Temperature modulation of the visible and near infrared absorption and scattering coefficients of human skin

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1 Introduction

Localized reflectance measurements have been used to determine the absorption and reduced scattering coefficients ($\mu_a$ and $\mu'_s$) of tissues and turbid media. Light intensity as a function of distance, diffusion theory approximation, or statistical Monte Carlo simulations are usually utilized to estimate $\mu_a$ and $\mu'_s$, the effective attenuation coefficient ($\mu_{eff}$), and light penetration depth ($\delta$) of the tissue or the turbid medium.\(^1\)\(^-\)\(^4\)

We constructed a localized reflectance optical probe that had the ability to control and vary temperature within a small depth in the tissue. The probe had source-detector distances that ranged between 0.4 and 1.9 mm, which are close to those used by Bevilacqua et al.\(^7\) We report the design and performance of this probe and results of the effect of temperature changes on $\mu_a$ and $\mu'_s$ of intact human skin between 590 and 980 nm. Several aspects of the apparatus used in this study were reported.\(^5\)\(^-\)\(^9\)

Effects of temperature on the optical properties of tissue, thermal effects of laser excitation, and photoocoagulation have been of interest in the past few years.\(^10\)\(^-\)\(^19\) Transmittance increased and diffuse reflectance signal decreased when the surface temperature of ex-vivo human forearm skin or aortic specimens were elevated to 70 °C by laser excitation. The changes were primarily reversible until photoocoagulation occurred. Temperature effect on the birefringence of ex-vivo rat-tail tendon was reported and attributed to change in collagen conformation.\(^14\)\(^-\)\(^16\)

Abstract. We determine temperature effect on the absorption and reduced scattering coefficients ($\mu_a$ and $\mu'_s$) of human forearm skin. Optical and thermal simulation data suggest that $\mu_a$ and $\mu'_s$ are determined within a temperature-controlled depth of $\approx 2$ mm. Cutaneous $\mu'_s$ change linearly with temperature. Change in $\mu_a$ was complex and irreversible above body normal temperatures. Light penetration depth ($\delta$) in skin increased on cooling, with considerable person-to-person variations. We attribute the effect of temperature on $\mu'_s$ to change in refractive index mismatch, and its effect on $\mu_a$ to perfusion changes. The reversible temperature effect on $\mu'_s$ was maintained during more than 90 min. contact between skin and the measuring probe, where temperature was modulated between 38 and 22 °C for multiple cycles. While temperature modulated $\mu'_s$ instantaneously and reversibly, $\mu_a$ exhibited slower response time and consistent drift. There was a statistically significant upward drift in $\mu_a$ and a mostly downward drift in $\mu'_s$ over the contact period. The drift in temperature-induced fractional change in $\mu'_s$ was less statistically significant than the drift in $\mu_a$. $\Delta \mu'_s$ values determined under temperature modulation conditions may have less nonspecific drift than $\mu'_s$, which may have significance for noninvasive determination of analytes in human tissue.

Keywords: tissue spectroscopy; temperature modulation; thermal effects; noninvasive measurements.

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A reversible temperature effect on near-infrared (600 to 1050 nm) $\mu'_s$ of ex-vivo human dermis and subdermis, and linear variation of $\mu'_s$ with temperature were reported.\(^18\) A nonreproducible temperature effect and random distribution of $\mu_a$ values was observed when tissue temperature was changed between 25 and 40 °C.\(^18\)

We discuss the effect of heating and cooling intact human skin on the values of cutaneous $\mu_a$ and $\mu'_s$. $\mu_a$ and $\mu'_s$ in the wavelength range 550 to 980 nm increased on heating and decreased on cooling. The linear relationship between $\mu'_s$ and temperature was confirmed. As both $\mu_a$ and $\mu'_s$ of skin decreased by lowering the temperature, the calculated light penetration depth ($\delta$) increased as temperature was lowered, indicating cooling-induced transparency of human skin. We studied the optical effects of prolonged probe-skin interaction and the resultant signal drift. We performed single-person and interperson measurements. We determined the fractional change in $\mu_a$ and $\mu'_s$, as temperature was modulated over 90-min contact time between skin and the optical probe. The observations are interpreted based on the dependence of blood perfusion and refractive index mismatch on temperature.

2 Instrumentation

The apparatus used in this study comprises a light source module, a tissue interface module, and a signal detection
module as shown schematically in Fig. 1. These three modules were interconnected through a branched optical fiber bundle. Light from a 5-W incandescent lamp (Gilway Technical Lamps, Woburn, Massachusetts) powered by a constant voltage source was modulated at 150 Hz by an optical chopper coupled to a lock-in amplifier (Stanford Research Systems, Sunnyvale, California). The light beam was then passed through one of six 10-nm bandpass filters assembled in a filter wheel. A portion of the filtered light was diverted by a beam splitter and focused onto a silicon photodiode to generate a reference signal, which was used to correct for long-term drift in lamp intensity. The rest of the filtered light beam was focused onto one end of the illuminating fiber housed at the source tip of the fiber bundle. Several filters were used throughout the course of the study, including filters with central wavelengths at 550, 560, 590, 660, 750, 800, 900, 950, and 980 nm. During any one experiment one to six filters were used.

The probe tip contained one light introduction fiber and six light collection fibers that were mounted in a 3-mm outer-diameter stainless steel tube, which was situated at the center of a 2-cm-diam, 6.25-mm-thick, aluminum disk shown in Fig. 1. A thermoelectric element was mounted on the backside of the disk and connected to a temperature controller (both from Marlow Industries, Dallas, Texas). A thermocouple (Omega Engineering, Stanford, Connecticut) embedded in the disk was used to deliver a feedback signal to the temperature controller.

The optical probe tip, with source fiber and the six light collection fibers (400-µm low-OH silica, Fiberguide Industries, Stirling, New Jersey), and the aluminum disk were components of the temperature-controlled probe or the tissue interface module. The other ends of the light collection fibers were arranged in a circular pattern in the detector tip, in the detection module. The output of each of the six fibers was sequentially focused on a silicon photodiode using a stepper-motor-controlled rotating shutter. The filter wheel was rotated to select a filter. A series of optical measurements were performed while selecting a different source-detector distance by rotating the shutter. Light intensity versus source-detector distance was determined at the selected wavelength, and the process was repeated at the other wavelengths.

3 Calibration and Data Reduction

3.1 Measurement of the Optical Fibers Throughput

Light throughput of each fiber, $T_{f_j}$, was determined by illuminating the common tip of the fiber bundle and measuring the intensity of light transmitted of each fiber. The 5-W source lamp and room light were turned off. A Lieca spotting tungsten lamp was mounted on a lamp stand 2-m away from the probe head, and was aligned to uniformly illuminate the probe and surrounding aluminum disk. Light throughput for fibers 1 through 6 was determined by switching the shutter position through a LabView® routine. The procedure was repeated three times with the spotting tungsten lamp being slightly moved every time. At each spotting lamp position, signal for every fiber was divided by signal for fiber 1. Then such ratios were averaged for three lamp positions to give a throughput factor for each fiber. Typical source-detector distances were: $r_1 = 0.44$, $r_2 = 0.78$, $r_3 = 0.92$, $r_4 = 1.22$, $r_5 = 1.40$, and $r_6 = 1.84$ mm. Typical ratios of light throughput, $T_{f_j}$, for different fibers were: 1.00, 0.948, 0.961, 0.969, 0.993, and 0.943, respectively, with ±2% variation.

3.2 Light Source Calibration and Data Reduction

In addition to the use of a reference detector, we performed direct measurement of the illuminating intensity at all wavelengths before or after each reflectance measurement and designated this measurement as a “control run.” A photodiode mounted in a fixture was placed in touch with the probe tip of the fiber bundle and connected to a wide dynamic range amplifier (Melles Griot, Boulder, Colorado). The lamp intensity at each wavelength defined as $S(c)$ was measured at the exit...
of the source fiber as the signal from the amplifier at wavelength $\lambda_i$. The reference detector signal was $R(c)_i$ at the same $\lambda_i$. This setup was used to detect long-term change in the output of the tungsten lamp. The lamp was replaced when its output $S(c)_i$ decreased by more than 20%. The values of $S(c)_i$ and $R(c)_i$ were used in the data reduction step.

Two data files were generated for each measurement, one for a sample run (sample) and the other for a lamp intensity (control) run. Both files had two columns of data: the signal detector intensity and the reference detector intensity. The output of each measurement was: $S(s)_ij$, signal for filter $i$ and aperture $j$ for the sample, $R(s)_ij$, the reference signal for filter $i$ and aperture $j$ combination, $S(c)_i$, signal for filter $i$ control run, $R(c)_i$, the reference signal for filter $i$ control run, and $Tf_j$, the throughput factor for fiber $j$, determined in Sec. 3.1. The corrected localized reflectance of the sample for wavelength (filter) $i$ and source-detector distance (aperture) $j$ is:

$$R(r_i, \lambda_j) = \frac{\{S(s)_{ij} / R(s)_{ij}\} \{R(c)_{i} / S(c)_{i}\}}{Tf_j}.$$ (1)

Dark current measurements were performed for both the sample and the reference detectors before each run and were found to be too small to correct.

### 3.3 Optical Calibration Using Tissue-Simulating Phantoms

Reflectance data for several solid and liquid phantoms were collected for all sampling distances and wavelengths. Scattering liquid phantoms had different concentrations of a lipid emulsion (10% Intralipid suspension), Pharmacia Incorporated, Clayton, North Carolina). Liquid tissue-simulating phantoms had similar composition to those used in previous studies. Absorbing tissue-simulating phantoms contained 0.0 to 5.0 g/DL hemoglobin and 0.65% lipid solution. Tissue-simulating solution phantoms were placed in a 50-mL centrifuge vial covered with a polyethylene film (15-µm-thick commercial Clingwrap brand, Dow Chemical Company, Midland, Michigan) and secured around the mouth of the vial using a rubber band. The vial was inverted on top of the optical probe and reflectance measurements were performed at each source-detector distance. Opal glass (Edmund Scientific, Barrington, New Jersey) plastic rods loaded with titanium dioxide and a pigment to generate white, and different levels of red colors of different absorption and scattering properties, were used as solid phantoms. The liquid phantoms were used for calibration and solid phantoms were repeatedly run to check measurement reproducibility.

### 4 Simulation Studies

#### 4.1 Monte Carlo Simulation of Photon Trajectories in Dermal Tissue

We performed Monte Carlo simulation studies using a program provided by Jacques. The calculations are similar to what we reported in previous communications. Simulations were performed according to probe geometry and the following inputs. A 400-µm pencil beam was injected in the tissue model, and the number of photons injected was 200,000 per run. Light was assumed to propagate from fiber ($n = 1.5$) into tissue ($n = 1.4$). Thickness of the tissue layer was set from 5 to 25 mm. Light emitted at each of the 400-µm light collection fibers was calculated for a matrix of a number of $\mu_a$ and $\mu_s$ combinations. The resultant $\log R_i$ versus $\log(R_i/R_0)$ was plotted as a grid for each wavelength $\lambda_j$. Here, $R_i$ is a simplified expression of $R(r_i, \lambda_j)$ of Eq. (1), which represents the corrected reflectance at a distance $r_i$ and $R_0$ represents the corrected reflectance at a distance $r_f$ from the source. The constant $\mu_a$ and $\mu_s$ points were connected to form grid lines in the $\log R_i$ versus $\log(R_i/R_0)$ space. Reflectance signals at each source-detector distance were determined for a set of lipid suspensions of different concentrations: hemoglobin solution in lipid suspension, opal glass, and plastic rods polymerized to incorporate different levels of the scattering and absorbing pigment. Using the optical probe geometry described in Sec. 2, the Monte Carlo grid generated by the calculations and by graphing $\log R_i$ versus $\log(R_i/R_0)$ is shown in Fig. 2. $R_1$ was the diffuse reflectance signal measured at fiber 1 and $R_6$ was the diffuse reflectance signal measured at fiber 6.

The experimental values were overlapped on the Monte-Carlo-generated grid, and $\mu_a$ and $\mu_s$ of the reference solutions were determined by the use of tables generated from the grid as shown in Fig. 2, where the lines are calculated grid lines and the numbers are the $\mu_a$ and $\mu_s$ values. The symbols at the bottom of the grid are the data points for hemoglobin in 0.65% lipid suspension measured at six wavelengths. The circles and diamonds on the right-hand corner are for lipid suspensions at six wavelengths. The (x) symbols in the upper right-hand corner of the grid are for solid plastic phantoms containing titanium dioxide. The (x) symbols on the left center of the grid are for opal glass. The squares are for an orange/red plastic phantom that contained titanium dioxide and a red pigment. Triangles are data points for human skin.

![Figure 2](https://www.spiedigitallibrary.org/journals/Journal-of-Biomedical-Optics/dApril-2003/Vol.8-No.2/193)
and a red pigment. Triangles are data points for human skin.

Determination of $\mu_a$ and $\mu'_s$ values from spatially resolved (localized) reflectance measurement using a fiber optics probe with a small source detector distance was previously discussed.\textsuperscript{5,6,9}

4.2 Modeling of Thermal Response of Human Skin

A model was developed to simulate the temperature response of human skin on contact with the probe disk. The model was constructed in finite-element formalism using the FIDAP software package (Fluid Dynamics Analysis Program, Fluent Incorporated, Lebanon, New Hampshire). The generalized heat diffusion equations were solved for a cylindrical geometry comprising the 2-cm-diam aluminum disk and a 6-cm-diam section of human tissue that was assumed to be 3 cm deep. The top surface of the disk was maintained at a set temperature value between 44 and 20 °C. The initial skin surface temperature was calculated to be approximately 32.2 °C by the model. Initial tissue temperature was calculated to be 32.7 and 33.0 °C at a depth of 1 and 2 mm below the skin surface, respectively. The initial temperatures corresponded to steady-state conditions assumed for a resting human forearm, exposed to a room environment at 22 °C.

4.2.1 Data input and assumptions for the thermal model of human skin

The equations governing the thermal model required input of thermal properties of the disk material and the adjoining tissue, as well as thermal boundary conditions surrounding the system geometry. The 3-cm-deep tissue section was divided into three discrete layers, each having a unique set of thermal properties. The top, or "skin" layer, was assumed to be 2 mm thick and comprised mainly of dermal tissue. The middle layer was assumed to be 3 mm thick and comprised mainly of adipose tissue. The bottom 25-mm layer was assumed to consist mainly of muscle tissue having a fixed body core temperature of 37 °C along its lower boundary. Actual thickness of each layer may vary from person to person. Thin sublayers such as the epidermis (approximately 100 $\mu$m thick) were not individually defined within the main layers. This was primarily because we considered that their impact on the overall tissue temperature calculations would have been negligible, and partly because thermal property data for these subdermal layers are not available in the literature. The physical relationship between the tissue boundaries used, the thermal calculation, and the geometry of the optical system is shown in Fig. 3.

Inputs of the thermal model are listed in Table 1. Literature data for human tissues were used when available. Specific heat values were not found for human skin or adipose tissue. Data from similar animal tissues were substituted in the calculation as an approximation. Metabolic heat values also were not found for skin or adipose tissue. We assumed that the metabolic heat values to have relatively small contribution of heat produced in the skin.

Heat associated with local blood perfusion was incorporated in the model by assuming the blood entered the tissue at 37 °C and exited at the calculated tissue temperature level. The corresponding quantity of heat transferred to the tissue by the blood was expressed as:

$$Q = WC_p[T_{core} - T_{tissue}] = WC_p[37 - T_{tissue}], \quad (2)$$

where $W$ is the local blood perfusion rate from Table 1, $C_p$ is the specific heat value of blood (3840 J/kg °C) and $T_{tissue}$ is the local tissue temperature. Perfusion units in Table 1 incorporate a mass flow (kg/s) of blood, which was obtained by multiplying the volumetric units given in published data by the blood mass density (1060 kg/m$^3$).

Thermal calculations of tissue temperature demonstrate that the skin surface rapidly reaches the temperature of the highly conductive aluminum disk surrounding the optical probe. The rapid change creates large temperature gradients in the dermal layer, causing heat conduction to locally dominate other sources of energy such as that resulting from blood perfusion and cellular metabolism. Thermal conductivity was, therefore, the dominant property governing the skin temperature distribution near the probe. Thermal conductivity values within hydrated tissues (skin, muscle, etc.) generally vary by small amounts, as main component of these tissues is water. Therefore, only minor inaccuracies are expected in the model by assuming the sublayers had conductivity values identical to the main layers. Values for the tissue-specific heat were less certain but were not expected to compromise accuracy of the thermal modeling results after the initial 30 s of contact with the probe. Within the first 30 s, the rate of tissue temperature change is greatest and therefore the specific heat values can substantially impact the relationship between temperature and time. Thin sections (sublayers) of tissue, however, have insignificant mass and subsequently any differences between actual and assumed specific heat values were assumed to have negligible effect on the calculated temperature values.

A complete set of thermal boundary conditions was applied to the model cylindrical geometry. The lower boundary of the muscle layer (3 cm below the skin surface) was held at 37 °C to represent body core temperature. The radial surfaces of the tissue cylinder (6 cm diam) and the aluminum disk (2 cm diam) were assumed to be adiabatic. The top surface of the disk was held at one of the specified control temperatures. The entire disk material was considered to have the same temperature prior to contact with the skin. A heat transfer coefficient of 20 W/m$^2$ °C was applied to the exposed skin.
surface to account for gray-body radiation exchange plus a minor amount of ambient air convection (on the order 1 m/s) at an environmental temperature of 22 °C. The effects on heat transfer caused by sweat effusion and evaporation were not accounted for in the model.

### 4.2.2 Output of the thermal model of human skin

Using the boundary conditions and the thermal properties in Table 1, the model calculated an initial tissue temperature distribution with 32.2 °C on the skin surface. Initial tissue temperatures were expected to represent a resting human forearm that is subjected to 22 °C noncirculating room air. On contact with the disk, the model simulated skin temperature versus time (starting with the initial tissue temperature distribution), as plotted in Figs. 4 – 6.

During the period following contact between the disk and the skin surface, the model calculated the rate at which heat was transferred by conduction between the aluminum disk and the skin. The heat conduction caused a continuous temperature change in the tissue during a 240-s contact period, during which the disk temperature remained essentially uniform and constant. At 240 s from the onset of interaction between the aluminum disk and the skin, the tissue approached thermal equilibrium with the disk and surrounding environment, making longer contact periods unnecessary for the model simulation. Figures 4 – 6 plot the calculated temperature history of the skin tissue corresponding to each case of disk temperature. Results are plotted for three tissue depths along the disk centerline location and at a radial distance of 5 mm from the disk centerline. The centerline and the 5-mm radial locations differed from each other by a maximum of 0.2 °C during the contact period.

Figure 4 illustrates the results when the temperature of the disk was set at 34 °C, which is close to the temperature of the skin surface (32 to 33 °C). The dermis temperature at 1 and 2 mm reached equilibrium quickly and remained constant at a value close to the surface temperature. The equilibrium temperatures were calculated to be very close at the two depths.

Figure 5 illustrates the simulation results when the temperature of the disk was set at 38 °C. The skin surface temperature was at the disk temperature (38 °C). The dermis temperature, initially at ~33 °C at both 1 and 2 mm depth “warmed up” to the surface temperature, approaching 38 °C and equilibrating after approximately 120 s. The calculated equilibrium temperature at a depth of 1 mm differed from its value calculated at a depth of 2 mm in the tissue. At a disk

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<table>
<thead>
<tr>
<th>Model component [thickness]</th>
<th>Thermal conductivity (W/m °C)</th>
<th>Mass density (Kg/m³)</th>
<th>Specific heat (J/kg °C)</th>
<th>Metabolic heat (W/m³)</th>
<th>Blood perfusion (Kg/s-m³)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Disk-Aluminum [6 mm]</td>
<td>170(1)</td>
<td>2780(1)</td>
<td>880(1)</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>Skin layer [2 mm]</td>
<td>0.39(2)</td>
<td>1116(3)</td>
<td>3200(4)</td>
<td>0(5)</td>
<td>2.4(6)</td>
</tr>
<tr>
<td>Adipose layer [3 mm]</td>
<td>0.25(2)</td>
<td>916(3)</td>
<td>2500(7)</td>
<td>0(5)</td>
<td>0.45(6)</td>
</tr>
<tr>
<td>Muscle layer [25 mm]</td>
<td>0.50(2)</td>
<td>1040(3)</td>
<td>3720(8)</td>
<td>673(9)</td>
<td>0.70(6)</td>
</tr>
</tbody>
</table>

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![Fig. 4](https://www.spiedigitallibrary.org/journals/Journal-of-Biomedical-Optics/images/Fig4.png)

**Fig. 4** Model results of the dependence of cutaneous temperature on contact time with a disk at a constant temperature of 38 °C. The solid lines are for the depth along the centerline, the dotted lines are for the depth along 5 mm from the center.
temperature much lower than the skin temperature, i.e., 20 °C, the dermal layer “cooled down” to approach the skin surface temperature as shown in Fig. 6. It reached as asymptotic value after approximately 120 s from the interaction between the probe and the skin, with a larger constant difference between the asymptotic value and the skin surface temperature. In all three plots in Figs. 4, 5, and 6, the solid lines are for the depth along the centerline, the dotted lines are for the depth along 5 mm from the center. There was no significant difference between the calculated temperature values along the centerline of the disk and at 5 mm away from the center of the disk, which covered twice the area under the optical fibers where the optical measurements are performed. The small difference in calculated temperature at the centerline and at 5 mm away from center can be explained by the ratio of the diameter of the temperature-controlled disk to this 5-mm distance. The diameter of the disk was 20 mm and may be able to overcome skin temperature differences and create temperature equilibrium conditions up to 5 mm away from the center of the disk. Reducing the diameter of the temperature-controlled disk to 10 mm had no effect on the calculated temperatures. A disk diameter of 8 mm showed a difference between the temperatures at the two points.

The results of the thermal simulations over multiple temperatures are summarized in Figs. 7(a) and 7(b) for 1 and 2 mm depth in the tissue. Thermal simulations show that it is possible to control tissue temperature at these depths in the tissue. However, there is an offset between the probe and tissue temperatures. The magnitude of the offset depends on the initial probe temperature. The offset between probe and tissue temperatures decreased as the contact time between the probe and skin increased. At a probe temperature of 20 °C, tissue temperature was controlled at a 1 mm depth with an offset of +3 °C after 60 s of contact time with the disk. The offset approached +2 °C after 240 s of contact. At 2 mm depth the offset was +5.3 °C at 60 s and decreased to +3.8 °C at 240 s. At 44 °C tissue temperature was controlled at a 1 mm depth with an offset of −1.9 °C after 60 s of contact time with the disk. At 40 °C, the offset approached −1.1 °C after 240 s of contact. At 2 mm depth, the offset was −4.5 °C at 60 s and decreased to −2.1 °C after 240 s of contact with the probe. Thermal modeling suggests that dermal temperature can be controlled within a depth of 2 mm into the skin and at a radial distance up to 5 mm from the center of the disk (optical probe). Equilibrium between the temperature controlled probe and the skin is reached after 60 to 240 s depending on the difference in their starting temperatures.

5 Experimental Results and Discussion

5.1 Experiments on Human Subjects

We performed four sets of experiments on human subjects using test protocols and informed consent forms approved by the Institution Review Board. All subjects were healthy volunteers from the research laboratories at Abbott Park, Illinois.

In the first set of experiments, localized reflectance of the skin of 29 volunteers was measured at six wavelengths ranging between 550 and 900 nm, and at a constant probe temperature of 34 °C, which is close to the average skin temperature. The data were used to calculate $\mu_s$ and $\mu'_s$ for a limited population. The data are presented and discussed in Sec. 5.2.

In the second experiment, localized reflectance of the skin of five volunteers was measured at 590 nm while the probe temperature was varied between 22 and 41 °C. The limits of
this temperature range were selected to vary between room temperature and a maximum temperature tolerated by all volunteers. The data were used to determine the effect of person-to-person variability on the temperature dependence of $\mu_a$, $\mu'_s$, and $\delta$ at three wavelengths. These data are discussed in Sec. 5.4.

In the fourth experiment, we investigated the effect of long-term interaction between the probe and the skin on the values of $\mu_a$ and $\mu'_s$. Localized reflectance of the skin of three volunteers was continuously measured for 90 min, while the probe temperature was repetitively stepped between 22 and 38 °C for 15 temperature cycles. These data are presented and discussed in Sec. 5.5.

5.2 Determination of $\mu_a$ and $\mu'_s$ of Human Skin at a Constant Temperature

In this experiment, reflectance measurements were performed on the dorsal forearms of 29 subjects (including four with dark skin). $\mu'_s$ and $\mu_a$ were calculated from the reflectance data and the calibrated grid in $\log_{10} R_d/R_t$ versus $\log_{10} R_t$ shown in Fig. 2. The average values of $\mu'_s$ and $\mu_a$ for all individuals at each wavelength were used to calculate photon-reduced mean free path [MFP$^r = 1/(\mu_a + \mu'_s)$] and light penetration depth ($\delta = 1/\mu_{\text{eff}} = 1/(\mu_a + \mu'_s))$.

The calculated MFP$^r$ (mm) for the 29 subjects at 34 °C was 0.62 mm at 550 nm, 0.72 mm at 590 nm, 0.88 mm at 650 nm, 1.03 mm at 750 nm, 1.1 mm at 800 nm, and 1.23 mm at 900 nm. Thus MFP$^r$ has the same magnitude as the source-detector distances, indicating a limited number of multiple scattering events per photon. The value of $\delta$ was 0.72 mm at 550 nm, 0.92 mm at 590 nm, 1.42 mm at 650 nm, 1.67 mm at 750 nm, 1.92 mm at 800 nm, and 2.04 mm at 900 nm. Thus $\delta$ is within 2 mm, where the thermal model predicts temperature control of the tissue and also where cutaneous microcirculation is reported to occur.

5.3 Temperature Dependence of $\mu_a$ and $\mu'_s$ of Individual’s Skin

In the second experiment, localized reflectance of the skin of five light-skin subjects was measured at 590 nm with the dorsal side of the forearm in constant contact with the temperature-controlled probe. Temperature was switched back and forth among three values between 25 and 41 °C. Plots of the regression lines for $\mu'_s$ versus probe temperature are shown in Fig. 8.

Plots of $\mu'_s$ versus temperature show no hysteresis in its thermal response, and the data fit straight lines. Data are labeled by subjects A through F. Subjects A, B, D, and F were Caucasian, Mediterranean, or Oriental, ranging in age between 35 and 58 years. Plots A and C are for the same subjects tested at two different times. The fitted slope ($\delta \mu'_s/\delta T$) for individual subjects was 0.053 ± 0.0094 cm$^{-1}/\degree$C. The intercept and the slope of the regression lines of $\mu'_s$ versus probe temperature plots varied from subject to subject. There is also difference in intercept for different measurements on the same subject, which is probably due to differences in repositioning the probe on skin. The dependence $\mu'_s$ on temperature for the intact human skin is similar to that reported for ex-vivo skin. The behavior of $\mu_s$ of intact human skin as a function of temperature is different from that of the $\mu'_s$. Figure 9 shows a plot of $\mu_a$ at different temperatures, deter-
mined at 590 nm and using the same temperature controlling steps used to generate the data in Fig. 8. Measurements are designated A through F in both Figs. 8 and 9. Data used for plots A and C were from measurements performed on the same subject. Plots A and C were from measurements performed on the designated A through F in both Figs. 8 and 9. Data used for the same subjects in Fig. 7. Data points for subjects B, C, and D are connected to show the direction of change in the temperature-controlled probe used in this study covers a wide area of the vasculature that is greater than the area probed by the fibers, which are 400 μm in diameter and any of them can be located on the top of these identified heterogeneous vascular structures or a void area in-between. Thus, while each of the fibers collected a localized signal, blood perfusion is affected by the response of the neighboring vascular structures to temperature changes.

We now attempt to interpret the results of the second experiment on temperature dependence of μ′s of the skin of the five subjects shown in Fig. 8. Scattering of a biological tissue containing spherical scattering centers is approximated by Graaff et al. and simplified by Chance et al. as

\[
\mu'_s = 3.28 \pi a^2 \rho (2 \pi a n_{\text{medium}}/\lambda)^{0.37} (m - 1)^{2.09} ,
\]

(3)

where \(a\) is the average radius of scattering particles, \(\rho\) is the number concentration of particles, \(n_{\text{medium}}\) is the refractive index of the medium, \(\lambda\) is the wavelength of light, and \(m = n_{\text{scattered}}/n_m\) is the mismatch of the refractive index. Equation (3) can be expressed in terms of the volume fraction of the scatterer in the tissue as

\[
\mu'_s = 2.46(\varphi/\pi)(2 \pi a n_{\text{medium}}/\lambda)^{0.37} (m - 1)^{2.09} ,
\]

(4)

where \(\varphi = 1.33 \pi a^2 \rho\) is the volume fraction of scattering centers in tissue. It is possible to use Eq. (4) to attempt interpretation of the temperature dependence of \(\mu'_s\). Temperature can affect \(\mu'_s\) by changes in one of the terms \(a\), \(\rho\), or \(m\).

One possible interpretation of the temperature effect on \(\mu'_s\) is that scatter size \(a\) is changed, while the density \(\rho\) is unaffected. This interpretation is based on the assumption of re-

For the case of subject B, starting at 34 °C and raising the temperature to 41 °C, \(\mu'_a\) increases (as shown by the arrow) by \(\approx 33\%\). Subsequent cooling to 25 °C results in a smaller increase in \(\mu'_a\). Heating again to 34 °C and then to 41 °C resulted in a further increase in \(\mu'_a\). One more cooling step to 25 °C resulted in further increase in \(\mu'_a\). Returning back to the starting temperature of 34 °C resulted in a net increase in \(\mu'_a\). The same behavior can be seen in the connected data points of subject D. Temperature cycling for subject C was performed between 25 and 34 °C and shows similar behavior except for a much limited increase in \(\mu'_a\), and an actual decrease on first cooling from 34 to 25 °C. Still, the final value of \(\mu'_a\) on going back to 34 °C was higher than its starting value.

Dependence of \(\mu'_a\) data of intact human skin in Fig. 9 first shows the short-term irreversibility of \(\mu'_a\) values on heating to or above 38 °C. Thus \(\mu'_a\) generally increased on heating and decreased on cooling. Second, the values of consecutively determined \(\mu'_a\) zigzagged upward. There is a cumulative effect of multiple times of heating and cooling. The value of \(\mu'_a\) depends on the thermal history of the skin. The upward zigzagging drift of \(\mu'_a\) can be explained by the pooling of blood in the cutaneous capillaries as temperature was changed.

Laser Doppler flowmetry (LDF) measurements indicated enormous variation in the red blood cells flux from comparable sites in the same individual and even for the same site at different times. Correlation between LDF patterns and morphology of the underlying vasculature identified 1 mm² areas of vascular “territories” surrounded in part by relatively avascular areas. The temperature-controlled probe used in this study covers a wide area of the vasculature that is greater than the area probed by the fibers, which are 400 μm in diameter and any of them can be located on the top of these identified heterogeneous vascular structures or a void area in-between. Thus, while each of the fibers collected a localized signal, blood perfusion is affected by the response of the neighboring vascular structures to temperature changes.

We now attempt to interpret the results of the second experiment on temperature dependence of \(\mu'_a\) of the skin of the five subjects shown in Fig. 8. Scattering of a biological tissue containing spherical scattering centers is approximated by Graaff et al. and simplified by Chance et al. as:

\[
\mu'_s = 3.28 \pi a^2 \rho (2 \pi a n_{\text{medium}}/\lambda)^{0.37} (m - 1)^{2.09} ,
\]

(3)

where \(a\) is the average radius of scattering particles, \(\rho\) is the number concentration of particles, \(n_{\text{medium}}\) is the refractive index of the medium, \(\lambda\) is the wavelength of light, and \(m = n_{\text{scattered}}/n_m\) is the mismatch of the refractive index. Equation (3) can be expressed in terms of the volume fraction of the scatterer in the tissue as:

\[
\mu'_s = 2.46(\varphi/\pi)(2 \pi a n_{\text{medium}}/\lambda)^{0.37} (m - 1)^{2.09} ,
\]

(4)

where \(\varphi = 1.33 \pi a^2 \rho\) is the volume fraction of scattering centers in tissue. It is possible to use Eq. (4) to attempt interpretation of the temperature dependence of \(\mu'_s\). Temperature can affect \(\mu'_s\) by changes in one of the terms \(a\), \(\rho\), or \(m\).
versible swelling of collagen fibers as a result of temperature change, which was suggested for the case of birefringence changes in rat-tail tendon and ex-vivo human skin scattering changes with temperature. In this case a 3% change in \( a \) leads to a 7.5% increase in \( \mu_s' \), and a 10% increase in \( a \) leads to a 22.5% increase in \( \mu_s' \). However, this assumption of increasing scatterer size while the density remains the same only holds if scatterers are sparsely distributed and their volume fraction is low, which is unlikely the case of packed skin structure, thus rendering this interpretation implausible.

A second possible interpretation based on Eq. (4) is a change in the dimension of the scatterer \( a \) as tissue thermally expands, while the volume fraction of scatterers in tissue remains unaffected. In this case 3% increase in \( a \) leads to a 1.8% decrease in \( \mu_s' \), and 10% increase in \( a \) leads to a 5.8% decrease in \( \mu_s' \). Thus the increase in the size of scattering particles while the volume fraction stays the same leads to a decrease in \( \mu_s' \), which is contrary to the observed data. This interpretation is also based on the assumption of reversible swelling of collagen fibers as a result of temperature change, as was suggested for the rat-tail tendon and ex-vivo human skin.

A third possible interpretation is a change in \( \rho \) on heating or cooling, which leads to physically unattainable results, as heating will decrease \( \rho \) due to tissue expansion, and hence will lead to a decrease in \( \mu_s' \) as temperature is raised. As \( \mu_s' \) values increase with temperature, this interpretation is also implausible.

We offer an interpretation that was presented in a preliminary report, and is based on the effect of temperature on refractive index mismatch \( m \), and hence \( \mu_s' \). \( n_{\text{water}} \), determined at 589 nm decreases nonlinearly with increasing temperature. Since the interstitial fluid (ISF) contents are more than 90% water, it can be assumed that similar to water, \( n_{\text{ISF}} \) decreases by 0.25% when the temperature rises from 20 to 45 °C. We simulated the change \( \Delta \mu_s' \% \) as a function of temperature under different refractive index mismatch \( m = n_{\text{scatterer}}/n_{\text{ISF}} \) conditions. We assumed that the protein content of ISF is equivalent to that of 3.5% serum, and thus \( n_{\text{ISF}} = 1.3351 \) at 20 °C. We applied a 0.25% decrease in \( n_{\text{ISF}} \) between 20 and 45 °C. We assumed that the value of \( n_{\text{scatterer}} \) was independent of temperature between 20 and 45 °C. We used two limits for \( n_{\text{scatterer}} \), 1.37 and 1.4, which are close to the reported average value of refractive index of the ex-vivo dermis of \( n = 1.4 \), and the value of \( n = 1.43 \pm 0.2 \) reported for the upper dermis human arm skin. A plot of the calculated \( \Delta \mu_s' \% \) based on the aforementioned assumptions is shown in Fig. 10. The calculated values of \( \Delta \mu_s' \) increase as temperature increases. The data points fall within the lines determined by the boundary values of \( m \), indicating that the assumptions used in this calculation closely describe temperature dependence of \( \Delta \mu_s' \) of cutaneous tissue. The depth in cutaneous tissue where temperature is controlled and varied is \( \sim 2 \) mm, and the light penetration depth in tissue \( \delta \) is estimated to vary in the range 0.72 at 550 nm to 2.04 mm at 900 nm. This 2 mm depth encompasses the blood capillaries, upper plexus, lower plexus, and the interconnecting arterio-venous Anastomoses (shunts), which take part in the body temperature regulation.

Changing the temperature of this cutaneous region by heating and cooling will change blood flow into the capillaries by opening and closing the arterio-venus shunts, thus affecting \( \mu_s' \) of the cutaneous tissue.

We interpret the temperature dependence of \( \mu_s' \) as resulting from the physiological response of the vascular bed surrounding the optical measurement areas. We interpret the observed temperature dependence of \( \mu_s' \) as a physical phenomenon relating to temperature effect on the refractive index mismatch between the cutaneous fluid medium and cutaneous scattering centers.

5.4 Effect of Interperson Variations on the Temperature Dependence of the Mean of Optical Properties of Human Skin

We performed optical measurements on seven Caucasian and Oriental subjects at two set temperatures. The skin was allowed to equilibrate for 3 min at 38 °C and the optical signal was recorded. The probe temperature was lowered to 22 °C over a 1-min period, and allowed to equilibrate for two min. Then another set of measurements was performed at 22 °C. The \( \mu_a \) and \( \mu_s' \) values were determined for each temperature and wavelength and were used to calculate \( \mu_{\text{eff}} \) and hence \( \delta \) and MFP’’. As shown in Fig. 11, all \( \mu_a \) and \( \mu_s' \) increased and all \( \delta \) values decreased as temperature was elevated from 22 to 38 °C.

As shown in Fig. 11, \( \Delta \mu_a = \mu_a(38 \degree \text{C}) - \mu_a(22 \degree \text{C}) > 0 \), \( \Delta \mu_s' = \mu_s'(38 \degree \text{C}) - \mu_s'(22 \degree \text{C}) > 0 \), while \( \Delta \delta = [\delta(38 \degree \text{C}) - \delta(22 \degree \text{C})] \) is negative for each of the seven subjects. Statistical analysis of the mean values of \( \mu_a \), \( \mu_s' \), and \( \delta \) is given in Table 2. The student t-test on the sample distributions at the two temperatures indicates that the relationship \( \mu_a(38 \degree \text{C}) > \mu_a(22 \degree \text{C}) \) holds across all individuals with greater than 99% confidence, while the relationship \( \mu_s'(38 \degree \text{C}) > \mu_s'(22 \degree \text{C}) \) holds with less confidence. Structural differences between individual’s skin and positioning of the probe on the skin may have greater effect on the signal than the temperature-induced change in \( \mu_s' \). The resultant effect of temperature on both \( \mu_a \) and \( \mu_s' \) at the three wavelengths is that \( \delta(22 \degree \text{C}) > \delta(38 \degree \text{C}) \), with greater than 95% confidence.
The mean free path MFP (22 °C) > MFP (38 °C) at 590 nm, but with much less confidence at 750 and 950 nm.

Increase in light penetration depth in tissue (\(\delta\)) on cooling is of particular interest, as it may allow mapping lower dermal layers (~200 \(\mu m\) deeper) by lowering probe temperature. It can also be used to limit optical measurements to the upper layers by heating the skin and using an optical probe of small source detector distances. Temperature dependence of light penetration depth in skin makes it necessary to perform measurement of skin optical properties at a constant temperature. This will minimize errors due to temperature dependence of light penetration in tissue. Controlling skin temperature is necessary to defining the depth from which reemitted light is being collected.

5.5 Temperature Modulation of \(\mu_a\) and \(\mu'_a\) of Skin Over Prolonged Interaction Between the Optical Probe and Skin

5.5.1 Time-behavior of thermal modulation plots

Studies of probe-skin contact over a long period of time are relevant to tracking of glucose change during a meal tolerance test or glucose clamp studies. Increase in glucose concentration decreases tissue \(\mu'_a\). Glucose-independent decrease in \(\mu'_s\) was also reported. We collected reflectance data continuously over 90 min of probe-skin contact and determined \(\mu_a\) and \(\mu'_a\) as temperature was repetitively stepped between 22 and 38 °C for 15 temperature modulation cycles. Each cycle comprised the following steps: skin was equilibrated for 2 min at a probe temperature of 22 °C, temperature was raised to 38 °C over the course of 1 min, and maintained for 2 min, then lowered to 22 °C over a 1-min period. At each temperature limit (during the 2-min window), four optical data packets were collected and values of \(\mu_a\) and \(\mu'_a\) were determined.

Three light-skin volunteers were tested after 12 h of fasting and their glucose levels were determined before and after each experiment and were close to 90 mg/dL. Prior to each experiment, the subject washed his arm with warm water and dried it by blotting with a paper towel. A coupling fluid (silicone oil) was applied to the surface of the probe and on the arm. Plots are shown in Figs. 12, 13, and 14 for these subjects.

Plots of the time course of temperature modulation reveal several interesting features. First, the fast reversible step change in \(\mu_a\) and \(\mu'_a\) for repeated heating and cooling cycles. Second, the step function is more prominent for \(\mu'_a\) than \(\mu_a\). Third, the \(\mu_a\) and \(\mu'_a\) plots over time are independent of each other. While \(\mu_a\) generally increases in time, \(\mu'_a\) either decreases or stays the same. Finally, the time course of the 590-nm \(\mu_a\) varied from subject to subject.

Since we have suggested that the temperature effect on \(\mu_a\) and \(\mu'_a\) are due to different phenomena (Sec. 5.3), the similarity of these plots raises the possibility of incomplete separation between \(\mu_a\) and \(\mu'_a\). We studied the effect of temperature modulation on \(\mu_a\) and \(\mu'_a\) of skin-simulating phantoms. We used a 5-mm stack of commercial ham slices as a phantom with no interstitial fluid, no blood, and no blood circulation. There was no temperature modulation in \(\mu'_a\) values. The temperature response of \(\mu_a\) and \(\mu'_a\) of ham slices was similar to that of opal glass and other solid phantoms. We used a 1.4-cm-thick skin section of freshly euthanized pig as a phantom with interstitial fluid, stationary blood, and no blood circulation. The values of \(\mu_a\) determined at several sites of the skin were 0.8 to 1.5 cm\(^{-1}\). The change \(\Delta \mu_a\) between 22 and 38 °C was 0.0 to 0.1 cm\(^{-1}\), leading to a fractional change \(\Delta \mu_a/\mu_a\) of approximately \(0.5\times10^{-2}\) °C\(^{-1}\). The range of values for nine human subjects was 2 to 3 cm\(^{-1}\) at 590 nm. \(\Delta \mu_a\) (38 to 22 °C) was \(0.4\pm0.12\) cm\(^{-1}\), leading to \((\Delta \mu_a/\mu_a)\) of \(\approx1\times10^{-2}\) °C\(^{-1}\). The change in \(\mu'_a\) was different. The \(\mu'_a\) values determined at several sites of the pig skin was 10 to 14 cm\(^{-1}\). The change \(\Delta \mu'_a\) between 22 and 38 °C was 0.9 to 1.0 cm\(^{-1}\), leading to a fractional change \(\Delta \mu'_a/\mu'_a\) of \(0.5\times10^{-2}\) °C\(^{-1}\). The range of values for nine human subjects was 9 to 11 cm\(^{-1}\) at 590 nm and \(\Delta \mu'_a\) (38 to 22) was 0.68±0.20 cm\(^{-1}\), leading to \((\Delta \mu'_a/\mu'_a)\) of \(\approx0.46\times10^{-2}\) °C\(^{-1}\). The pig skin showed the same change in \(\mu'_a\) and a smaller change in \(\mu_a\) compared to that of intact human skin. The diminished temperature response of \(\mu_a\) of ham slices and ex-vivo pig skin compared to intact human skin may be due to the absence of blood circulation. The different response of skin-simulating phantoms to temperature modulation from that of intact human skin suggests that there is sufficient separation between \(\mu_a\) and \(\mu'_a\). This is further confirmed by the response of the two coefficients when the skin is heated above 41 °C and cooled. Temperature steps...
were applied. While $\mu_a$ showed a reversible response, $\mu_s^8$ showed a consistent increase, the two optical parameters were independent.

**5.5.2 Calculation of the drift in optical parameters on probe-skin contact**

The first two subjects had similar profiles that slightly differed from that of the third. We performed a student t-test on the data of the first two subjects using temperature modulation cycles 1 through 5 and 11 through 15. We used cycles 3 through 6 and 11 through 14 for the third subject. We calculated long-term effects of skin-probe interaction as front-to-end drift in $\mu_a$ and $\mu_s^8$. Comparisons were made among $\mu_a$ and $\mu_s^8$ at 22 and 38 °C calculated for the first 30 min and the last 30 min from the start of probe-skin contact. The time lapse between the mean of the early five cycles and the

<table>
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<th>Optical property or probability of overlap</th>
<th>Wavelength</th>
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<tbody>
<tr>
<td></td>
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<tr>
<td>$\mu_a$ (cm$^{-1}$) at 22 °C</td>
<td>2.372±(0.282)</td>
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<tr>
<td>$\mu_a$ (cm$^{-1}$) at 38 °C</td>
<td>2.869±(0.289)</td>
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<tr>
<td>$\rho([\mu_a(38,^\circ\text{C})=\mu_a(22,^\circ\text{C})]^{1,2}$</td>
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</tr>
<tr>
<td>$\mu_s^8$ (cm$^{-1}$) at 22 °C</td>
<td>9.191±(0.931)</td>
</tr>
<tr>
<td>$\mu_s^8$ (cm$^{-1}$) at 38 °C</td>
<td>9.613±(0.894)</td>
</tr>
<tr>
<td>$\rho([\mu_s^8(38,^\circ\text{C})=\mu_s^8(22,^\circ\text{C})]^{1,3}$</td>
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</tr>
<tr>
<td>$\Delta \delta$ (μm)=[$\delta(22,^\circ\text{C})-\delta(38,^\circ\text{C})$]</td>
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<td>$\rho(\delta(22)-\delta(38,^\circ\text{C})^{1,2}$</td>
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<tr>
<td>$\Delta \text{MFP}^8$ (μm)=[$\text{MFP}^8(22,^\circ\text{C})-\text{MFP}^8(38,^\circ\text{C})$]</td>
<td>65(±16)</td>
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<tr>
<td>$\rho(\text{MFP}^8(22,^\circ\text{C})-\text{MFP}^8(38,^\circ\text{C})^{1,3}$</td>
<td>0.0431</td>
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</table>

Fig. 12 Effect of temperature modulation on $\mu_a$, $\mu_s^8$ of subject 1: (a) modulation of $\mu_a$ at the 590 nm (top), 800 nm (center), and 950 nm (bottom); (b) modulation of $\mu_s^8$ at 590 nm; and (c) modulation of $\mu_s^8$ at 800 nm (▼) and at 950 nm (●).

Fig. 13 Effect of temperature modulation on $\mu_a$ and $\mu_s^8$ of subject 2: (a) modulation of $\mu_s^8$ at the 590 nm (top), 800 nm (center), and 950 nm (bottom); (b) modulation of $\mu_a$ at 590 nm; and (c) modulation of $\mu_a$ at 800 nm (▼) and at 950 nm (●).
late five cycles was 1 h. In all temperature-modulation cycles, \( m_a \) and \( m_s \) at 38 °C were larger than \( m_a \) and \( m_s \) at 22 °C.

The drift in the absorption and scattering coefficients was calculated for subjects 1 and 2 as: 

\[
\% \text{Drift} = 100 \left( \frac{\mu_a(11 \pm 15)-\mu_a(1 \pm 5)}{\mu_a(1 \pm 5)} \right)
\]

The quantity \( \mu_a \) is either of \( m_a \) or \( m_s \), and the numbers in parentheses indicate the range of the temperature modulation cycles on which the coefficients were averaged. The mean values of \( m_a \) and \( m_s \) over five temperature modulation cycles were also used to calculate within-cycle fractional changes \[ \left( \frac{\Delta \mu_a}{\mu_a} \right)_T \] and \[ \left( \frac{\Delta \mu_s}{\mu_s} \right)_T \], and the drift in these fractional change values. Within-cycle fractional changes \[ \left( \frac{\Delta \mu_a}{\mu_a} \right)_T \] and \[ \left( \frac{\Delta \mu_s}{\mu_s} \right)_T \] were calculated as:

\[
\left( \frac{\Delta \mu_a}{\mu_a} \right)_T = \frac{\mu_a(T_1 °C) - \mu_a(T_2 °C)}{\mu_a(T_1 °C)} \Delta T.
\]

\[
\left( \frac{\Delta \mu_s}{\mu_s} \right)_T = \frac{\mu_s(T_1 °C) - \mu_s(T_2 °C)}{\mu_s(T_1 °C)} \Delta T.
\]

where the temperature \( T_1 \) was 38 °C and \( T_2 \) was 22 °C. The fractional change in \( m_a \) is related to the temperature effect on the refractive index by a model similar to that used for calculating the data points in Fig. 10.

### 5.5.3 Drift in \( m_a \) and in \( m_s \) and in \( \frac{\Delta \mu_a}{m_a} \) and \( \frac{\Delta \mu_s}{m_s} \)

The front-to-back drift in \( m_a \) and in \( m_s \) values on prolonged skin-probe contact is summarized in Table 3. The mean of \( m_a \) over five temperature modulation cycles drifts upward by +0.79% to +25.5% (+0.79×10^{-2} to +25.5×10^{-2}) over the 1-h mean time between the first and last cycles. This increase in \( m_a \) on prolonged contact with the probe can be explained by blood pooling in the region of contact between the skin and probe. The drift in \( m_s \) over the same contact period is much smaller, ranging between +0.5% and −3.25% (+0.5×10^{-2} and −3.25×10^{-2}) and is mostly negative.

We examined the statistical significance of the drift using the student t-test. The calculated probability that the mean values for the first five cycles and the last five cycles (11 to 15) are from the same population indicates no overlap (i.e., statistically significant drift) between front to back values of \( m_s \) and \( m_a \) for subject 1 at both temperatures limits. This also applies for subject 2 at 38 °C for all wavelengths and for the case of subject 2 at 22 °C at 800 and 950 nm. Data for subject 3 showed overlap (i.e., no statistically significant drift) be-

### Table 3 Drift in \( m_a \) and \( m_s \) as a result of continuous probe-skin contact. 1. Drift is statistically significant. The probability that the mean values for the first five cycles and the last five cycles (11 to 15) are from the same population, \( p \), \( [\mu(1-15)-\mu(1-5)]/\mu(1-5) \) ranges from 0.0000 to 0.0052. 2. Drift is statistically significant, \( p \), \( [\mu(1-15)-\mu(1-14)]/\mu(1-14) \) ranges between 0.0000 and 0.0004 except for \( m_a \) at 590 nm, where \( p \) was 0.127 and 0.025 for 22 and 38 °C, respectively. Drift is calculated as \( 100[\mu(1-14)-\mu(3-6)]/\mu(3-6) \). Drift is less statistically significant, \( p \), \( [\mu(3-6)-\mu(1-14)]/\mu(1-14) \) ≤ 0.036, except \( m_s \) at 590 nm and 38 °C, where \( p = 0.323 \).

<table>
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<th>At 38 °C</th>
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</table>
between the initial and final values of \( \mu_a \) and in \( \mu'_t \). The magnitude of the drift and the statistical significance of it varied from person to person.

The calculated \( [\Delta \mu_a/\mu_a]_T \) and \( [\Delta \mu'_t/\mu'_t]_T \) for the three subjects are given in Table 4 for a \( \Delta T \) of 16°C. \( [\Delta \mu_a/\mu_a]_T \) values varied in the range \(+1.49 \times 10^{-3} {\text{mmol glucose per mmol Hb}}\) to \(+8.73 \times 10^{-3} {\text{mmol glucose per mmol Hb}}\) for the three subjects. The calculated drift in \( [\Delta \mu_a/\mu_a]_T \) was statistically significant for subjects 1 and 2 (no overlap between initial and final values) and less significant (some overlap) for subject 3. Except for the 950-nm wavelength for subject 1, the front-to-back drift in \( [\Delta \mu_a/\mu_a]_T \) ranged between \(-0.18 \times 10^{-3} \) to \(+0.18 \times 10^{-3} \).

The drift in \( [\Delta \mu_a/\mu_a]_T \) over time is generally positive. Since \( \mu_a \) increases as a function of probe-skin contact, this fractional value should decrease. Thus an increase in \( [\Delta \mu_a/\mu_a]_T \) overwhelms the possible decrease in the fractional value due to increase in \( \mu_a \). It is noticeable from Figs. 12, 13, and 14 that \( [\Delta \mu_a/\mu_a]_T \) increases as a function of contact time, suggesting strong dependence of \( \mu_a \) on the thermal history of the sample. \( [\Delta \mu'_t/\mu'_t]_T \) varied in the range \(+3.1 \times 10^{-3} \) to \(+7.01 \times 10^{-3} \). The front-to-back drift in \( [\Delta \mu'_t/\mu'_t]_T \) ranged between \(+1.1 \times 10^{-3} \) to \(+0.19 \times 10^{-3} \) per hour. Similar to the case of \( [\Delta \mu_a/\mu_a]_T \), \( [\Delta \mu'_t/\mu'_t]_T \) also increases as a function of time (positive drift). Since \( \mu'_t \) decreases as a function of probe-skin contact, this fractional value should increase unless \( \Delta \mu'_t \) values decrease faster than \( \mu'_t \). The fractional change \( [\Delta \mu'_t/\mu'_t]_T \) drifts in an opposite direction to that of \( \mu'_t \).

We now discuss the significance of the drift (or conversely the overlap of initial and final values) of these calculated parameters for prolonged time on the noninvasive determination of analytes in the human body. Glucose induces \( 5 \times 10^{-4} \) to \( 1.0 \times 10^{-3} \) per mmol/L fractional decrease in \( \mu'_t \) for deep human tissue between 500 and 900 nm. \( 31-33 \) Monte Carlo simulations on phantoms suggest that the calculated effect of 1 mmol glucose or 0.1°C on \( \mu'_t \) (i.e., \( \Delta \mu'_t \)) is \( \sim -1 \times 10^{-3} \). The present study suggests that a 1°C change in cutaneous temperature induces a fractional change in \( \mu'_t \), \( [\Delta \mu'_t/\mu'_t]_T \), equivalent to 5.8 to 11.6 mmol/L of glucose (104 to 209 mg/dL). Additionally, \( [\Delta \mu'_t/\mu'_t]_T \) varies according to the lengths of probe-skin contact time. Front-to-back drift in \( \mu'_t \) of cutaneous tissue is large compared to the reported glucose-induced change in the \( \mu'_t \). \( 31-33 \) The statistical significance of the drift as gauged by the \( p \) values varied by subject and by wavelength. The magnitude of nonspecific drift due to the probe-skin interference will hamper attempts for noninvasive measurements of analytes by tracking changes in \( \mu'_t \) values.

### Table 4 Variation and drift in mean fractional change \([\Delta \mu_a/\mu_a]_T \) and \([\Delta \mu'_t/\mu'_t]_T \) as a result of 90 min probe-skin contact.

<table>
<thead>
<tr>
<th>Subjects, temperature modulation</th>
<th>Cycles and probability of overlap</th>
<th>Mean fractional change ([\Delta \mu_a/\mu_a]_T ) × 10³ (°C⁻¹)</th>
<th>Mean fractional change ([\Delta \mu'_t/\mu'_t]_T ) × 10³ (°C⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subject 1: Cycles (1–5)</td>
<td>590 nm</td>
<td>2.79</td>
<td>2.69</td>
</tr>
<tr>
<td>Subject 1: Cycles (11–15)</td>
<td>800 nm</td>
<td>3.56</td>
<td>1.49</td>
</tr>
<tr>
<td>Drift (subject 1) = ([\Delta \mu(11–15) – \Delta \mu(1–5)])</td>
<td>950 nm</td>
<td>0.77</td>
<td>–0.80</td>
</tr>
<tr>
<td>Subject 1: (p[\Delta \mu(1–5) = \Delta \mu(11–15)])</td>
<td>590 nm</td>
<td>0.0000</td>
<td>0.0663</td>
</tr>
<tr>
<td>Subject 2: Cycles (1–5)</td>
<td>590 nm</td>
<td>3.24</td>
<td>4.30</td>
</tr>
<tr>
<td>Subject 2: Cycles (11–15)</td>
<td>800 nm</td>
<td>4.22</td>
<td>5.16</td>
</tr>
<tr>
<td>Drift (subject 2) = ([\Delta \mu(11–15) – \Delta \mu(1–5)])</td>
<td>950 nm</td>
<td>0.98</td>
<td>0.86</td>
</tr>
<tr>
<td>Subject 2: (p[\Delta \mu(1–5) = \Delta \mu(11–15)])</td>
<td>590 nm</td>
<td>0.0000</td>
<td>0.0000</td>
</tr>
<tr>
<td>Subject 3: Cycles (3–6)</td>
<td>590 nm</td>
<td>5.30</td>
<td>8.55</td>
</tr>
<tr>
<td>Subject 3: Cycles (11–14)</td>
<td>800 nm</td>
<td>6.37</td>
<td>8.73</td>
</tr>
<tr>
<td>Drift (subject 3) = ([\Delta \mu(11–14) – \Delta \mu(3–6)])</td>
<td>950 nm</td>
<td>1.07</td>
<td>0.18</td>
</tr>
<tr>
<td>Subject 3: (p[\Delta \mu(3–6) = \Delta \mu(11–14)])</td>
<td>590 nm</td>
<td>0.0148</td>
<td>0.0115</td>
</tr>
</tbody>
</table>

6 Conclusions

The temperature-controlled probe detects photons that are remitted from different depths in the tissue down to \( \equiv 2 \) mm. Thermal modeling suggests that temperature of the tissue volume that is optically sampled can be controlled at a probe temperature up to 44°C and down to 20°C.

For a given subject, \( \mu'_t \) is linearly dependent on temperature. Between-person \( \mu'_t \) is distinguishably larger at 38 than at 22°C at the wavelengths we studied. Between-person \( \delta \) is distinguishably higher at 22 than at 38°C. The major contribution to differentiation in the near-IR light penetration depth \( \delta \) in tissue is the change in blood \( \mu_a \). In general, \( \delta \) increased on cooling and was modulated over a large number of temperature cycles.
We studied the thermal aspects of probe-skin interaction. We attempted to separate the short-term temperature effect from the long-term effect of the probe-skin interaction. We interpret the short-term reversible change in $\mu_a$ to blood perfusion. We attribute the long-term upward drift in $\mu_a$ to increased blood pooling in the dermis.

We attribute the short-term change in $\mu'_a$ to the effect of temperature on $n_{SFG}$. We attribute the long-term decrease in $\mu'_a$ during skin-probe contact to structural factors in the skin. Hydration of the protein fibers may cause a slow and consistent decrease in $\mu'_a$ due to change dimensions of the scattering centers at constant volume fraction.

We recognize three effects of probe-skin interaction. First, there is a short-term reversible within-temperature-modulation cycle of $\mu_a$ and $\mu'_a$. Second, superimposed on this is a long-term drift in $\mu_a$ and $\mu'_a$. Third, there is a change in $[\Delta \mu_a/\mu_a]$ and $[\Delta \mu'_a/\mu'_a]$ on prolonged probe-skin interaction that varies for each individual and wavelength.

The instantaneous and reversible temperature dependence of $\mu'_a$ over a number of cycles suggests that it is a physical phenomenon related to the effect of temperature on the refractive index mismatch between cutaneous scattering centers and surrounding medium. Temperature dependence of $\mu_a$ results from physiological response of the vascular bed and surrounding areas.

We compared the effect of temperature change and probe-skin interaction on the measured and calculated effect of glucose on cutaneous scattering coefficients. The effect of glucose concentration is within the range of change in $\mu'_a$ induced by 1 °C-temperature change and natural drift at a constant temperature.

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We acknowledge the help of Ronald Hohs in electronic design, Dr. Arnold Stalder in optical design, Dr. Wolfgang Haller at the National Institute for Science and Technology for construction of the solid phantoms, and Dr. Steven Jacques (Oregon Graduate Research Institute) for the Monte Carlo program. We appreciate the encouragement of Professor Jan Greves (University of Twente, the Netherlands) and Dr. James Babb, Abbott Laboratories.

References
30. A. Knuttel and M. Boehlau-Godau, “Spatially confined and tempo-


