the measured phase was equal to that measured in its absence.

Results and Discussion

θ Resulting from the Presence of an Absorber at Varying Positions

Figure 5 illustrates the dependence of AO measured at 30.48, 41.91, 49.53, and 99.0 MHz on the z position of the 2.5-mm-radius cylindrical absorber. Consistent with the theory described above, the measured value of θ was nearly zero when the absorber was located far from the source-detector plane (i.e., when the absorber z position was > 3.5 cm when $\rho = 2$ cm, > 4.5 when $\rho = 4$ cm, and > 5.5 cm when $\rho = 5$ cm). However, as the absorber moved closer to the source-detector plane, the photons that traveled the longest optical path lengths were extinguished from the migration process, resulting in a negative value of θ . As the absorber became located even closer, the results suggest that detected photons traveled around the absorber with increasing probability, reversing the θ trend to the point at which its presence caused no change ($\theta = 0$). A further approach of the absorber resulted in the elimination of photons that traveled the shortest optical path lengths, which caused a positive θ value. The trends experimentally demonstrated have been successfully simulated in two-dimensional simulations.³³ Comparison of the figures show that at greater ρ the absorber was detected by $|\theta| > 1^\circ$ at greater z distances away from the source and detector. In addition, the largest negative θ occurred for greater absorber z positions at greater ρ . This result is consistent with the increased penetration depths associated with increased ρ .¹⁸ Figure 6 summarizes the measurements of θ at 99 MHz as a function of the absorber position at varying ρ .

Note that previous multipixel θ images obtained at 34 and 134 MHz provided detection of a 3-mm-diameter absorber located 1.2 cm (in the z dimension) from the detection plane from approximately $+1^\circ$ and -5° changes in θ , respectively. At 76 MHz no change in phase shift θ occurred because of the presence of the absorber.¹⁶ These imaging results are consistent with

the trends exhibited in the current experimental data and reported Monte Carlo simulations.

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θ Resulting from the Presence of an Absorber at Varying Modulation Frequencies

Figure 5 also illustrates that the absorber was detected by negative θ values at closer z positions at 99 MHz than at 30, 42, or 49 MHz. The result suggested a diminished depth of photon penetration with increased modulation frequency. The relationship between θ resulting from the presence of the absorber at $z = 0.5$ cm and the modulation frequency is best illustrated in Fig. 7. At low modulation frequencies (<50 MHz) the presence of the absorber was detected by positive phase-shift changes, indicative of optical path lengthening. At modulation frequencies greater than 50 MHz the presence of the absorber caused negative phase shifts. Therefore for this system the ZCF was 50 MHz. The relationship between θ and the modulation frequency is consistent with the reduction in photon-sampling volumes and the increased contribution of photon migration between the absorber and source and detector at greater modulation frequencies.

In Table 1 we show that for a fixed source-detector separation, the position at which the absorber caused no change in phase shift was dependent on modulation frequency. At low modulation frequencies there was no change in phase shift when the absorber was located at depths of 1 cm or more. As the modulation frequency increased, the absorber position at which there was no phase-shift change was located closer to the source and detector. This result is consistent with the probabilistic arguments presented in Fig. 3, which show a diminished contribution of photons traveling along optical paths in moderate- to high-frequency photon-migration measurements. In addition, as the source-detector separation was increased, the absorber position associated with no change in phase shift was located at greater distances from the source and detector. Again this observation is consistent with increased penetration depths of the most probable path of migrating photons. These results demonstrate that there should be a unique relationship between ZCF and the absorber position for (1) a known source-detector separation, (2) a known absorber volume and z dimension, and (3) known optical properties of the medium.

M_r Resulting from the Presence of an Absorber at Varying Positions

Figures 8(a), 8(b), and 8(c) illustrate the experimental measurements of M_r at $\rho = 2, 4$, and 5 cm, respectively, as a function of the absorber position at varying modulation frequencies. At all frequencies and source-detector separations, the following trends occurred: (1) M_r initially increased from a value of one as the absorber moved closer to the source and detector, indicating a reduction in volume available for photon migration (or a reduced probability for photon migration over long optical path lengths). As the photon density wave became restricted to smaller volumes, the degree of demodulation lessened. (2) As the absorber approached further (at absorber positions closer than $z \approx 1$ cm for a source-detector separation of $\rho = 2$ cm, $z \approx 2$ cm for $\rho = 4$ cm, and $z \approx 2.5$ cm for $\rho = 5$ cm), M_r diminished to values of less than 1 and subsequently began to increase. The reduction in M_r is expected since there is enhanced probability for photon transport around the absorber at positions closer to the source and detector. At this position the absorber effectively increases

the volume interrogated by the photon-density wave and increased the degree of its demodulation. At the closest absorber positions M_r increases again, probably because of another reduction in the volume available for photon migration as the absorber blocks source and/or detected light. (3) Finally, at increased modulation frequencies the magnitude of M_r increased. M_r must be investigated further to understand the increase in M_r at small distances from the source and detector. Nonetheless this phenomenon may not play an important role in photon-migration imaging because the goals for imaging optical heterogeneities several millimeters to centimeters deep in tissues may not involve such measurements.

Conclusions

It is probable that the development of biomedical optical imaging will progress first toward a new and economical screening tool for detecting and localizing diseased tissues. Subsequent development will be toward a complete diagnostic tool for the characterization of diseased tissues based on optical properties. Previous attempts to solve the inverse-imaging problem specify the entire problem of detection, localization, and characterization of optical heterogeneities through computer-intensive algorithms. Such approaches have value for the development of NIR imaging as a diagnostic tool but may overspecify the simpler problem for NIR imaging as a screening tool. Here we have taken a different approach to investigating how frequency-domain photon-migration imaging measurements may provide direct information for detection and localization of an optical heterogeneity. Specifically the unique relationship between ZCF and the absorber position demonstrated here suggests an approach for detection and localization of obscured optical heterogeneities involving multifrequency scanning from kilohertz to hundreds of megahertz. On tuning the modulation frequency to one in which the optical heterogeneity disappears from θ images or measurements, information on the object x , y , z position may be possible from measurements in the x - y plane. Information regarding x - y localization and geometry may also be available from M_r images whose resolution may be tuned by increasing the modulation frequency.¹⁶ In addition, we have recently demonstrated that the changes in photon-migration and frequency-domain measurements of θ and M_r are highly dependent on the photon-survival probability [i.e., $\exp(-\mu_a/\mu_s')$ (Refs. 33 and 34)] of the optical heterogeneity and the surrounding medium. For example, Monte Carlo simulations illustrate that the presence of heterogeneity with a low photon-survival probability (i.e., a perfect absorber in which $\mu_a \rightarrow \infty$) causes an increase in modulation and a reduction in the phase shift at a specified position and modulation frequency. Yet when the heterogeneity is assigned optical properties associated with a high photon-survival probability (i.e., a transparent or nonabsorbing heterogeneity in which $\mu_s' \rightarrow \infty$ or $\mu_a = 0$), a decrease in modulation and an increase in the phase shift occur.³³ Thus the direction and magnitude of the phase shift and modulation changes during multifrequency scanning may provide information pertaining to the relative optical property differences between a heterogeneity and the surrounding media.

Finally, the results here illustrating detection and localization are based on measurements that show a change in photon migration from where the heterogeneity is present and absent. Since the absence condition is not feasible for biomedical optical screening, an alternative

method must be employed to impose a change in photon migration. Further consideration of contrast agents and/or multiwavelength measurements is necessary for the development of NIR biomedical optical imaging by use of time-dependent measurements of photon migration.

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Appendix A:: Nomenclature

c	Speed of light.
f	Modulation frequency of incident and detected light.
f^*	Dimensionless modulation frequency, $f^* = f/f^*$.
L	Optical path length.
$\langle L \rangle$	Mean of the distribution of optical path lengths.
μ_s^*	Mean isotropic scattering length ($= 1/\mu_s^*$).
M	Amplitude modulation of emitted light with respect to the incident.
M_{absence}	Amplitude modulation of emitted light from a homogeneous medium.
M_{presence}	Amplitude modulation of emitted light in the presence of an embedded absorber.
M_r	Modulation ratio, $= M_{\text{presence}}/M_{\text{absence}}$.
n^*, n', n_{tot}	Number of scattering events or scattering steps.
$P_s(\mathcal{X}, \zeta, n)$	Probability that a photon is emitted at a source sample position $(\mathcal{X}, \zeta)$ in n scattering steps.
$P_D(\mathcal{X}, \zeta, n)$	Probability that a photon is emitted at a detector sample position $(\mathcal{X}, \zeta)$ in n scattering steps.
$P(\mathcal{X}, \zeta, n)$	Probability that a photon is emitted at a source and detected at the detector sample position $(\mathcal{X}, \zeta)$ in n scattering steps.
t	Photon time of flight.
x, y, z	Directions of the Cartesian system.
ZCF	Zero-crossing frequency at which $\theta = 0$.
θ	Phase shift of emitted modulated light with respect to the incident light.
θ_{absence}	Phase shift of emitted light from a homogeneous medium.
θ_{presence}	Phase shift of emitted light in the presence of an embedded absorber.
θ	Phase-shift difference, $\theta_{\text{presence}} - \theta_{\text{absence}}$.
ρ	Source-detector separation.
ρ^*	Dimensionless source-detector separation, $= \rho/l^*$.
μ_s^*	Isotropic scattering coefficient (length^{-1}).
μ_a	Absorption coefficient (length^{-1}).
$\mathcal{X}, \zeta$	Nondimensional x and z directions $\mathcal{X} = x/l^*$ and $\zeta = z/l^*$.

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Fig. 1.

Schematic describing the analysis of $P(\chi, \zeta, n_{\text{tot}})$, the probability for photon visitation at point (χ, ζ) for photons emitted at S and detected at D .

Fig. 2.

Probability for photon sampling at point $(0, \zeta)$ at time step $n_{\text{tot}}[P(\mathcal{X}, \zeta, n_{\text{tot}})]$ versus the dimensionless depth ζ ($= z/l^*$) midpoint between the source and detector. Curves for $P(\mathcal{X}, \zeta, n_{\text{tot}})$ are drawn for varying n_{tot} between 480 and 1480 steps. The separation between the source and detector ρ^* ($= \rho/l^*$) was 40 isotropic scattering lengths.

Fig. 3. Probability for photon sampling at point $(0, \zeta)$ from a source modulated at frequency $f^* [P(\mathcal{X}, \zeta, f^*)]$ versus the dimensionless depth $\zeta (= z/l^*)$ midpoint between the source and detector. Curves for $P(\mathcal{X}, \zeta, f^*)$ are drawn for positions at varying dimensionless modulation frequencies f^* ($f^* = f/f^*$). The separation between the source and detector $\rho (= \rho/f^*)$ was 40 isotropic scattering lengths.

Fig. 4.
Schematic of the experimental instrumentation and setup; see text for details.

Fig. 5.

Experimental measurements of the phase-shift difference $\Delta\theta (= \theta_{\text{presence}} - \theta_{\text{absence}})$ (degrees) versus absorber position (z , centimeters) at modulation frequencies of 30.48, 41.91, 49.53, and 99.0 MHz at (a) $\rho = 2$ cm, (b) $\rho = 4$ cm, and (c) $\rho = 5$ cm. The absorber radius was 2.5 mm.

Fig. 6.

Experimental measurements of the phase-shift difference $\Delta\theta (= \theta_{\text{presence}} - \theta_{\text{absence}})$ (degrees) versus absorber position (z , centimeters) at a modulation frequency of 99 MHz and source-detector separations of 2, 4, and 5 cm. The absorber radius was 2.5 mm.

Fig. 7. Experimental measurements of the phase-shift difference $\Delta\theta (= \theta_{\text{presence}} - \theta_{\text{absence}})$ (degrees) versus modulation frequency (megahertz) for an absorber position at $z = 0.5$ cm. The source-detector separation was 3 cm, and the absorber radius was 2.5 mm.

Fig. 8.

Experimental measurements of the modulation ratio $M_r (= M_{\text{presence}}/M_{\text{absence}})$ versus absorber z position at modulation frequencies of 30.48, 41.91, 49.53, and 99.06 MHz at (a) $\rho = 2$ cm, (b) $\rho = 4$ cm, and (c) $\rho = 5$ cm. The absorber radius was 2.5 mm.

Table 1.Experimental Values of ZCF and Absorber Position as a Function of ρ

ZCF (MHz)	Absorber Position (mm)		
	5 cm	$\rho = 4$ cm	2 cm
30.48	9.5	7.4	—
41.91	—	6.8	—
49.53	10.0	6.7	—
60.96	9.3	6.4	5.1
99.06	8.5	6.5	4.7
114.3	—	6.2	4.6
118.11	7.1	—	—
129.54	—	6.0	—
148.59	7.0	5.6	3.7