New Optical Probe Designs for Absolute (Self-calibrating) NIR Tissue Hemoglobin Measurements.

Dennis M. Hucber, Sergio Fantini, Albert E. Cerussi, and Beniamino Barbieri

ISS Incorporated, 2604 North Mattis Avenue, Champaign, Illinois 61822

Laboratory for Fluorescence Dynamics
University of Illinois Urbana-Champaign, Urbana, Illinois 61801

ABSTRACT

Most of the instruments reported for near infrared absorption measurements in highly scattering media, suitable for noninvasive hemoglobin concentration measurements in living tissues, require some type of instrumental calibration, or knowledge of the initial concentrations of the two species. A new type of probe, which does not require calibration, has been developed. These new probes can provide a rapid and “absolute” measurement of absorption and scattering in tissue when combined with the “multi-distance” method developed, in part, at the Laboratory for Fluorescence Dynamics at the University of Illinois and ISS Incorporated. An initial evaluation of the performance and efficacy of these new probes has been performed using a frequency-domain ISS tissue oximeter. The new probes offer the convenience of not requiring calibration, and also provide more reliable measurements and greater long-term stability compared with the standard multi-distance design. The new probes rely on a symmetrical arrangement of light sources and detectors. These geometries allow for measurements that are, in theory, entirely independent of the intensity differences between light sources, phase differences between the multiple light sources, sensitivity differences between the detectors, and any optical coupling losses, including those at the probe sample interface.

Keywords: near-infrared spectroscopy, turbid medium, noninvasive tissue spectroscopy, absorption coefficient, reduced scattering coefficient

1. INTRODUCTION

Because light in the wavelength range 700-900-nm can penetrate several centimeters into tissues, it has been used to measure tissue optical properties and, by extension, the concentration of tissue constituents such as hemoglobin. Recent developments in near-infrared spectroscopy have increased its usefulness as a tool for the non-invasive in vivo measurement and monitoring of tissues, including the use of time-resolved spectroscopy and the corresponding development of the theoretical models which describe light propagation in turbid (highly scattering) media.

A tissue spectrometer, based on a frequency-domain multiple distance (FMDM) method, has been developed. The instrument contains multiple intensity-modulated laser-diode sources which are multiplexed to time-share a common detector. Simultaneous measurements of absorption and scattering in tissues (and other turbid media) can be made continuously and as quickly as 160-ms per measurement. The instrument does not require apriori knowledge of the path length (or differential path length factor, DPF) or of the scattering properties of the sample.

The standard (fiber-optically coupled) multi-distance probe (or scanner) directs the light emitted by multiple laser-diodes onto to the surface of (or into) the sample using separate fibers for each laser, and collects a fraction of the diffusely reflected light using a fiber-optic bundle coupled to a PMT light detector. The emitting ends of the source fibers are positioned such that they define several source-detector separation distances for each wavelength. The FMDM method requires that light...
intensities and phases be measured at two or more source-detector separation distances. The change in measured intensity and phase as a function of distance from the source is used to calculate the absorption coefficient (μ₀) and reduced scattering coefficient (μ's).

Unfortunately, the use of multiple light sources with the FDMD method requires the performance of an instrumental calibration. The multiple laser-diodes do not emit with equal intensity or phase, and the optical-coupling efficiencies between the lasers and the fiber-optics, and between the fiber-optics and the sample are not equal. Therefore the probe (and the instrument) is calibrated by measuring a phantom of known optical properties before real measurements are performed.

Once calibrated the probe can be moved to the unknown sample (or tissue) where “calibration corrections” can be applied during the measurement. In practice, a calibration is required each time a probe is connected to the instrument, since the coupling efficiency of the fiber-optic connections may vary, and once each morning. This is not only inconvenient, but two (possible inaccurate) assumptions are made, 1) that the relative intensities and phases of the sources will remain constant, and 2) that there will be no changes in the optical coupling efficiencies. While the effects of these assumptions are somewhat alleviated by the use of a redundant number of sources, the possibility of small errors exists. In addition, thermally induced laser-diode intensity and phase changes can cause significant signal drift (low frequency noise).

A new type of probe has been developed, which does not “require” a calibration step and which provides more stable long-term measurements. The new probes are referred to below as “absolute probes”, since they are capable of “absolute” analysis. Like the standard multi-distance probes, the new absolute probes have no moving parts and can be used to make very rapid measurements. The new probes require at least two detectors and two emitters (per wavelength) arranged in a symmetric pattern that allows for the calibration free measurement of the relative intensity and phase of modulated light at two (or more) source-detector distances.

These “absolute probes” can make measurements which are independent of the intensity differences between light sources, phase differences between the multiple light sources, sensitivity difference between the detectors, and any optical coupling losses (including those at the probe sample interface) provided that four basic assertions hold true: 1) the detector response is linearly with photon flux, 2) there is no inter-channel (detector or source) cross talk, 3) the intensity of the light does not affect the phase measurements (no phase-amplitude cross talk), and 4) the sample is macroscopically homogeneous. While these assumptions are common to many spectroscopic measurements (including the standard multi-distance probe), the absolute nature of these new probes make deviations from these assertions unusually important. Therefore, while an absolute probe does not require calibration, in some cases, accuracy may be insured using a calibration method similar to that used for standard multi-distance probes. Once calibrated, the absolute probe can still make measurements which are largely free of the errors and drift associated with changes in the output of the individual laser-diodes, changes in the optical-coupling efficiency between the source fibers and the surface (skin) of the sample and other factors. A prototype absolute probe has been constructed and tested on solid phantoms. The results are compared to those obtained by a moving source reference method.

Another possible mode of use for the absolute probe can be called “calibration-in-place”. An absolute probe can be placed on an unknown (but reasonably homogeneous) sample and the optical properties of the sample measured. Once the initial properties are determined, calibration factors and terms can be calculated to equalize the response of the two detectors and the probe can be used as two or more overlapping probes to detect changes in the sample, or half the probe can be used for faster measurements. The second of these options was demonstrated.

2. THEORY AND INSTRUMENTATION

2.1 The Frequency-Domain Multiple-Distance Method

As light travels outward from a source in an infinite highly scattering medium, the radiant energy density, U, observed (or measured) at a point in the medium decreases exponentially with increasing distance from the source as a function of distance. The rate of change in U with distance depends on the optical properties of the medium. In frequency-domain instruments the intensity of the light source(s) is sinusoidally modulated, a “photon density wave” can be said to propagate
through the medium outward from the source. The variation in time of radiant energy density at any point in the medium can be described by the equation,

\[ U(t) = U_{AC} \sin(2\pi ft + \Phi_U) + U_{DC} \]

(1)

\( U_{AC} \) is the amplitude of the modulation in the energy density, \( f \) is the frequency of the modulation, \( \Phi_U \) is the phase, and \( U_{DC} \) is the average radiant energy density. Both \( U_{AC} \) and \( U_{DC} \) decrease as a function of the distance between the observed point and the source. While a photon density wave travels with a constant frequency and speed, the speed of the photon density wave is slower than the speed of light in the medium. The phase (or phase shift) at a fixed time, increases with distance according to the propagation speed of the photon density wave. The rate of change in \( U_{AC}, U_{DC} \) and \( \Phi_U \) with distance all depend on the optical properties of the medium including \( \mu_a \) and \( \mu'_a \) and index of refraction (\( n \)). Therefore, the optical parameters of the sample can be related to changes in the amplitude of the modulation (ac), average (dc), and phase shift (\( \Phi \)) of the signal produced by a light detector (assuming linear response) as a function of distance from a point source. Fishkin and Gratton\( ^{16} \) have derived the following relationships for FDMD measurements in an effectively infinite medium. These equations are based on the “diffusion approximation” of the transport equation:

\[ \ln(\text{dc } r) = r S_{\text{dc}}(\mu_a, \mu'_a) + \ln(\text{dc } D, K_{\text{dc}}) \]

(2)

where \( S_{\text{dc}} = -\left( \frac{\mu_a}{D} \right)^{1/2} \)

(3)

\[ \Phi = r S_{\Phi}(\mu_a, \mu'_a, \omega, \nu) + \ln(\Phi_{\text{dc}}, K_{\text{dc}}) \]

(4)

where \( S_{\Phi} = \left( \frac{2}{3D} \right)^{1/2} \left[ 1 + \left( \frac{\omega}{\sqrt{\mu_a}} \right)^2 \right]^{-1/2} \)

(5)

\[ \ln(\text{ac } r) = r S_{\text{ac}}(\mu_a, \mu'_a, \omega, \nu) + \ln(\text{ac } D, K_{\text{ac}}) \]

(6)

where \( S_{\text{ac}} = -\left( \frac{\mu_a}{D} \right)^{1/2} \left[ 1 + \left( \frac{\omega}{\sqrt{\mu_a}} \right)^2 \right]^{-1/2} \)

(7)

where \( \nu \) is the speed of light in the medium, and \( \omega \) is the angular frequency of the modulation. \( D \) is the diffusion constant, where \( D = 1/(3\mu_a + 3\mu'_a) = 1/(3\mu'_a) \). \( K_{\text{ac}} \) and \( K_{\text{dc}} \) are constants that depend on source intensity, modulation depth and detector sensitivity factors, and \( K_{\Phi} \) is the relative phase of the source plus any phase shifts external to the sample. To facilitate the multi-distance method, equations 2, 4 and 6 are written so that the quantity on the left of the equality has a linear dependence on \( r \). \( \ln(\text{dc}), \ln(\text{ac}) \) and \( \ln(\Phi) \) (which are independent of \( r \)) define the intercept of the lines, and \( S_{\text{dc}}, S_{\text{ac}}, \) and \( S_{\Phi} \) define the slopes. Note that the slopes are independent of instrumental factors.

Once \( S_{\text{dc}}, S_{\text{ac}}, S_{\Phi} \) have been calculated based on measured intensities, phases and distances, \( \mu_a \) and \( \mu'_a \), and can be recovered by solving the system of simultaneous linear equations defined by the three slope functions. Since there are two unknowns and three equations any two slopes need to be used. However, the tiny difference between \( S_{\text{dc}} \) and \( S_{\text{ac}} \) observed in tissues using 110-MHz modulation prevents the practical use of this pair. The \( S_{\text{dc}} \) and \( S_{\text{ac}} \) pair is often preferred, since \( S_{\text{ac}} \) is much less affected by room light. Although, these equations have some limitations\( ^{16,17} \), the have been experimentally verified in media with “tissue like” optical properties using modulation frequencies between 100 to 200-MHz and distances much larger than the photon mean free path (\( \mu >> \mu'_a \)).\( ^{14,16,18} \)

While this solution works well when the light source(s) and detector(s) can be immersed into the sample, this is not often practical for tissue measurements. A similar set of equations can be derived for the geometry of a semi-infinite media bounded by a flat plane.\( ^{10,17,18,20,22} \)
\[
\ln(\text{ac} \ r^2) = r S_{\text{ac}} (\mu_a, \mu'_a, \sigma, \nu) + \ln(\text{ac}) \quad (8)
\]
\[
D = r S_{\phi} (\mu_a, \mu'_a, \sigma, \nu) + \ln(\phi) \quad (9)
\]
\[
\ln(\text{sc} \ r^2) = r S_{\text{sc}} (\mu_a, \mu'_a, \sigma, \nu) + \ln(\text{sc}) \quad (10)
\]

Note that while \( S_{\text{sc}} \) and \( S_{\text{ac}} \) are the same as those in equations 1, 3 and 5, \( r \) has changed to \( r' \) on the left side of equations 8 and 10. These equations (8-10) are approximations of a more complicated set of equations given by Fantini et al.\textsuperscript{11} The approximate equations are valid when \( r (\frac{3 \mu_a}{\mu'_a})^2 \gg 1 \). The value of \( r (\frac{3 \mu_a}{\mu'_a})^2 \) typically ranges from 2 and 8 in tissue when source-detector distance are 1.5 - 4.5 cm. While the more exact equations given in reference 17 may be slightly more accurate, they require the use of iterative calculations, so are less suitable for rapid real time computations. For simplicity and convenience, we will refer to the more exact solutions as the “exact semi-infinite” solution and equations 8-10 as the approximate semi-infinite solution.

Figure 1. A simplified diagram showing a standard probe connected to the oximeter. The standard probe has two lines of source output ports, defining four source detector separation distances for both wavelengths.

Once \( \mu_a \) has been determined at two or more near-infrared wavelengths, the Hemoglobin concentrations can be determined based on the Beer-Lambert law and the assumption that hemoglobin and water are the dominant absorbers in the tissues measured.

If \( \mu'_a \) is known to be constant, equations 8 or 10 can each be solved directly for \( \mu_a \) requiring only that \( S_{\text{ac}} \) or \( S_{\text{sc}} \) be measured. This approach can be useful, because phase noise is often the dominant noise source in frequency-domain spectroscopic measurements.

2.1 Oximeter

An ISS dual channel oximeter model 96208 was used to test prototype probes of the new design and to compare their performance with standard multi-distance (single source) probes. The ISS oximeter is a frequency domain, near-infrared, tissue spectrometer which measures tissue absorption and scattering coefficients using the multi-distance frequency domain method described above. The instrument has been described previously in the literature.\textsuperscript{20,21}

The oximeter contains two banks of multiplexed sources, each bank (or channel) contains eight laser diodes (only one bank, a maximum of eight laser-diodes, was used in this study) and two photomultipliers (PMT) detectors. With a standard multi-distance probe, the two detectors are used separately, each servicing its own probe. To support an absolute probe, both detectors are used with each probe. Probes (or scanners) are optically coupled to the oximeter's laser-diode sources by large core (> 200um) fiber-optics using SMA type connectors. Larger (3mm diameter or smaller) fiber-optic bundles are used to guide light from the probes to the PMTs. Each oximeter can be made with a variety of emission wavelengths, the instrument used contained laser-diodes that emit at either approximately 750 or 830-nm (four of each wavelength per source bank).
The laser-diodes are intensity modulated at 110-MHz and they are multiplexed such that only one source in each bank is "on" at a time. Generally all eight sources are cycled on and off continuously while the instrument is operating. Approximately 3-nsec is required to turn each diode on, therefore there is a brief period, just after each source is switched on, during which no measurements are made. While the source-switching period can be adjusted between 5-nsec and several seconds, in practice each source is usual "on" for 20 to 100-nsec. This allows good compromise between speed and measurement duty factor. While each source is on, the ac, dc and \( \Phi \) are measured. At the end of each source cycle, the ac, dc and \( \Phi \) values are used to calculate \( \mu_a \) and \( \mu'_s \) (at both wavelengths) and hemoglobin concentration values. Typically, when all eight sources are used each measurement cycle is 160 ms. It is possible to make measurements more quickly, but only at a significant reduction in duty factor. The results of many cycles can be averaged to improve the signal-to-noise ratio when high speed is not required. Since living tissue is dynamic, a short measurement cycle, followed by averaging is preferred.

The oximeter is controlled by a Windows 95/98 computer. The software allows for real-time acquisition, calculation, display and storage of all the "raw" (ac, dc and \( \Phi \) values) data, \( \mu_a, \mu'_s, S_l, S_{lc}, S_{dc}, S_{ldc} \) and the hemoglobin values; oxygenated hemoglobin concentration, deoxygenated hemoglobin concentration, total hemoglobin concentration and \( S_lO_2 \) (tissue hemoglobin oxygenation or mole fraction oxygenated hemoglobin).

2.2 Standard Multiple-Distance/Multiple-Source Probes

The standard multiple distance probes contain two rows of source fibers and one detector bundle (see the bottom left of Figure 1). There are four emitters connected to 750-nm lasers and four connected to 830-nm lasers. The standard probe positions emitters at 4 distances (rather than only 2) so that a small change in the optical-coupling of any one channel will have little effect on the result. Some of the emitters are optically filtered to reduce the light intensity emitted. This is done so that the light intensity reaching the detector from all of the emitters is roughly equal during the measurement of a typical sample (the closest emitters are filtered the most etc.). This limits the linear-dynamic-range required of the FMT and other electronics.

![Source 1 and Source 2 diagram](image)

Figure 2. A schematic representation of the source and detector positions on an absolute probe. The points from which light is emitted and collected are indicated with circles. The distances \( S_a = S_b \) and \( L_a = L_b \), but \( S \neq L \). The distances labeled X and Y can be any size providing this the symmetry is maintained. For simplicity, this figure shows only one emitter pair. In general, one pair would be included for each wavelength.

This type of probe requires an instrumental calibration because the sources are not equal in phase, intensity or modulation depth (even if no filters are used). Also there may be unequal electronic phase shifts, unequal optical phase shifts in the fiber-optics and other (unequal) optical losses. Note that the intercepts \( (In_e, In_{dc}, In'_{dc}, \text{and} \ In'_{ac}) \) of equations 2, 6, 8 and 10 depend on the optical properties of the medium and on the source intensities, detector sensitivities and various optical coupling efficiencies. Also, \( In_0 \) and \( In'_{ac} \), in equations 4 and 9 depend on various phase shifts introduced by the optics and electronics. Therefore, the intercept functions are not constant for the various sources.

The calibration procedure involves placing a probe on the surface of (or immersing it in) a phantom of known optical properties. The relative intensities and phases of the signals arising from each source are measured and compared to the theoretically expected intensity ratios and phase differences. Corrective, calibration factors and terms are calculated and stored. Once a probe is calibrated, it can be moved to the unknown tissue (or sample) and the ac, dc and \( \Phi \) measured for each source. The correction factors and terms are then applied, and \( S_{lc}, S_{dc}, \text{and} \ S_{ldc} \) are calculated based on the calibration-corrected ac, dc and \( \Phi \) values.
2.3 Absolute Probes in Absolute-Mode

In absolute probes, at least two detectors and two sources (per wavelength) are arranged symmetrically such that at least two source-detector separation distances are defined. In Figure 2, two sources are located near the center of the probe, with the sources toward the edge. Source 1 is placed $S_a$ (S=short) from detector A and $L_a$ (L=long) from detector B, and Source 2 is placed $S_b$ from detector B and $L_b$ from detector A. $S_a$ must be equal to $S_b$, and $L_a$ must be equal to $L_b$, such that the two source detector separations (S and L) are defined redundantly. For simplicity, Figure 2 indicates the position of only one pair of sources. Additional pairs can be added as long as $S_a = S_b$, $L_a = L_b$, and $S + L$. The distance (S and L) need not be equal for each pair. (Recall that the sources are multiplexed so that only one source is on at any given time.) Figure 3 shows three other possible arrangements of sources and detectors. Unfortunately, the dynamic range of the measurements cannot be easily reduced with optical filters, as it often is in the standard probes. This puts practical limits on the range of distances that can be used.

Before examining how the absolute probe works we point out that to measure $\text{SI}_{dc}$ we only need to measure the ratio of the dc values at two source-detector distances. Note that if we write equation 8 for two sources positioned at $r_1$ and $r_2$ respectively, and then subtract the resulting two equations:

\[
\ln(d_{c1} r_1^2) - \ln(d_{c2} r_2^2) = r_1 \text{SI}_{dc} - r_2 \text{SI}_{dc} + \ln(d_c) - \ln(d_c) \quad \text{or, } \quad \text{SI}_{dc} = \frac{\ln\left(\frac{d_{c1}}{d_{c2}}\right) + 2 \ln\left(\frac{r_1}{r_2}\right)}{r_1 - r_2}
\]  

(A similar equality can be found for $\text{SI}_{dc}$ using equation 2). It is equally simple to show that only the ratio of the dc values at two distances is required to calculate $\text{SI}_{dc}$, and that $S_b$ can be calculated from the phase difference between two source-detector distances. Therefore to measure $\text{SI}_{dc}$, $\text{SI}_{ac}$ and $S_b$ (and by extension $\mu_1$ and $\mu'_1$) we need only measure the intensity ratios and phase difference between two distances.

The absolute probe design requires no calibration because it can measure these intensity ratios and the phase differences in a manner which is independent of many instrumental factors. To show this mathematically, we consider the simple probe shown in Figure 2. First we note that the signals produced by detectors A and B while source 1 is on are given by:

\[
dc_{1,A} = I_1 C_1 Y_A C_A dc_S \quad \text{and}
\]

\[
dc_{1,B} = I_1 C_1 Y_B C_B dc_L,
\]

where $dc_{1,X}$ is the measured dc signal due to source 1 on detector X (A or B), $I_1$ is the intensity of source 1, $Y_X$ is the sensitivity of the detector X, $C_1$ is the combined coupling-efficiency for light from source 1 into the sample, $C_X$ is the coupling-efficiency for light out of the sample and into detector X. $C_1$ and $C_A$ include losses inside the light-guides. $dc_S$ is the...
hypothetical dc signal at the shorter of the two distances \( S \), if the detector sensitivity were unity, the intensity of the source were unity and there were no intensity losses. \( dc_1 \) is the hypothetical dc signal at the longer of the two distances \( L \), if the detector sensitivity was unity, etc. In other words, \( dc_1 \) and \( dc_2 \) are what the dc signals would be if there were no losses and the intensity of every source was exactly 1. Therefore, \( dc_1/dc_2 \) is the exactly equal to \( U_1 \) (at distance \( L \))/\( U_2 \) (at distance \( S \)). When source 2 is on,

\[
dc_{2,A} = l_2 C_2 Y_A C_A dc_L \quad \text{and} \quad \quad (14)
\]

\[
dc_{2,B} = l_2 C_2 Y_B C_B dc_S , \quad \quad (15)
\]

where the subscript "2" indicates source 2. Next we point out that,

\[
\ln \left( \frac{dc_1}{dc_2} \right) = \ln \left[ \left( \frac{dc_1}{dc_S} \right)^2 \right] = \frac{1}{2} \ln \left[ \left( \frac{dc_1}{dc_S} \right) \left( \frac{dc_1}{dc_S} \right) \right] , \quad \quad (16)
\]

Finally we solve equations 12-15 for \( dc_2 \) and \( dc_1 \) and substitute the resulting expressions into equation 16:

\[
\ln \left( \frac{dc_1}{dc_2} \right) = \frac{1}{2} \ln \left[ \frac{dc_{2,A} dc_{1,B} C_1 C_2 Y_A C_A Y_B C_B B}{dc_{1,A} dc_{2,B} C_1 C_2 Y_A C_A Y_B C_B B} \right] = \frac{1}{2} \ln \left( \frac{dc_{2,A}}{dc_{1,A}} \right) \quad \text{or} \quad \frac{dc_1}{dc_2} = \sqrt{\frac{dc_{2,A}}{dc_{1,A}}} . \quad \quad (17)
\]

By similar reasoning it is just as easily shown that:

\[
\ln \left( \frac{ac_L}{ac_S} \right) = \frac{1}{2} \ln \left[ \frac{ac_{2,A} ac_{1,B}}{ac_{1,A} ac_{2,B}} \right] \quad \text{or} \quad \frac{ac_L}{ac_S} = \sqrt{\frac{ac_{2,A}}{ac_{1,A} ac_{2,B}} , \quad \quad (18)
\]

and \( \Phi_L - \Phi_S = \frac{1}{2} \left[ \Phi_{2,A} + \Phi_{1,B} - \Phi_{1,A} - \Phi_{2,B} \right] . \quad \quad (19)\]

We stress that equations 17-19 are (as promised) free of any instrumental terms or factors, but that the 4 basic assertions listed in the introduction were implicitly assumed. We can also add a fifth assertion; the probe distances are exactly accurate and all the symmetry is correct. Any deviations from the ideal, will cause some degree of error in the values of \( (ac/L) \), \( (dc/L) \), \( (\Phi_L - \Phi_S) \) etc. We refer to the use of an absolute probe, without calibration, as the "absolute-mode" of operation.

2.4 Absolute Probes in Calibrated-Mode

It is possible to calibrate a probe of this design against known optical properties in a manner similar to that used to calibrate a standard probes. In this mode, the probe is placed on (or in) a phantom of known optical properties, and the intensity ratios and phase differences are measured according to equations 17-19. These values are compared to the values calculated (from theory) for the known optical properties. Corrective intensity ratio factors and corrective phase difference terms are calculated. Then during the measurement of an unknown, these factors and terms can be applied to the measured intensity ratios and phase differences. The advantage of a "calibrated" absolute probe over a standard probe is that the measurement is still independent of changes in many instrumental factors.

2.5 Calibrate-In-Place Mode

A potentially useful mode of operation for the absolute probe can be termed "calibrate-in-place". In this mode the probe can be used in absolute-mode long enough to measure the initial optical properties of a sample. Then once the optical properties are known, the correction factors and terms can be calculated to equalize the response of the detectors and sources. With these calibration corrections applied, one absolute probe can be used as several calibrated probes (with some overlap in the probe volume, esp. as in Figure 3C). Alternatively only one source of each wavelength could be used. This could facilitate faster measurements because full hemoglobin measurements could be made with only two sources. Furthermore, if the absorption and/or scattering at only one wavelength is required, then one source could be left on continuously, and full
FDMD measurements could be made with no dead time (duty factor of 1). The advantage over a standard multiple-distance probe is twofold. No calibration phantom is required, and the probe does not have to be moved after calibration. Therefore, there is less chance that the optical-coupling will change between the calibration step and the measurement.

3. EXPERIMENTAL

Several experiments were carried out to evaluate the efficacy and performance of these new probes. First a prototype absolute probe was used to measure 4 solid phantoms (with a range of optical properties) the results were compared to values determined using a single moving source method as outlined below. Second the same probe was used to evaluate the "noise" (random measurement error), especially very low frequency noise and thermal drift. Third the theory summarized by equations 3, 5, 7, 8-10 and 17-19 was used to evaluate several possible sources of systematic error that affect accuracy. Finally a second prototype probe was used to make very rapid measurements on a human head demonstrating the "calibrate-in-place" mode.

3.1 Measurement on Solid Phantoms

Four solid phantoms were measured using a reference method and using an absolute probe. The phantoms were constructed from silicone rubber compound (3M Silicones RTV615), TiO₂ rutile powder (average particle size 2-5 microns, Alfa Aesar) and carbon black, and formed into rectangular blocks (8 x 12 x 7 cm).

The reference method involved two emitters (750 and 830-nm) that were moved across the surface of the block relative to a single light collector. The emitter (400-micron core fibers-optics) tips were held approximately 0.5-mm above the surface of the block at all times. (Past experience has demonstrated that this procedure produces very reproducible results.) The input tip of a collector light guide (a 3 mm core fiber-optic bundle) was held in contact with the blocks and surrounded by -10-mm of black tape to block light from entering the phantom near the detector. Part of the input area of the collector bundle was masked with tape to limit the effective area to a 1-mm wide strip perpendicular to the motion of the emitter fibers. The emitter fibers were moved in line with the collector using a precision X-Y-Z translational stage (Techno XYZ, New Hyde Park, N.Y.). An ISS oximeter was used to provide the light sources (750 and 830-nm laser-diodes), the detector (PMT) and detector electronics. The emitters were moved in 1-mm increments to define source-detector distances between 1.5 and 3.2-cm. The signals were averaged in each position for 5 seconds. The scan was repeated at least 7 times on each block. The data was analyzed using the FDMD method with both the exact and approximate semi-infinite solutions.

The absolute probe was configured as shown in Figure 3A. The probe was constructed of black plastic (5 x 7-cm). The tips of the emitter fibers (400-micron core) were allowed to protrude from the bottom (working) side of the probe by approximately 0.5 mm. The collector bundles were 3-mm core glass fiber bundles. The probes distances were 5, 7, and 9 cm. (See Figure 2) for both pairs of emitters. The ISS oximeter was used, and data was averaged on each block for about minute. The data was analyzed using the approximate semi-infinite solutions in absolute-mode and in the calibrated-mode after calibration against one of the phantoms. The absolute probe was calibrated against properties of the phantom determined by the reference method using the exact solution, but the approximate solution was always used with the absolute probe.

To evaluate the long-term stability and low frequency noise characteristics of the absolute probe, it was used to measure a solid phantom over a 14 hour period. For comparison a standard probe (1.75-3.25-cm distance range) was also used to measure the same phantom. Data was averaged for 64 seconds per measurement during these tests.

3.2 Evaluation of Potential Errors

A complete analysis of possible systematic errors affecting FDMS and this new type of probe is behind the scope of this study. However, some insight into the relative importance of the potential error sources is essential when using any absolute method of analysis. Therefore the effect of various error sources on the calculated optical properties and hemoglobin concentrations were calculated based on the approximate semi-infinite solution. The results were tabulated as percent errors in S₂, S₆, S₅, µₐ, µₛ, [HbO₂], [Hb], total [Hb] and S₂O. Two typical absolute probes and one standard multi-distance probe were compared. See the Results and Discussion sections below.
3.3 Measurement of Human Head, a Demonstration of the Calibrate-In-Place Mode for High Speed Measurements

An absolute probe configured as shown in Figure 3C was built. Construction was similar to that described for the absolute probe described in section 3.1. The distances were S=3.08-cm and L=4.98-cm for the 820-nm source pair and S=3.07-cm and L=4.19-cm for the 750-nm pair. The bottom (working side) of the probe was lined with soft rubber foam to conform to the shape of the head. The probe was placed approximately 5-cm above the left eyebrow of a male volunteer. After the probe was strapped in place, (with an elastic strap) it was operated in two modes for one second each. First the absolute-mode (multiplexing all four sources) was used to make measurements at 96-ms per measurement. Finally, the probe was operated with only one source (830-nm) making one measurement every 4.8-ms. The average μ_{a} and μ_{s}'s measured during absolute-mode operation was used to “calibrate” the detectors during the single source measurements.

4 RESULTS AND DISCUSSION

4.1 Measurement of Solid Phantoms

The results of the solid phantom experiments are summarized in Figure 4. The first bar in each graph (MsA) is the value measured with the reference method using the approximate semi-infinite solution. The second bar (AbA) is the value measured with the absolute probe (in absolute-mode) using the approximate semi-infinite solution. Note that there is good agreement between these two methods. The third bar is the value measured with the reference method using the exact semi-infinite solution (MsE), and the forth bar (CaA) is the value measured by the absolute probe after it was calibrated on block A. There is good agreement in μ_{a} between the reference method using the exact solution and the absolute probe in calibrated-mode for all phantoms. The calibration procedure does not work as well for the μ_{s}'s values. This observation has also been made using standard multi-distance probes. In both cases, the best practice is to calibrate the probe on a phantom with optical properties that nearly match those of the sample (tissue) to be measured.

Figure 5 shows some of the results of the long-term stability tests. The hemoglobin concentrations in Figure 5C are based on the measured μ_{a} values, but the phantoms did not contain any actual hemoglobin. Clearly the absolute probe provides much more stable measurements. The very low-frequency noise (in the standard probe signal) is mostly due to thermally induced drifts in the intensity and phase of the laser-diodes. Although all laser-diodes are experiencing the same small changes in room temperature, there exist small differences in the magnitude of the effect in individual diodes, this causes a drift in the results obtained using the standard multi-distance probe. What appears to be a very small drift in the values measured using the absolute probe, may be due to actual changes in the optical properties of the silicone phantoms.

4.2 Evaluation of Potential Errors

In Tables 1 and 2 the calculated effects of selected potential error sources are listed. The first row in Table 1 and 2 lists the “correct” (base) values, all other rows indicate % errors. The slopes and optical properties are given at only 1 wavelength for brevity. Emphasis is given to the various types of possible source-detector distance errors.

The first type of distance error considered is an error in one of the short (S or S) in Figure 2) distances in involving the 750-nm source pair. The second type of distance error is an error in one of the short distances for both wavelength pairs, this type of error has little effect on S/D since it affacts both wavelength nearly equally. The third type is an equal error in all S distances. The forth type is an equal error in all S and L (see Figure 2) values. This error actually has a smaller influence because it involves no error in the range (L-S) of distances used. Finally, a group of distance errors that do not change the “average” distances used are considered. In other words, an error of equal magnitude but opposite direction such the averages [S'(S_{T}-S_{B})/2] and [L'=(L_{T}+L_{B})/2] are correct. Distance errors of this last type have very little effect (even very large errors). It is clear that the symmetry of the probe distances is not critical as long as the correct average S and L values are known and used. This is an important insight, since it may not always be convenient to use rigid probes where the source positions are precisely defined.
Figure 4. Measured absorption and scattering values for 4 solid phantoms/blocks. MsA = measured with the moving sources (reference method) using the approximate semi-infinite solution. AbA = measured with the absolute probe in absolute-mode using the approximate solution. MsE = measured with the reference method using the exact semi-infinite solution. Cab = measured with the absolute probe in calibrated-mode, calibrated on phantom A using the MsE values.
The tenth row indicates the result of a detector non-linearity that leads to a 2% intensity error over 1 order of magnitude. The last three errors listed in Table 1 do not vary with probe type (they affect any probe equally). The last error type is an error in the assumed 70% water concentration. Table 1 was created assuming probe distance and optical properties typical for head measurements. Table 2 was created using probe distances and optical properties typical for muscle measurements.

Table 3 shows similar error calculations for a standard multi-distance probe. The same hypothetical optical properties used for Table 1 were used for Table 3. The simulation involved calibration against a phantom with optical properties that nearly matched those of the sample (0.9 times μs and 1.1 times μr). Such a close match is not always possible but can be arranged in some cases. The simulated probe was treated as if it were constructed with optical filters that would have nearly equalized the measured intensities. This is why a non-linearity would have little consequence. Note how distance errors have a much smaller effect on a calibrated probe. These errors would be larger if the probe was calibrated on a less closely matched phantom, but they would still be smaller than those for absolute probes in absolute-mode. Also, note the effect of coupling efficiency changes and phase changes; these factors were not listed in Tables 1 and 2 because they do not directly result in measurement errors with an absolute probe.

4.3 Measurement of Human Head, a Demonstration of the Calibrate-In-Place Mode for High Speed Measurements

Figure 6 shows the Fast Fourier Transform (FFT) of μs, versus time for 1-second of high-speed measurements. This data was taken using only one source (830-nm), after calibration-in-place. The zero frequency component of the signal was removed by subtraction of the average μs value before the FFT was performed. This was done so that the higher frequency components could be clearly seen. A constant μs was assumed to improve the signal-to-noise for fast μs measurements. The FFT of μs measured on a solid phantom is also plotted to indicate the level of instrumental noise.

The signal at 1.8-Hz can be attributed to arterial pulsations. No 1.8-Hz signal was seen in the FFT of μr, (or in either of the phase measurements). A repetitive phase variation of 0.005° degrees would have been clearly detected, see Figure 7. Experiments that allow for phase-sensitive frequency filtering might allow even smaller repetitive signals to be detected.
Table 1. Some error sources for absolute probes. Assuming source detector distances of 3 and 4.2-cm, and optical properties of $\mu_a=0.10-\text{cm}^{-1}$ and $\mu'_a=10-\text{cm}^{-1}$ at 750-mm, and $\mu_a=0.11-\text{cm}^{-1}$ and $\mu'_a=9.5-\text{cm}^{-1}$ at 830-mm.

<table>
<thead>
<tr>
<th>&quot;Correct Values&quot;</th>
<th>$S_{ab}$</th>
<th>$S_{br}$</th>
<th>$\mu_a$</th>
<th>$\mu'_a$</th>
<th>$H_b$</th>
<th>$H_bO$</th>
<th>Total $H_b$</th>
<th>% $S_{ab}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Error type</td>
<td>Error</td>
<td>% Error</td>
<td>Error</td>
<td>% Error</td>
<td>% Error</td>
<td>% Error</td>
<td>% Error</td>
<td>% Error</td>
</tr>
<tr>
<td>One S distance at 750nm</td>
<td>1 mm</td>
<td>5.7</td>
<td>4.2</td>
<td>1.7</td>
<td>9.7</td>
<td>6</td>
<td>-1.8</td>
<td>0.39</td>
</tr>
<tr>
<td>One S distance at 750nm</td>
<td>0.1 mm</td>
<td>0.57</td>
<td>0.42</td>
<td>0.17</td>
<td>1</td>
<td>0.58</td>
<td>-0.18</td>
<td>0.04</td>
</tr>
<tr>
<td>One S at 750 and 830nm</td>
<td>0.1 mm</td>
<td>1.2</td>
<td>0.83</td>
<td>0.33</td>
<td>2</td>
<td>1.2</td>
<td>-0.36</td>
<td>0.08</td>
</tr>
<tr>
<td>All S distances</td>
<td>0.1 mm</td>
<td>1.2</td>
<td>0.83</td>
<td>0.33</td>
<td>2</td>
<td>1.2</td>
<td>-0.36</td>
<td>0.08</td>
</tr>
<tr>
<td>All S and L distances</td>
<td>0.1 mm</td>
<td>0.09</td>
<td>0</td>
<td>0.09</td>
<td>0.12</td>
<td>0.11</td>
<td>0.11</td>
<td>-0.002</td>
</tr>
<tr>
<td>All S and L, no change in ave.</td>
<td>0.1 mm</td>
<td>-0.03</td>
<td>0</td>
<td>-0.03</td>
<td>-0.03</td>
<td>-0.03</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>All S and L, no change in ave.</td>
<td>10 mm</td>
<td>-2.81</td>
<td>0</td>
<td>-2.94</td>
<td>-2.81</td>
<td>-3.8</td>
<td>-3.5</td>
<td>-3.6</td>
</tr>
<tr>
<td>Detector non-linearity</td>
<td>2%</td>
<td>-1.1</td>
<td>0</td>
<td>-1.2</td>
<td>-1.1</td>
<td>-1.2</td>
<td>-1.5</td>
<td>-1.5</td>
</tr>
<tr>
<td>Wavelength error at 750-850nm</td>
<td>1 mm</td>
<td>Na</td>
<td>Na</td>
<td>Na</td>
<td>3.1</td>
<td>-0.9</td>
<td>0.19</td>
<td>-1.09</td>
</tr>
<tr>
<td>Wavelength error at 830-850nm</td>
<td>1 mm</td>
<td>Na</td>
<td>Na</td>
<td>Na</td>
<td>-0.7</td>
<td>0.73</td>
<td>0.31</td>
<td>0.42</td>
</tr>
<tr>
<td>Water concentration</td>
<td>10%</td>
<td>Na</td>
<td>Na</td>
<td>Na</td>
<td>Na</td>
<td>3.1</td>
<td>3.8</td>
<td>3.6</td>
</tr>
</tbody>
</table>

Table 2. Some error sources for absolute probes. Assuming source detector distances of 1.75 and 2.75-cm, and optical properties of $\mu_a=0.15-\text{cm}^{-1}$ and $\mu'_a=5-\text{cm}^{-1}$ at 750-mm, and $\mu_a=0.165-\text{cm}^{-1}$ and $\mu'_a=4.7-\text{cm}^{-1}$ at 830-mm.

<table>
<thead>
<tr>
<th>&quot;Correct Values&quot;</th>
<th>$S_{ab}$</th>
<th>$S_{br}$</th>
<th>$\mu_a$</th>
<th>$\mu'_a$</th>
<th>$H_b$</th>
<th>$H_bO$</th>
<th>Total $H_b$</th>
<th>% $S_{ab}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Error type</td>
<td>Error</td>
<td>% Error</td>
<td>Error</td>
<td>% Error</td>
<td>% Error</td>
<td>% Error</td>
<td>% Error</td>
<td>% Error</td>
</tr>
<tr>
<td>One S distance at 750nm</td>
<td>1 mm</td>
<td>5.7</td>
<td>4.2</td>
<td>1.7</td>
<td>9.7</td>
<td>6</td>
<td>-1.8</td>
<td>0.39</td>
</tr>
<tr>
<td>One S distance at 750nm</td>
<td>0.1 mm</td>
<td>0.57</td>
<td>0.42</td>
<td>0.17</td>
<td>1</td>
<td>0.58</td>
<td>-0.18</td>
<td>0.04</td>
</tr>
<tr>
<td>One S at 750 and 830nm</td>
<td>0.1 mm</td>
<td>1.2</td>
<td>0.83</td>
<td>0.33</td>
<td>2</td>
<td>1.2</td>
<td>-0.36</td>
<td>0.08</td>
</tr>
<tr>
<td>All S distances</td>
<td>0.1 mm</td>
<td>1.2</td>
<td>0.83</td>
<td>0.33</td>
<td>2</td>
<td>1.2</td>
<td>-0.36</td>
<td>0.08</td>
</tr>
<tr>
<td>All S and L distances</td>
<td>0.1 mm</td>
<td>0.09</td>
<td>0</td>
<td>0.09</td>
<td>0.12</td>
<td>0.11</td>
<td>0.11</td>
<td>-0.002</td>
</tr>
<tr>
<td>All S and L, no change in ave.</td>
<td>0.1 mm</td>
<td>-0.03</td>
<td>0</td>
<td>-0.03</td>
<td>-0.03</td>
<td>-0.03</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>All S and L, no change in ave.</td>
<td>10 mm</td>
<td>-2.81</td>
<td>0</td>
<td>-2.94</td>
<td>-2.81</td>
<td>-3.8</td>
<td>-3.5</td>
<td>-3.6</td>
</tr>
<tr>
<td>Detector non-linearity</td>
<td>2%</td>
<td>-1.1</td>
<td>0</td>
<td>-1.2</td>
<td>-1.1</td>
<td>-1.2</td>
<td>-1.5</td>
<td>-1.5</td>
</tr>
<tr>
<td>Wavelength error at 750-850nm</td>
<td>1 mm</td>
<td>Na</td>
<td>Na</td>
<td>Na</td>
<td>3.1</td>
<td>-0.9</td>
<td>0.19</td>
<td>-1.09</td>
</tr>
<tr>
<td>Wavelength error at 830-850nm</td>
<td>1 mm</td>
<td>Na</td>
<td>Na</td>
<td>Na</td>
<td>-0.7</td>
<td>0.73</td>
<td>0.31</td>
<td>0.42</td>
</tr>
<tr>
<td>Water concentration</td>
<td>10%</td>
<td>Na</td>
<td>Na</td>
<td>Na</td>
<td>Na</td>
<td>3.1</td>
<td>3.8</td>
<td>3.6</td>
</tr>
</tbody>
</table>

Table 3. Some error sources for standard multi-distance probes. Assuming source detector distances of 3, 3.4, 3.8, and 4.2-cm and optical properties of $\mu_a=0.10-\text{cm}^{-1}$ and $\mu'_a=10-\text{cm}^{-1}$ at 750-mm, and $\mu_a=0.11-\text{cm}^{-1}$ and $\mu'_a=9.5-\text{cm}^{-1}$ at 830-mm.

<table>
<thead>
<tr>
<th>&quot;Correct Values&quot;</th>
<th>$S_{ab}$</th>
<th>$S_{br}$</th>
<th>$\mu_a$</th>
<th>$\mu'_a$</th>
<th>$H_b$</th>
<th>$H_bO$</th>
<th>Total $H_b$</th>
<th>% $S_{ab}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Error type</td>
<td>Error</td>
<td>% Error</td>
<td>Error</td>
<td>% Error</td>
<td>% Error</td>
<td>% Error</td>
<td>% Error</td>
<td>% Error</td>
</tr>
<tr>
<td>Position* of 750nm source</td>
<td>1 mm</td>
<td>-0.029</td>
<td>0.77</td>
<td>-0.82</td>
<td>0.75</td>
<td>-2.1</td>
<td>1.4</td>
<td>-0.22</td>
</tr>
<tr>
<td>Position* of 750nm source</td>
<td>0.1 mm</td>
<td>-0.029</td>
<td>0.77</td>
<td>-0.82</td>
<td>0.75</td>
<td>-2.1</td>
<td>1.4</td>
<td>-0.22</td>
</tr>
<tr>
<td>Position* of source at both λs</td>
<td>1 mm</td>
<td>-0.029</td>
<td>0.77</td>
<td>-0.82</td>
<td>0.75</td>
<td>-2.1</td>
<td>1.4</td>
<td>-0.22</td>
</tr>
<tr>
<td>Detector non-linearity</td>
<td>2%</td>
<td>0.0029</td>
<td>0</td>
<td>0.003</td>
<td>0.0029</td>
<td>0.0026</td>
<td>0.0049</td>
<td>0.0043</td>
</tr>
<tr>
<td>Coupling efficiency change*</td>
<td>2%</td>
<td>0.0029</td>
<td>0</td>
<td>0.003</td>
<td>0.0029</td>
<td>0.0026</td>
<td>0.0049</td>
<td>0.0043</td>
</tr>
<tr>
<td>Phase shift after calibration*</td>
<td>0.2 deg.</td>
<td>0</td>
<td>-1.0</td>
<td>1.1</td>
<td>-1.1</td>
<td>2.7</td>
<td>-1.9</td>
<td>0.29</td>
</tr>
</tbody>
</table>

*Involving the closest source
5 CONCLUSIONS

These results verify that the new type of probe described here (the absolute probe) can be used to make "calibration free" measurements of optical properties in homogeneous highly scattering media, and that good accuracy can be achieved despite the possibility of deviations from the assertions listed in the introduction and section 2.3. The results also demonstrate that these probes can be calibrated to increase confidence and accuracy while retaining improved long-term stability. More work is required to fully examine the accuracy of the method. For example, inhomogeneities in the sample have not been considered. But these probes show great potential for both fast non-invasive tissue hemoglobin measurements and long-term monitoring.

The authors would like to thank the National Institutes of Health for supporting this research. NIH grant # R44 RR08827-03.

REFERENCES


