In Vivo Noninvasive Temperature Measurement by B-Mode Ultrasound Imaging

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Objective. This study investigated the use of ultrasound image analysis in quantifying temperature changes in tissue, both ex vivo and in vivo, undergoing local hyperthermia. Methods. Temperature estimation is based on the thermal dependence of the acoustic speed in a heated medium. Because standard beam-forming algorithms on clinical ultrasound scanners assume a constant acoustic speed, temperature-induced changes in acoustic speed produce apparent scatterer displacements in B-mode images. A cross-correlation algorithm computes axial speckle pattern displacement in B-mode images of heated tissue, and a theoretically derived temperature-displacement relationship is used to generate maps of temperature changes within the tissue. Validation experiments were performed on excised tissue and in murine subjects, wherein low-intensity ultrasound was used to thermally treat tissue for several minutes. Diagnostic temperature estimation was performed using a linear array ultrasound transducer, while a fine-wire thermocouple invasively measured the temperature change. Results. Pearson correlations ± SDs between the image-derived and thermocouple-measured temperature changes were $R^2 = 0.923 ± 0.066$ for 4 thermal treatments of excised bovine muscle tissue and $R^2 = 0.917 ± 0.036$ for 4 treatments of in vivo murine tumor tissue. The average differences between the two temperature measurements were $0.87°C ± 0.72°C$ for ex vivo studies and $0.97°C ± 0.55°C$ for in vivo studies. Maps of the temperature change distribution in tissue were generated for each experiment. Conclusions. This study demonstrates that velocimetric measurement on B-mode images has potential to assess temperature changes noninvasively in clinical applications. Key words: hyperthermia; image analysis; noninvasive thermometry; speckle tracking; ultrasonics.
reproducibility and safety of hyperthermia treatment. Not only must the therapy adequately damage malignant tissue, but a precise safety margin of normal tissue surrounding the tumor should also be treated to ensure the efficacy of the technique.2

Medical imaging serves a critical role in thermal therapies because it can be used to identify diseased tissue, guide the placement of heating devices, and visualize the targeted tissue during and after therapy. Multiple imaging modalities have been evaluated as possible tools for quantification of the temperature change distribution in tissue during thermal treatment. These include ultrasound imaging, computed tomography (CT), and magnetic resonance imaging (MRI). Ultrasound methods are based on variation in acoustic speed, energy of backscattered pulsed ultrasound, and acoustic nonlinear parameter imaging; CT approaches are based on changes in density; and the forefront MRI technique is based on shifts in the proton resonance frequency. In contrast to MRI and CT, ultrasound benefits from its portability, accessibility, low cost, and compatibility with other medical equipment. Ultrasound is a nonionizing imaging modality that is capable of real-time image acquisition, which facilitates rapid monitoring of physiologic characteristics and temperature changes.

Previous studies of velocimetric ultrasound thermometry have been dominated by the analysis of temperature-induced echo shifts in raw radio frequency (RF) data rather than echo tracking on digital ultrasound images. Much of the early thermometry work was performed using simulations or tissue-mimicking models, and a recent study by Anand and colleagues demonstrated the use of the RF analysis technique to monitor temperature in 3-dimensional space in gel phantoms. Varghese and colleagues are one of few groups to apply the velocimetric RF analysis method in vivo. In contrast to RF analysis, the advantage of echo tracking in image space is that it can be conveniently used with any clinical scanner without the need to access raw RF data. A preliminary study by Abolhassani et al tested a velocimetric image analysis method in one tissue-mimicking phantom in which graphite powder acted as an ultrasonic scattering element and glycerin was added to achieve the speed of sound in biological tissue. Heating was performed using a resistor. The study demonstrated that in this ideal phantom, accuracy better than 0.5°C could be obtained. The application of velocimetric analysis to B-mode images, however, has not yet been evaluated in excised tissue, nor has it been assessed with ultrasound used for both therapeutic heating and diagnostic temperature measurement. Furthermore, it has not yet been demonstrated whether this method can be used to measure in vivo temperature changes in biological tissues with substantially more complex architecture and structure than tissue-mimicking material.

In this study, we assessed the feasibility of applying the velocimetric image analysis approach to monitor temperature changes in tissue, both ex vivo and in vivo. Our technique involves acquisition of B-mode ultrasound images of tissue undergoing low-intensity thermal therapy and postprocessing of the image data with a custom echo-tracking algorithm. First, we validate this velocimetric measurement method in excised bovine muscle tissue and then demonstrate the method’s utility in quantifying in vivo temperature changes in murine hyperthermia. In these experiments, ultrasound was used as both the therapeutic source of heat and the diagnostic mode of temperature change measurement. The objective was to investigate whether ultrasound thermometry by B-mode image analysis can effectively be extended to localized thermal treatments performed in preclinical and clinical studies.

Theory

The temperature estimation method in this study exploits temperature-induced shifts in echo patterns of digital ultrasound images to quantify regional temperature changes within a tissue sample undergoing localized thermal therapy. Two mechanisms for these echo shifts are (1) the thermal dependence of the speed of sound and (2) thermally induced physical expansion of the tissue sample. The former mechanism produces artificial displacements in the acquired images, whereas the latter produces physical or actual displacement of the tissue sample.
Artifactual shifts in the images due to the thermal dependence of acoustic speed can be explained as follows: when a cross-sectional ultrasound image of a tissue sample is displayed on a clinical scanner, the reconstruction of RF data with standard receiver beam-forming algorithms assumes that the speed of sound is constant throughout the field of view. As the tissue sample is heated over time, the temperature change induces nonuniformity of the speed of sound throughout the tissue sample. These temperature-induced changes in the speed of sound manifest as subtle alterations in the echo pattern of images acquired throughout the heating process. Previous studies have demonstrated an approximate linear relationship between temperature changes and variations in the speed of sound:

(1) \[ \beta = \frac{1}{c_0} \frac{dc}{dT}, \]

where \( c \) is the speed of sound; \( T \) is the temperature; and \( \beta \) is a material-dependent parameter that is constant for a temperature change of up to 10°C.\(^{20} \) The parameter \( \beta \) accounts for artifactual displacement in the ultrasound images during thermal therapy. The linear coefficient of thermal expansion, also a material-dependent parameter, accounts for physical displacement of the sample due to thermally induced expansion:

(2) \[ \alpha = \frac{1}{L} \left( \frac{dL}{dT} \right)_P. \]

Here, \( L \) is the linear dimension of the tissue, and \( (dL/dT)_P \) measures the proportion of the linear change in the dimension per degree Celsius at a constant pressure \( P \). The thermal expansion of tissue is a result of the combined thermal expansion of its constituents, and the expansion is often anisotropic because of the cellular organization of the tissue. For muscle tissue, the linear expansion of blood and myoglobin, a constituent protein of skeletal muscle, can be accounted for in the approximation of \( \alpha = 1.2 - 10^{-4} \text{C}^{-1} \).\(^{29} \)

Beginning with the integral form of the time-of-flight equation of a propagating ultrasound waveform, Simon et al\(^{21} \) derived an expression for the temperature change \( \delta T \) in a medium as a function of axial depth \( x \):

(3) \[ \delta T(x) = \frac{c_0}{2} \frac{1}{\alpha - \beta} \frac{\partial \delta t(x)}{\partial x}, \]

where \( \delta t(x) \) is the temperature-induced change in the time delay of the signal from a scatterer within the medium at axial depth \( x \). A factor \( k \) can be defined as a depth-invariant material-dependent parameter that accounts for both the thermal dependence of the acoustic speed and thermal expansion of the medium:

(4) \[ k = \frac{1}{\alpha - \beta}. \]

The formulation described by Equations 1 through 4 has been used in several studies to quantify temperature changes in phantoms and tissue from shifts in the RF echo signal.\(^{19-23} \) Abolhassani et al\(^{28} \) have since modified Equation 3 to express temperature changes in terms of displacement in the scatterer location on digital B-mode ultrasound images. The authors’ modification of Equation 3 assumes that the time shift \( \delta t \) produces a displacement \( \Delta d = \delta t \times c_0 \) in image space, where \( c_0 \) is the constant speed of sound used on the clinical scanner to reconstruct the digital image from time-domain RF data. In our studies, the \( c_0 \) value is 1540 m/s. The temperature change \( \Delta T \) at depth \( x \) of a particular A-line is related to scatterer displacement \( \Delta d \) by the following equation:

(5) \[ \Delta T(x) = k \cdot \frac{\partial (\Delta d)}{\partial x}, \]

where the material-dependent parameter \( k \) in Equation 5 indicates that \( \Delta d \) is a combination of both physical displacement of the tissue and artifactual displacement in the image due to the thermal dependence of the acoustic speed. Similar to Equation 3 presented by Simon et al,\(^{21} \) this model assumes a linear relationship between the temperature change and acoustic speed. Thus, by measuring the gradient of scat-
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ter displacement along the axial dimension, the temperature is mapped as described in the following section.

**Materials and Methods**

**Ex Vivo Studies**

An unfocused low-intensity therapeutic ultrasound transducer (Dynatronics Corp, Salt Lake City, UT) was fixed above a sample of bovine muscle tissue measuring 7 × 7 × 3 cm, as shown in Figure 1. The 2.2-cm-diameter transducer locally heated the sample for a duration of several minutes at 3 MHz with a continuous wave at 2 W/cm². The spatial-average temporal-average intensity was measured by radiation force balance. A thermocouple with 0.5°C accuracy (Omega Engineering, Inc, Stamford, CT), inserted into the tissue under image guidance, was used to record the temperature at 15-second intervals. A 10-MHz linear array ultrasound transducer (Ultrasonix Medical Corp, Vancouver, British Columbia, Canada) acquired digital B-mode images of the tissue sample at 15-second intervals, after which the thermal therapy was momentarily paused to prevent interference between the therapeutic and imaging beams (Figure 1). First, this thermal treatment was performed on 1 tissue slice (sample A) to determine the material-dependent parameter \( k \) of bovine muscle, and then the experiment was repeated on 4 additional slices (samples B–E) to demonstrate experimental reproducibility. During thermal treatment of the last 2 tissue samples (D and E), both B-mode and RF data were acquired to compare the results of noninvasive thermometry by B-mode image analysis and by RF data analysis.

**Image Analysis**

Digital B-mode images were processed offline in MATLAB (The MathWorks, Natick, MA) after thermal treatment. The temperature change was determined by tracking the echo displacement relative to the baseline image at each location and time point, differentiating the displacement along the axial direction as indicated by Equation 5, and applying a 2-dimensional filter to the resulting derivatives. The following image-processing algorithm was used to analyze a sequence of B-mode images acquired during thermal therapy. The RF data acquired during 2 thermal therapies of ex vivo tissue were processed in a similar manner.

- A normalized cross-correlation algorithm was applied to the pixel intensity \( I \) of each pair of sequential images, \( I_{tn} = I(x, y, t_n) \) and \( I_{tn+1} = I(x, y, t_{n+1}) \), to determine the speckle displacement \( d \) at each location \((x, y)\) in image space at time \( t_n \). Here, \( t_n \) refers to the time at which the \( n \)th image frame was acquired, where \( n = 1, 2, \ldots, N \). The normalized correlation coefficient \( C \) was maximized with respect to displacement to determine the best match between a kernel of axial length \( k \) in an image shifted within a search window of axial length \( s \) in image \( I_{tn+1} \):

\[
C(m) = \frac{\sum_x \sum_y (I_{tn}(i, j) - \bar{I}_{tn}) \times (I_{tn+1}(i + m, j) - \bar{I}_{tn+1})}{\sqrt{\sum_x \sum_y (I_{tn}(i, j) - \bar{I}_{tn})^2} \times \sqrt{\sum_x \sum_y (I_{tn+1}(i + m, j) - \bar{I}_{tn+1})^2}}
\]

\[
d = \arg \max_{m \in [-s, s]} C(m).
\]

Here, \( I_{tn}(i, j) \) is the gray scale value of pixel \((i, j)\) in image \( I_{tn} \); \( \bar{I}_{tn} \) is the average gray scale intensity in the kernel; and \( I_{tn+1} \) is the average gray scale intensity in the search window. The displacement \( d \) is determined by the maximum of \( s \) coefficients computed in Equation 6. One value of \( d \) is assigned to each pixel of the image.

- The displacement values were thresholded, and only the values corresponding to a high normalized correlation coefficient greater than 0.80 were retained:

\[
d(x, y, t_n) = 0 \text{ if } C(x, y, t_n) \leq 0.80.
\]

- The cumulative absolute displacement \( |\Delta d(x, y, t_n)| \) was determined for each acquisition time \( t_n \) to measure the temperature change relative to the baseline image acquired at \( t_1 \):

\[
|\Delta d(x, y, t_n)| = \sum_{t=1}^{n} |d(x, y, t)|
\]

This produces a series of \( N \) cumulative displacement maps. The first of these maps is composed of all 0s, representing no displacement of \( I_{t1} \) with respect to itself. Note that the...
absolute displacement was computed because the thermally treated tissue expanded radially with respect to the center of heating. Because the direction of expansion was not accounted for in this experimental setup, we investigated only increases in temperature.

A 5 × 5 median filter was applied to each cumulative displacement map to mask erroneously large or small displacements and to smoothen the lateral displacement gradient. A gaussian filter was applied to each axial data line to regularize speckle motion and penalize high-frequency content of the displacement fields. A finite-differences filter was applied to each line to obtain estimates of $|\frac{\partial (\Delta d(x, y, t_n))}{\partial x}|$. The estimates of $|\frac{\partial (\Delta d(x, y, t_n))}{\partial x}|$ were multiplied by the known or measured material-dependent parameter $k$ to determine absolute temperature change $\Delta T(x, y, t_n)$ as in Equation 5.

An averaging filter was applied to each temperature map $\Delta T(x, y, t_n)$, and each map was then superimposed on the original gray scale image acquired at $t_n$. This final step is for display purposes; it provides a color-scaled distribution of temperature changes in image space that indicates the extent to which different areas of the tissue were thermally treated.

To accurately calibrate the color scale of the temperature maps $\Delta T(x, y, t_n)$ with the appropriate value of $k$, the first thermal treatment data set from sample A was used to quantify the $dc/dT$ value of the bovine muscle tissue. To compute this value, the thermocouple-measured temperature change, $\Delta T_{\text{TH}}$, was plotted as a function of the image-derived estimates of $|\frac{\partial (\Delta d(x, y, t_n))}{\partial x}|$. The slope, or $k$ value, of the line $\Delta T_{\text{TH}}$ versus $|\frac{\partial (\Delta d)}{\partial x}|$ determined by linear regression was applied to Equation 4 obtain $dc/dT$. With knowledge of $dc/dT$ from sample A, the absolute temperature change distribution was measured in the 4 subsequent thermal treatments of samples B through E. The average image-derived temperature change in a 40 × 40-pixel region of interest around the thermocouple was compared to the thermocouple-measured temperature for validation. A statistical comparison was performed by computing the Pearson correlation of the thermocouple and image-derived measurements. In addition, the averages and SDs in the absolute difference between the thermocouple and image-derived measurements were calculated.

**In Vivo Studies**

In 5 female nude mice (10–12 weeks of age; National Institute of Health, Bethesda, MD), 1 million human lung carcinoma cells (A549; American Type Culture Collection, Manassas, VA) were injected subcutaneously on the right flank. Once the tumors had grown to at least 1 cm in one dimension, each mouse was prepared for imaging by placement in an induction box containing 2% to 3% isoflurane in oxygen. When induction was complete, the mouse was removed from the induction chamber, and anesthesia was maintained by 1% to 2% isoflurane in oxygen inhaled through a nose cone. The tumor was then insonated at 3 MHz for 4 to 6 minutes. A CL10-5 linear transducer (HDI 5000; Philips Healthcare, Bothell, WA), oriented perpendicular to the therapeutic beam, acquired B-mode images at a rate of 10 frames per second throughout the experiment. A thermocouple was inserted into the center of the tumor via image guidance to monitor the temperature change throughout the treatment. During each 30-second interval of thermal therapy, the therapeutic probe was activated for 20 seconds and then inactivated for 10 seconds to allow for image acquisition in the absence of interference between the therapeutic and imaging beams. To minimize motion due to the respiratory cycles of the mouse, we tracked the mean gray scale intensity of the images acquired in the 10-second period during which therapy was paused.

![Figure 1. Setup of the ex vivo thermal treatment experiment.](image-url)
Assuming that the periodicity of the echogenicity profile was a marker of rhythmic motion of the subject, we selected an image corresponding to a peak in the echogenicity profile at approximately 6 to 8 seconds into the pause for echo-tracking analysis. This image selection was performed for each pause phase in the thermal therapy imaging cycle. In effect, this process served as a form of retrospective respiratory gating.

The same procedure outlined above in the “Image Analysis” section was used to process the sequences of images acquired during thermal treatment of the 5 mice. Because $dc/dT$ of tumor tissue in vivo was unknown, the procedure discussed for computing $dc/dT$ of bovine muscle tissue was applied to the first of 5 in vivo experiments. The thermocouple-measured temperature change $\Delta T_{\text{thermocouple}}$ was plotted as a function of the gradient of axial displacement $[\partial(\Delta d)/\partial x]$, and the $k$ value of the tumor tissue was determined by linear regression. This value was applied to Equation 4 to obtain $dc/dT$, which was subsequently used to calibrate the image-derived temperature change measurements in each of the following 4 in vivo treatments.

Because an unfocused ultrasound transducer was used for therapy, diffuse heating occurred in regions within and surrounding the tumor tissue. To illustrate a region of interest in the B-mode images containing the tumor, the tumor boundaries were segmented by the user-guided automated boundary detection algorithm. In this algorithm, the user defines the tumor boundary in one image, and the information is propagated from one frame to the next for automated boundary detection. Radial brightness profiles are extracted from the images at equispaced rays from the center of gravity of the initial boundary, and the updated boundary is determined by detecting the large gray scale gradient between the hypoechoic interior of the tumor and the hyperechoic tumor edge. The temperature maps showing an outline of the segmented tumor were superimposed in color on the original B-mode images acquired during therapy.

## Results

### Ex Vivo Studies

The thermocouple and image-derived temperature change measurements for the thermal treatment of samples B through E of bovine muscle tissue are presented in Figure 2. In these graphs, the lines indicate the thermocouple-measured temperature change, and the dots refer to the image-derived temperature change. The bottom two graphs include results of noninvasive thermometry by RF analysis, indicated by gray crosses. The image- and RF-derived measurements were calibrated using $dc/dT = 0.82 \text{ m/s/°C}$, which was calculated from the thermal treatment of sample A as described in the “Image Analysis” section of “Materials and Methods.” The Pearson correlations indicating the strength of correlation between measured and image-derived estimates were $R^2 = 0.859$ and 0.989 for samples B and C, respectively. For samples D and E, the Pearson correlations were $R^2 = 0.874$ and 0.971 by B-mode analysis and $R^2 = 0.980$ and 0.867 by RF analysis. The averages ± SDs in the difference between the thermocouple-measured and image-derived temperature change were $1.19 \pm 1.01$ °C and $0.46 \pm 0.28$ °C for samples B and C, respectively. For samples D and E, the averages ± SDs were $1.00 \pm 1.22$ °C and $0.82 \pm 0.37$ °C by B-mode analysis and $0.94 \pm 0.67$ °C and $0.81 \pm 0.55$ °C by RF analysis. These results are summarized in Table 1.

Maps illustrating the temperature change distribution in sample C are presented in Figure 3. In this series of images, the color map displays the local temperature change, which is superimposed on the original gray scale image. The color bar indicates the temperature change in degrees Celsius. Note that the therapeutic transducer locally heated the tissue sample at the bottom right of the imaging plane. Slight axial extension of the heated region toward the base of the image frame, marked by a white arrow in the last image in Figure 3, can be attributed to the presence of sharp lateral temperature gradients, which produce acoustic aberrations distal to the region of heating with respect to the imaging probe. Similar temperature change maps were obtained for all thermal treatment experiments in bovine muscle tissue.

### In Vivo Studies

The thermocouple and image-derived temperature change measurements for the thermal treatment of 4 mice are presented in Figure 4. In these graphs, the lines indicate the thermocouple-
measured temperature change, and the dots refer to the image-derived temperature change. The image-derived measurements were calibrated using $dc/dT = 2.34 \text{ m/s/°C}$, which was calculated from the thermal treatment of the first of 5 mice as described in the “In Vivo Studies” section of “Materials and Methods.” The Pearson correlations, averages, and SDs in the difference between the thermocouple and image-derived measurements are listed in Table 1. Specifically, the SDs in the absolute difference between the thermocouple and image-derived measurements were 0.56°C, 0.68°C, 0.21°C, and 0.76°C for the in vivo experiments. These SDs are comparable to a previous in vivo thermometry study based on local frequency shifts in the spectrum of reflected A-lines, where an SD of 0.5°C was reported.

To verify that the outcome is consistent regardless of which mouse is chosen for calibration, the measured temperature change $\Delta T_{TH}$ is plotted as a function of the image-derived estimate of $|\partial (\Delta d) / \partial x|$ for each of the 5 in vivo experiments (Figure 5). This graph indicates a strong linear correlation ($R^2 = 0.91$) between the thermocouple-measured temperature change and the axial

![Figure 2](https://example.com/figure2.png)

**Figure 2.** Temperature changes measured as a function of time for 4 thermal treatment experiments in bovine muscle tissue (samples B–E). The solid lines represent the thermocouple-measured temperature change, and the gray dots represent the corresponding image-derived temperature change. The bottom two graphs (samples D and E) show temperature change estimates derived from raw RF data, shown as gray crosses.

### Table 1. Comparison Between Thermocouple-Measured and Image-Derived Temperature Change

<table>
<thead>
<tr>
<th>Study Type</th>
<th>$R^2$</th>
<th>Average Difference, °C</th>
<th>SD, °C</th>
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<tbody>
<tr>
<td>Ex vivo</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.859</td>
<td>1.19</td>
<td>1.01</td>
</tr>
<tr>
<td></td>
<td>0.989</td>
<td>0.46</td>
<td>0.28</td>
</tr>
<tr>
<td></td>
<td>0.874 (B), 0.980 (RF)</td>
<td>1.00 (B), 0.94 (RF)</td>
<td>1.22 (B), 0.67 (RF)</td>
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<tr>
<td></td>
<td>0.971(B), 0.866 (RF)</td>
<td>0.82 (B), 0.81 (RF)</td>
<td>0.37 (B), 0.55 (RF)</td>
</tr>
<tr>
<td>In vivo</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>0.922</td>
<td>0.93</td>
<td>0.56</td>
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<tr>
<td></td>
<td>0.914</td>
<td>0.83</td>
<td>0.68</td>
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<tr>
<td></td>
<td>0.960</td>
<td>0.76</td>
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<tr>
<td></td>
<td>0.873</td>
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The averages and SDs are computed on the distribution of the absolute difference between the thermocouple and image-derived measurements. B indicates B-mode image analysis; and RF, analysis of RF data.
displacement gradient, a direct measure of the temperature change. When $\Delta T_{TH}$ versus $|\partial(\Delta d)/\partial x|$ is plotted separately for each individual experiment, the average $dc/dT$ is $2.32 \pm 0.092$ m/s/°C, with $R^2$ ranging from 0.851 to 0.960. Note that several of the data points in Figure 5 exceed a $\Delta T$ of 10°C, the limit at which $\beta$ remains constant and the relationship $\Delta T$ versus $|\partial(\Delta d)/\partial x|$ is linear. If the data points for which $\Delta T > 10°C$ are excluded from this plot, the resulting $k$ value indicates a minor change in $dc/dT$ of 0.01 m/s/°C. This finding suggests that inclusion of data points for which $10°C < \Delta T < 17°C$ does not introduce nonlinearities in the relationship $\Delta T$ versus $|\partial(\Delta d)/\partial x|$ that significantly alter temperature map calibration.

With knowledge of $dc/dT$ in murine tumor tissue, the value of $k$ can be used to scale the image-derived measurements of $|\partial(\Delta d)/\partial x|$ and produce a series of temperature maps illustrating the evolution of temperature changes during therapy. Figure 6 shows the quantitative temperature change distribution in the second of 5 murine tumors treated with thermal therapy. Similar to Figure 3, the temperature change is displayed as a color map superimposed on the corresponding gray scale image. Because the validation of this technique corresponds to $\Delta T < 20°C$, any image-derived temperature changes that exceed this threshold are displayed as the brightest yellow on the color scale. Figure 6 shows that, in this particular experiment, the temperature of the tumor tissue increased rapidly at several “hot spots” in the periphery. The presence of cool and hot spots may be due to the diffuse ultrasound field used as our heat source. Because the in vivo thermal treatments were easiest to perform in the near field of the therapeutic probe, there is not a straightforward characterization of the heating field. Although slow movement of the therapeutic probe over the tumor may have generated a more uniform heating pattern, the therapeutic probe was kept stationary during therapy to avoid the interference of motion artifacts in our thermometry analysis.

**Discussion**

The results of this study demonstrate that velocimetric measurement on digital B-mode images shows promise in noninvasively quantifying temperature changes in clinical applications of hyperthermia. Our work with murine subjects indicates that this analysis, which had been explored thus far only in vitro, can be effectively applied to temperature estimation performed ex vivo and in vivo. This technique does not require any dose of ionizing radiation to the subject; it is compatible with the use of other medical equipment; and it can be easily implemented using technology that is widely accessible. With future improvements in the computational speed of speckle tracking, real-time temperature monitor-
ing can be achieved because of the capability of real-time ultrasound image acquisition.

The image-processing algorithm that monitors temperature by tracking echo shifts in digital B-mode images produces results of consistent quality on two different clinical scanners: the Sonix RP (Ultrasonix Medical Corp) and the HDI 5000 (Philips Healthcare). The method does not require access to raw RF data. Preclinical animal studies demonstrate that this echo-tracking technique can be used to noninvasively measure temperature changes in vivo. However, the application of the technique requires appropriate motion reduction strategies. Even a simple form of respiratory gating, as implemented here, allows for tracking of temperature-induced speckle movement without overwhelming interference from nonthermal motion. Although the deviation in our image-derived temperature estimates may have been caused by ignoring the effects of nonthermal motion of the subject or variable lipid content of the treated tissues, the discrepancy in image-derived and thermocouple-measured temperatures may also be understood in terms of the limited sensitivity of the fine-wire thermocouple, which has accuracy of 0.5°C. Differences between the thermocouple and image-derived measurements may be attributed to small misalignment of the thermocouple and the imaging plane during thermal treatment or to degradation of speckle characteristics in the

Figure 4. Thermocouple-measured (solid lines) and image-derived (dots) temperature change estimates for the in vivo thermal therapies. Each graph corresponds to the thermal treatment of 1 mouse.

Figure 5. Thermocouple-measured temperature change, $\Delta T_{th}$, plotted as a function of the image-derived estimate of $|\partial (\Delta d/\partial x)|$ for 5 murine tumor thermal treatments. The slope of this line provides the average value of $k$, defined in Equation 4.
image due to thermocouple insertion. Minor improvements of the experimental setup alone could likely enhance the results of this in vivo analysis.

Unlike approaches that estimate temperature changes based on shifts in harmonically related resonances of the power spectral density of the RF data, the velocimetric model makes no assumption that the biological tissue is a semiregular lattice of discrete scatterers. However, this model requires the presence of multiple stationary reflectors in the tissue and assumes uniform sound speed, \( c_0 \), in the absence of a temperature change, which is believed to produce inaccuracies in temperature estimation of inhomogeneous tissue. It should additionally be noted that in water and in most tissue media, the speed of sound \( c \) increases with increasing temperature. In fatty tissue, \( c \) decreases with increasing temperature. The model used in this study assumes a linear relationship between the temperature change and the speed of sound. The extent of the inaccuracies due to these assumptions is unknown, and it was therefore critical to extend this velocimetric image analysis from thermometry experiments performed in vitro to thermometry performed in vivo to explore the scope of validation. Our results support the following conclusions: (1) digital image analysis may perform as well as RF-based methods on the basis of the statistical metrics used in this study, and (2) imaged-derived temperatures are highly correlated with direct measurements made via a thermocouple. Temperature can be measured noninvasively within \( 0.92^\circ C \pm 0.27^\circ C \) of the actual temperature, as indicated by the average difference between thermocouple and image-based measurements in both ex vivo and in vivo studies. Although the average difference is higher than the reported results of other noninvasive methods (\( 0.34^\circ C \pm 0.36^\circ C \) for in vivo MRI thermometry and \( 0.5^\circ C \) for ultrasound thermometry based on RF analysis), the accuracy of the B-mode thermometry method was assessed relative to a less precise invasive method with an average error of \( 0.5^\circ C \).

Maass-Moreno et al. pointed out notable differences between in vitro and in vivo thermal treatment experiments that must be addressed. During therapy, a potential source of change in tissue volume is thermal hyperemia, the tendency of vessels to dilate and accommodate increased blood flow when heated. Increased blood flow may, in turn, produce engorgement and unpredicted contributions to \( \Delta d \) in Equation 5. The extent of this volume expansion depends on multiple factors, including the presence and uniformity of vessels in the therapy field and the distensibility of the vascular bed of the tissue. Song indicated that this tissue swelling differs from thermal expansion in that the time course of the hyperemic response may be too long to render this effect significant during thermal treatment. Moreover, not only does tumor tissue exhibit a limited hyperemic response to heating, but perfusion also markedly decreases at tem-
peratures as low as 42°C. Because the accuracy of our temperature estimation in vivo was similar to that observed ex vivo, we expect that thermal hyperemia did not undermine the validity of the ultrasound thermometry analysis.

Several challenges to this thermometry technique include tracking of temperature-induced motion artifacts in the presence of nonthermal movement of the subject, aberrations in temperature changes distal to the heated region produced by the thermoacoustic lens effect, the necessity of known material-dependent parameters to accurately calibrate temperature change measurements in potentially inhomogeneous tissue, the reduction of the computational time involved in echo tracking for online applications, and validation of the spatial distribution of temperature measurement.

Several modifications can be made to further minimize the error in our technique and address the challenges listed above. First, respiratory gating can be performed during the experiment, so that image acquisition is triggered to a specific phase of the subject’s respiratory cycle. Second, a more sophisticated filter can be used to smoothen and differentiate the displacement values obtained via echo tracking. Simon et al suggested using the Remez algorithm to design an equiripple filter such that the choice of the spatial cutoff frequency controls the amount of ripple in temperature estimates. This serves to reduce the artifacts produced by the thermoacoustic lens effect at the expense of spatial resolution. Alternatively, the use of ultrasound spatial compound imaging has been shown to reduce ripple artifacts caused by sharp lateral gradients.

Third, echo tracking can be optimized by comparing the performance of cross-correlation to alternative methods using nonrigid image matching. A preliminary study indicates improved accuracy and a shortened computational time when optical flow, as opposed to cross-correlation, is used to track speckle displacement. The use of a high-sensitivity thermocouple array, rather than a single thermocouple, would contribute to optimization of the tracking algorithm by evaluating the spatial accuracy of temperature change estimation. In addition, more systematic characterization of the material-dependent parameters of various tissue types would promote the application of this velocimetric image-processing approach to an increased number of clinical scenarios. For application to nonsuperficial thermal treatments, the noninvasive thermometry technique can be evaluated using lower-frequency probes to achieve greater depth penetration at the possible expense of decreasing the spatial resolution of temperature estimation.

The results of this study warrant further validation of noninvasive thermometry by B-mode image analysis. As the state of multimodality imaging and noninvasive thermometry progresses, noninvasive thermometry methods such as ultrasound imaging and MRI can be compared to verify the spatial distribution of temperature change measured using the two approaches. Further validation studies will explore the limitations of temperature monitoring by B-mode imaging, including characterization of spatial resolution and the temperature range over which this approach reliably monitors thermal therapy.

References


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