Published in final edited form as:

Ultrasound Med Biol. 2016 May ; 42(5): 1182–1192. doi:10.1016/j.ultrasmedbio.2016.01.007.

Improved Estimation of Ultrasound Thermal Strain Using Pulse Inversion Harmonic Imaging

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Abstract

Thermal (temporal) strain imaging (TSI) is being developed to detect the lipid rich core of atherosclerotic plaques and fatty liver disease. However, the effects of ultrasonic clutter on TSI have not been considered. In this study, we evaluated whether pulse inversion harmonic imaging (PIHI) could be used to improve estimates of thermal (temporal) strains. Using mixed castor oil-gelatin phantoms of different concentrations and artificially introduced clutter, we showed that PIHI improved the signal-to-noise ratio of TSI by an average of 213% or 52.1% as compared to 3.3 MHz and 6.6 MHz imaging. In a phantom constructed using human liposuction fat in the presence of clutter, the contrast-to-noise ratio was degraded by 35.1% for PIHI as compared to 62.4% and 43.7% for 3.3 MHz and 6.6 MHz imaging, respectively. These findings were further validated using an *ex vivo* carotid endarterectomy sample. PIHI can be used to improve estimates of thermal (temporal) strain in the presence of clutter.

Key terms

Thermal strain imaging; temporal strain imaging; non-invasive ultrasound thermometry; oil-gelatin phantom; ultrasound clutter; pulse inversion harmonic imaging

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Introduction

Ultrasound thermal strain imaging (TSI), which has previously been referred to as temporal strain imaging, is a non-invasive imaging technology that relies on the temperature dependence of the speed of sound as a means of differentiating fatty tissue from water-based tissue. In water-based tissues, the speed of sound increases with increasing temperature and in lipid-based tissues, the speed of sound decreases with increasing temperature. This relationship is quantified with the material constant λ , which has units of $\%/^{\circ}\text{C}$ and is a measure of the percentage change in sound speed per unit change in temperature (Seo et al. 2011a). For water-based tissues like normal liver or muscle, λ has been found to be between 0.06 and 0.13 $\%/^{\circ}\text{C}$. For lipid-based tissues like abdominal fat, λ is typically between -0.13 and -0.20 $\%/^{\circ}\text{C}$ (Duck 2013). Clinically, TSI development is focused on non-invasive detection of fatty liver disease and identification of lipids in atherosclerotic plaques (Mahmoud et al. 2013; Mahmoud et al. 2014).

In TSI, a reference ultrasound image is acquired. Next, custom beamforming and pulse sequences are used to generate a high intensity ultrasound beam that will homogeneously increase the temperature in the target region by $<2^{\circ}\text{C}$. The temperature rise is the result of absorption of the propagating ultrasound wave. After the heating sequence is completed, a post-heating ultrasound image is acquired. As a result of the change in the sound speed, there is an apparent displacement (time-shift), u , between received echoes in the reference and post-heating images. The derivative of this displacement in the ultrasound wave propagation (axial) direction is the “thermal (temporal) strain,” $\frac{du}{dz}$, and is proportional to the change in sound speed. In water-based tissue, this strain is negative and the tissue appears to undergo relative compression. In lipid-based tissue, the strain is positive and the tissue appears to undergo relative expansion. For the small temperature changes utilized in TSI, the temperature induced mechanical strain resulting from volumetric tissue expansion is approximately an order of magnitude smaller than the apparent strain resulting from the change in sound speed and can typically be ignored (Seo et al. 2011a). Thus, the governing equation for TSI is:

$$\frac{du}{dz} = -\lambda \Delta T, \quad (\text{Eq. 1})$$

where ΔT is the temperature change. In TSI, u is tracked by comparing the reference and post-heating images and $\frac{du}{dz}$ is calculated from the measured displacement.

Ultrasound non-invasive thermometry (NIT) is based on the same underlying physics as TSI but incorporates three major differences. First, NIT is typically used to assess temperatures changes during high intensity focused ultrasound (HIFU) or radiofrequency (RF) ablation procedures which result in temperature changes on the range of 2°C to $>20^{\circ}\text{C}$ (Liu and Ebbini 2010; Seo et al. 2011b). In this temperature range, it is no longer acceptable to ignore thermally induced mechanical strains. As a result, the governing equation is modified to

$$\frac{du}{dz} = (\alpha - \lambda) \Delta T, \quad (\text{Eq. 2})$$

where α is the coefficient of thermal expansion. Finally, in NIT, the material constants are assumed to be known parameters. The temperature change is found by dividing the measured strain by the material constants.

Both TSI and NIT have been tested using *in vivo* animal models and *ex vivo* tissue preparations (Ding et al. 2015; Kim et al. 2008; Lai et al. 2010; Liu et al. 2008; Mahmoud et al. 2014; Miller et al. 2004). For TSI, these studies have aimed at identifying the lipid rich core of atherosclerotic plaques or quantifying hepatic steatosis. The clinical applications of NIT are focused on monitoring of thermal ablation as well as theranostic approaches utilizing temperature-sensitive drug delivery vehicles. However, a number of challenges are present in human patients that are absent or less flagrant in small animal models. One major challenge is clutter-based degradation of image quality (Lediju et al. 2008). Clutter is a general term for noise that serves to decrease image contrast. One prominent source of clutter in patients is near-field reverberation. Near-field reverberation occurs because the propagating ultrasonic wave is reflected multiple times within superficial tissue layers (e.g. skin, subcutaneous fat). These multiple reflections corrupt echoes arriving from deeper tissue structures and appear as a haze in the ultrasound image (Dahl and Sheth 2014). One well-established method commonly used in clinics to reduce clutter is harmonic imaging. Harmonic imaging exploits the non-linear propagation of the fundamental wave and images the resulting second harmonic signal. The second harmonic signal is typically weak in the near-field and reaches its peak intensity in the focal zone which helps to mitigate near-field clutter artifacts (Christopher 1997).

Recently, groups have shown that harmonic imaging can not only be used to improve traditional B-mode images, but can also be used to improve ultrasound-based displacement tracking which serves as the basis for acoustic radiation force and shear wave imaging (Doherty et al. 2013; Song et al. 2013). Doherty et al found that pulse inversion harmonic imaging (PIHI) provided the greatest improvement to ultrasonically tracked displacements. In the pulse inversion technique, two 180° phase-shifted (inverted) fundamental pulses are transmitted sequentially. Summation of the received signals from these pulses results in cancellation of the fundamental signal and amplification of the harmonic. Because TSI measures strain calculated from ultrasonically tracked apparent displacements, we hypothesized that PIHI could also be applied to improve estimation of apparent strains produced as result of temperature-induced changes in the speed of sound. In this article, we experimentally show that PIHI can be used to improve the quality of thermal (temporal) strain images using a variety of relevant imaging phantoms and *ex vivo* human tissue samples. Conventionally, NIT uses the same ultrasound tracking scheme as TSI. As such, we point out potential applications and parallels of this work to NIT when they are relevant.

Material and Methods

Ultrasound TSI Pulse Sequence

The pulse sequence and timing for the TSI sequence is diagrammed in Fig. 1a. PIHI was incorporated into the imaging portions of the TSI pulse sequence using a research ultrasound platform with an external HIFU power supply (Vantage, Verasonics Inc, Kirkland, WA) using an ATL L7-4 transducer (Philips Healthcare, Amsterdam, Netherlands) (Christopher

1997). The -6 dB bandwidth of this transducer was measured to be $3.3 - 6.6$ MHz. The overall sequence consists of imaging, followed by a heating phase, and ends with a post-heating imaging phase. There is a 35 ms pause between the first imaging phase and the beginning of the heating phase and a 500 μ s pause between the end of the heating and the beginning of the final imaging phase. These pauses are inserted to allow time for data transfer and power supply switching.

During imaging, the transducer was first excited with a 3.3 MHz pulse. RF backscattered echoes were received on 128 parallel channels at a sampling frequency of 41.7 MHz and saved for offline processing. This process was repeated for a 180° phase-shifted (inverted) 3.3 MHz excitation, and again for two 6.9 MHz excitations with the same phase. All excitations were 2.5 cycles long. It was found that by using excitations at 3.3 MHz and 6.9 MHz, the center frequency (f_c) of the received echoes was approximately 3.3 MHz and 6.6 MHz. This was likely due to the convolution of the excitation pulses with the impulse response of the transducer. This transmit/receive sequence was repeated at each of the 83 imaging lines and corresponded to an imaging time of approximately 30 ms. The spacing between lines was 0.298 mm. The f-number for both transmit and receive focusing was set to 1.97 in order to minimize beamforming errors due to changes in sound speed.

We previously described specialized beamforming utilizing two temporally interleaved beams with multiple lateral foci that could be used to achieve homogeneous heating in a trapezoidal region from $20 - 30$ mm axially (Fig. 1b) (Nguyen et al. 2014a). In this article, the heating sequence has been modified slightly in order to improve the overall efficiency. The excitation frequency was shifted to 4.6 MHz as a compromise between attenuation and transducer bandwidth. The first beam utilizes four lateral foci and is transmitted for 32 cycles. Immediately afterwards, a second beam utilizing three lateral foci is transmitted for 32 more cycles. The seven foci are spaced to provide a broad, homogeneous heating region. Finally, a variable length waiting period with no transmits is utilized. These three events repeat until the total heating duration is equal to two seconds (Fig. 1a, "Heating"). Because the duration of the heating sequence is fixed at two seconds, the length of the waiting period determines the overall duty cycle during the heating period. Unless otherwise specified, the duty cycle was fixed at 5% and corresponded to a wait time of 264 μ s. The excitation amplitude used for heating was 15 V and corresponds to a peak negative pressure of approximately 2.3 MPa in degassed water which was measured using an ONDA HGL0200 bullet type hydrophone at the heating focus of 25 mm axially.

Offline Ultrasound TSI Signal Processing

3.3 MHz fundamental data was generated by *subtracting* the RF data from the first pair of phase-inverted 3.3 MHz transmits. PIHI data was generated by *summing* the RF data from the first pair of phase-inverted 3.3 MHz transmits. 6.6 MHz fundamental data was generated by *summing* the RF data from the pair of in-phase 6.6 MHz transmits. RF data were beamformed using delay-and-sum beamforming and Hilbert transformed in order to obtain IQ data. Apparent axial displacements between the reference and post-heating frame were calculated using Loupas' estimator with a kernel size equal to 1.5 wavelengths (Loupas et al. 1995). For all quantitative calculations, displacement data was median filtered with a 0.10 X

0.89 mm (axial X lateral) median filter and the apparent axial strain was calculated using a 2 mm 1st order Savitzky-Golay filter. For display purposes, the strain was calculated using a 3 mm 1st order Savitzky-Golay filter. In this article, TSI acquisition refers to both the ultrasound pulse sequence and these offline signal processing steps.

In vitro Gelatin-Castor Oil Phantom Experiments

Mixed gelatin-castor oil phantoms were fabricated in order to mimic fat-water mixtures that might be found in hepatic steatosis of heterogeneous atherosclerotic plaques. The phantom fabrication technique was modified from a recipe previously reported in the literature (Nguyen et al. 2014b). The respective volumes of water and sulphonated castor oil (Spectrum Chemical, CA, USA) were first calculated in order to obtain a castor oil percentage ranging from 0 – 50% (vol/vol). Then, based off of the total volume, 5% (wt/vol) gelatin from porcine skin (Sigma Aldrich Co., MO, USA) and 2% (wt/vol) cellulose (Sigma Aldrich Co., MO, USA) were weighed. The percentages of gelatin and cellulose, which provide underlying structure and ultrasound scatterers respectively, were fixed for all phantoms in this article. The gelatin was added to cold water, allowed to mix, and then heated to approximately 65°C or until the solution became clear. Then, the cellulose was added to the solution and allowed to thoroughly mix. Finally, castor oil was slowly added to the mixture in order to ensure homogeneous mixing. The mixture was allowed to cool while maintaining constant stirring. When the mixture approached approximately 35°C, the mixture was poured into a pre-chilled plastic mold in a –20°C freezer. The phantom was allowed to cure at this temperature for an additional hour. After the phantom cured, it was carefully removed from the plastic mold and placed on sound attenuating rubber for imaging. In this study, the sound speed of the phantoms were not modulated using n-propanol. The scatterer concentration used produced fully developed speckle in all phantoms. Throughout this article, degassed ultrasound gel was used to couple the transducer to the phantom and the transducer was held using a clamp.

A gelatin (0% castor oil) phantom was allowed to come to room temperature and TSI was performed with heating duty cycles ranging from 0.1 – 10% (11 total duty cycles). At each duty cycle, TSI was performed at 5 different locations within the phantom resulting in a total of 55 TSI measurements for the 0% castor oil phantom. Following imaging, one temperature measurement was obtained for each duty cycle using a Type T needle sheathed thermocouple (MT-23/5, Thermoworks, UT, USA) connected to a data logger (Quadtemp, Thermoworks, UT, USA). The thermocouple was accurate to within ± 0.1 °C and had a time constant of 25 ms. For all temperature measurements in this article, the tip of the thermocouple was placed at an axial depth of 25 mm and a lateral position of 0 mm (centered). The thermocouple was aligned as shown in Fig. 1b in order to reduce temperature underestimation.

TSI was also performed on 6 more homogeneous gelatin-castor oil phantoms with castor oil concentrations from 0 – 50%. Imaging occurred immediately after the phantoms were removed from the freezer because phantoms with high castor oil concentrations were mechanically unstable at room temperature. TSI was performed at 5 different spatial locations in each phantom with and without clutter. Clutter was introduced by placing layers

of copper wire mesh (Fly-Bye Bird Control Products, WA, USA) between the transducer face and the phantom (Lediju et al. 2008). A single layer of mesh has pore sizes of approximately 0.5 X 0.5 cm (Fig. 1c). The level of noise generated by the copper mesh was measured relative to an anechoic background and found to be 27.5 dB, 28.9 dB, and 31.6 dB for 2, 3, and 4 layers of mesh respectively. For the gelatin-castor oil phantoms, two layers of clutter were used. A total of 60 TSI measurements were made across 6 phantoms. The theoretical elevational beamwidth of the ultrasound heating beam was 1.01 mm at 4.6 MHz which made thermocouple measurements across different phantoms sensitive to elevational misalignment errors. In order to obtain a better temperature estimate, 5 temperature measurements with and without clutter were obtained for each castor oil concentration. Between temperature measurements, the ultrasound probe was raised, lowered, and re-aligned with the thermocouple. As a result, the variation in the temperature measurements incorporate error inherent to the thermocouple ($<0.1^{\circ}\text{C}$) as well as errors relating to the alignment of the ultrasound probe and thermocouple. A total of 60 temperature measurements were taken across 6 phantoms.

Liposuction Phantom

Liposuction fat was collected from a patient undergoing body contouring surgery for massive weight loss. University of Pittsburgh Internal Review Board (IRB) exemption was obtained for the use of human tissues because they met criteria for medical waste. Following liposuction, fat was centrifuged at 1286g for 3 minutes in order to remove aqueous and oil layers from intact adipose tissue. A two layered phantom was constructed. Both layers were approximately 25 mm thick. The top layer was a gelatin layer and the bottom layer was fresh liposuction fat.

TSI was performed with 0, 2, and 4 layers of copper mesh. A motor-controlled translation stage was used to raise and lower the transducer so that clutter could be introduced without disturbing the imaging plane. In this manner, paired, cluttered and clutter-free images were obtained. An independent set of paired clutter-free images were obtained for 2 and 4 layers of clutter. This process was repeated at 5 spatial locations corresponding to a total of 20 TSI acquisitions.

Ex vivo Carotid Endarterectomy (CEA)

Informed consent to obtain a surgical specimen was granted by a 73 year old female asymptomatic patient with $>70\%$ carotid stenosis previously scheduled to undergo CEA surgery. During surgery, the atherosclerotic plaque, intimal, and some medial portions of the carotid artery were removed. This sample was retrieved and frozen at -80°C with University of Pittsburgh IRB approval. At a later date, the sample was defrosted and embedded in a gelatin phantom.

TSI was performed and images were acquired every 0.5 – 1 mm along the short axis of the artery using the translation stage. Spatially matched imaging with and without clutter was performed using 0 and 3 layers of clutter. Following imaging, the CEA sample was carefully extracted from the gelatin and immersed in formalin. The sample was sectioned every 1 mm

along the short axis and stained using Oil red O staining. B-mode ultrasound was compared to histology to find matching sections.

TSI Quality Metrics and Statistical Analysis

Three metrics were used to evaluate the quality of TSI images in this article. First, the experimentally measured value for λ was calculated as

$$\lambda_{exp} = \frac{1}{\Delta T} \frac{du}{dz} \quad (\text{Eq. 3})$$

where T is the temperature measured by thermocouple, and $\frac{du}{dz}$ is the experimentally calculated apparent strain. When multiple temperature measurements were available, this error was propagated into the final calculated value of λ_{exp} . When multiple temperature measurements were not available, the error was assumed to be 27% which is experimentally measured variation for a gelatin (0% castor oil) phantom heated using a 5% duty cycle. This metric provides a temperature-normalized measure of the relative biases of each TSI tracking mode. In addition, it is an important parameter for NIT measurements because it must to be known in order to calculate the absolute temperature change.

Homogeneous regions, were evaluated using signal-to-noise ratio (SNR) which was defined as

$$SNR = \frac{|\mu|}{\sigma} \quad (\text{Eq. 4})$$

Here, μ and σ are the mean and standard deviation of the apparent strain within the heated region which is represented by red dashed lines in Fig. 1b.

For inhomogeneous regions, the contrast-to-noise ratio (CNR) was used to measure image contrast and overall image quality. It was defined as

$$CNR = \frac{\mu_{lipid} - \mu_{water}}{\sqrt{\sigma_{lipid}^2 + \sigma_{water}^2}} \quad (\text{Eq. 5})$$

Here, μ_{lipid} and μ_{water} represent the mean apparent strain within the lipid and water-based regions respectively. Similarly, σ_{lipid} and σ_{water} represent the standard deviation of the apparent strain within the lipid and water-based regions. Lipid and water-based regions were chosen such that they had the same area.

The percent true and false positive areas were also measured. The percent true positive area was the percentage area with positive strain greater than 0.1% strain within a known *lipid region*. Conversely, the percent false positive area was the percentage area with positive strain greater than 0.1% strain within a known water-based (gelatin) region. The 0.1% threshold was chosen in order to prevent small, unreliable strains from influencing the calculation.

A one-tailed, paired t-test was used to evaluate statistical differences. The difference between PIHI and 6.6 MHz tracking and between PIHI and 3.3 MHz tracking was evaluated.

For the liposuction phantom, differences in the strain estimates before and after addition of clutter were also evaluated. Thus, a maximum of three comparisons were used in order to minimize the probability of type I error. A paired t-test was used because all three tracking methods were implemented within a single pulse sequence such that the temporal and spatial variation in the targets was negligible. In the case of the liposuction phantom, a mechanical translation stage was used to ensure spatially matched images before and after the addition of clutter. A one-tailed test was used because the goal was only to test the hypothesis of whether PIHI tracking improved strain estimates as compared to 6.6 MHz and 3.3 MHz tracking. In all cases, when $p < 0.05$, differences were considered significant. MATLAB R2014a (MathWorks Inc., MA, USA) was used for all statistical analyses.

Results & Discussion

Homogeneous Gelatin Phantom Experiment

Fig. 2 shows the results for the homogeneous gelatin (0% castor oil) phantom imaged using TSI. The heating duty cycle was varied from 0.1 – 10% which produced monotonically increasing T on the range of $<0.10 - 0.78^\circ\text{C}$. A single temperature measurement was taken for each duty cycle in order to avoid potential damage to the transducer at higher duty cycles. Fig. 2a shows λ_{exp} as a function of T . We observe that λ_{exp} converges to a value of approximately $0.13 \text{ }^\circ\text{C}^{-1}$ for $T > 0.26^\circ\text{C}$ (4% duty cycle). Although λ_{exp} converged to a smaller value for fundamental as compared to PIHI tracking, the difference was not statistically different. Prior studies using ultrasound tracking simulations have shown that a 5–10% bias in displacement can be expected in the presence of stationary clutter or lower tracking transmit frequencies (Palmeri et al. 2006; Pinton et al. 2006). The reason statistically significant bias was not reproduced in this study was likely the result of uncertainty in the thermocouple measurements. In the context of the literature, these data suggest that if a large enough region is chosen, a wide range of tracking frequencies will provide estimates of the apparent strain that agree within 5–10% of each other.

Fig. 2b shows SNR as a function of T . The region over which the SNR was calculated is shown in Fig. 1b and has an area equal to 125 mm^2 . We observe that the trend in SNR parallels the trend in λ_{eff} . In other words, for $T > 0.26^\circ\text{C}$, the measured SNR appears to converge to a fixed value for all imaging modes. In addition, PIHI tracking improves the SNR by an average of 21% and 124% as compared to the 6.6 MHz and 3.3 MHz tracking respectively for $T > 0.10^\circ\text{C}$ (Fig. 2b, $p < 0.05$). This improvement is largely due to decreased spatial variation in the PIHI tracked thermal strain image. The decreased spatial variation is likely due to two factors which have improved the variance in the original displacement estimate. First, the PIHI tracking beam has a f_c close to 6.6 MHz. Doubling the f_c relative to the 3.3 MHz tracking pulse will decrease the variance in the displacement tracking (Palmeri et al. 2006). In addition, because the pulse inversion signal is proportional to the square of the fundamental signal, the PIHI tracking beam will have side lobes that are 6 dB lower than the side lobes for the 6.6 MHz tracking beam and will reduce off-axis scattering and further decrease variance in the displacement estimate. For $T < 0.10^\circ\text{C}$, the apparent strain has a very small magnitude such that there is effectively no signal which likely reflects a lower temperature limit for reliable TSI and NIT measurements.

Additionally, for NIT applications, the data in Fig. 2 suggest that if a large region of interest ($>100 \text{ mm}^2$) is chosen, temperature estimation will agree within 5–10% over a range of tracking frequencies. However, if the goal is to estimate the temperature rise in a small region of interest ($<10 \text{ mm}^2$), higher tracking frequencies and PIHI will provide more precise estimates of the temperature change. These tradeoffs might be important to consider in the case of monitoring of HIFU liver ablation where the penetration achieved with a lower frequency might need to be balanced against accurate temperature estimation within a pre-defined treatment area.

Gelatin-Castor Oil Phantom Experiment

Fig. 3a shows λ_{exp} as a function of castor oil percentage. Similar to Fig. 2a, the value of λ_{exp} is not statistically different for fundamental and PIHI tracking. This is true both with and without clutter. As previously noted, simulations have shown that a small degree of bias is introduced in the presence of stationary echoes or lower tracking frequencies (Palmeri et al. 2006; Pinton et al. 2006). In spite of these simulations, Doherty et al also experimentally found that clutter corrupts displacement tracking primarily by introducing variance to the estimate (Doherty et al. 2013). Our findings are consistent with those findings because the derivative of an unbiased or minimally biased estimate should also be unbiased or minimally biased.

The literature reports that for pure castor oil, λ is approximately $-0.13 \text{ }^\circ\text{C}^{-1}$ at room temperature (Timme 1972). We found that small percentages of castor oil initially resulted in an increase in sound speed as compared to pure gelatin. This means that the speed of sound further increases with increasing temperatures. Above 10% castor oil, λ_{exp} decreases monotonically for increasing castor oil concentrations. While this finding was unexpected, Nguyen et al reported a similar trend between absolute sound speed and castor oil concentration suggesting that our measurement might be a consistent physical phenomena resulting from low concentrations of oil in gelatin (Nguyen et al. 2014b).

Miller et al previously used a mixture model to simulate the effects of mixtures of materials on sound speed properties (Miller et al. 2004). Our data suggests that for low percentage mixtures, Miller et al's model might not be valid because their model only produced monotonic changes in sound speed properties. These are empirical findings and, to the best of the authors' knowledge, no thorough investigations of the physics underlying the relationship between λ and material composition have been completed (Duck 2013). Additionally, our data suggest that for NIT applications where ablation is being performed in the presence of fatty liver disease, temperature underestimation is likely if λ is not appropriately adjusted. Further studies are required in order to fully understand the properties of tissue mixtures that might be found in fatty liver disease or atherosclerotic plaques.

Fig. 3b shows the SNR as a function of castor oil percentage. Like Fig. 2b, it shows that, in the absence of clutter, PIHI tracking results in a better SNR than fundamental tracking. With the introduction of clutter, all tracking schemes suffer a decrease in SNR. However, PIHI tracking still provides a SNR which is the same or better than 6.6 MHz fundamental tracking without clutter. In the absence of clutter, PIHI tracking improves the SNR by an average of

36.4% and 145% as compared to 6.6 MHz and 3.3 MHz tracking respectively (Fig. 3b, $p < 0.05$). In the presence of this clutter, the advantage of PIHI tracking grows to an average SNR improvement of 52.1% and 213% as compared to 6.6 MHz and 3.3 MHz tracking respectively (Fig. 3b, $p < 0.05$). For the 50% castor oil phantom, the apparent strain and, as a result, the SNR are very small for all tracking modes. Although the trend is consistent when compared with the other phantoms, a larger number of independent images would be required to detect a significant difference.

The data from Fig. 2 and Fig 3, when taken together, show that for $T > 0.26^{\circ}\text{C}$, the SNR is correlated with λ_{exp} but not further increases in T . Interestingly, these data suggest that only a small temperature increase well below American Institute of Ultrasound in Medicine thermal safety limits of 1.5°C is necessary to achieve an optimal SNR. This is not only important from a safety standpoint, but also because it is expected that attenuation and phase aberration will decrease the maximum acoustic pressure of the heating beam *in vivo* resulting in a smaller maximum temperature rise. These data show that, for TSI, acceptable SNR might be achieved *in vivo* even with lower than expected temperature increases. This might be important for the feasibility of TSI in hepatic steatosis due to the depth of the liver and attenuation of the ultrasound beam from thick, superficial, abdominal fat. In addition to temperature rise generated, the inherent material properties, specific tracking configuration, and signal processing steps will determine the absolute SNR level.

Liposuction Phantom

Data for the liposuction phantom are presented in Fig 4, Fig 5, and Table 1. In Fig. 4, the B-mode and TSI images are shown for a single spatial location with no clutter and two layers of clutter. Black dashed lines indicate the -6 dB beamwidth for the heating beam. The processed liposuction fat is expected to be even more homogeneous than native adipose tissue and PIHI tracking provides a qualitatively smoother TSI estimate than fundamental tracking. This is true in the absence of clutter and with two layers of clutter. It can be seen that the magnitude of the apparent strain in the images with clutter is smaller than in the images without clutter. This is because the copper mesh attenuates the heating beam resulting in a smaller temperature increase. In gelatin phantom experiments, the temperature difference with and without clutter was found to be $0.030 - 0.13^{\circ}\text{C}$.

Fig. 5 and Table 1 show quantitative analyses for the liposuction data. Examples of lipid and water-based (gelatin) regions chosen for these calculations are shown in Fig. 4b. The interface between the liposuction fat and gelatin was not perfectly flat. As a result, the exact regions chosen varied slightly depending on the spatial location imaged. In Fig. 5, CNR, percent true positive area, and percent false positive area are presented for 0, 2, and 4 layers of clutter. The statistical analyses of the magnitude of differences between the different TSI tracking modes are shown in Table 1.

Clutter degrades the performance of all TSI tracking modes in the liposuction phantom in a manner consistent with what was seen previously for the gelatin-castor oil phantoms. Furthermore, as might be expected, the degree of performance degradation is correlated to the number of layers of clutter used. PIHI tracked TSI has suffers the smallest degradation to performance. It maintains the highest CNR, the highest true positive area, and the smallest

false positive area. This advantage grows when more layers of clutter are used. CNR is a useful metric to evaluate image contrast when the imaging target is inhomogeneous. This is relevant to TSI applications in which the goal is to differentiate a lipid rich core from the surrounding atherosclerotic plaque. The percent true and false positive areas are relevant metrics for atherosclerotic plaques, but are also relevant to imaging relatively homogeneous structures like livers with suspected fatty liver disease.

B-mode, TSI, and Oil red O histology images of the *ex vivo* CEA sample are shown in Fig. 6. In Oil red O staining, lipid rich regions appear red. Based on the histology, red and blue dashed boxes were drawn to mark lipid rich and lipid poor regions, respectively. Matching regions are marked in the TSI imaging. The 3.3 MHz tracking overestimates the extent of the lipid region within the artery and produces a large number of false positive lipid regions in the surrounding gelatin. In 6.6 MHz tracking, the number of false positive lipid regions is drastically reduced and it produces an accurate representation of the lipid deposits within the artery. The images in the third row are spatially matched to the second row, but were taken in the presence of three layers of clutter. After the introduction of clutter, 3.3 MHz tracking produces false positives within the artery in the blue dashed box. The homogeneity within the surrounding gelatin region is also degraded. In the 6.6 MHz tracked image, the lipid rich region marked by the red dashed box is lost. After the introduction of clutter, the PIHI tracked image is the most spatially comparable with the histology image and qualitatively provides best CNR between lipid and water-based regions within the artery. Overall, the CEA data show that the findings previously demonstrated using simplified phantom setups are applicable to real, complex tissues. PIHI-based tracking has potential to improve TSI and NIT estimates across a range clinical applications where clutter might affect image quality.

This study is subject to several limitations resulting from practical constraints in the experimental design. The first limitation was the bandwidth (3.3 – 6.6 MHz) of the ATL L7-4 transducer used in this study. Ideally, this transducer would have had a bandwidth that extended beyond 6.6 MHz. This is because the harmonic signal has a finite bandwidth which should be captured within the passband of the transducer. Although the ATL L7-4 was able to receive harmonic signals satisfactorily, a transducer with a broader bandwidth would be expected to provide further improved harmonic signal transduction which might further improve strain estimation.

Another limitation of this study is that experiments were conducted at 25°C in order to prevent the gelatin from melting. The absolute value of λ is known to change with temperature (Miller et al. 2002). Nevertheless, we found that for the gelatin (0% castor oil) phantom between approximately 5.0 – 20°C, λ was within $\pm 10\%$ of the mean value. For pure castor oil, λ was approximately constant between 25 – 40°C. These findings suggest that the data in Fig. 2a and Fig 3a are likely to be internally consistent. However, it is still possible that these values are different than the actual values of λ for *in vivo* water-based tissues. This concern is somewhat alleviated because data in Fig. 3b, show that PIHI tracking consistently improves performance for a wide range of λ . Nevertheless, reference values for λ should be carefully measured for NIT applications which deviate significantly from the temperature ranges provided for this study in order to obtain accurate estimates of λ .

Conclusion

Pulse inversion harmonic tracking is combined with thermal (temporal) strain imaging in order to improve estimates of apparent strains. The improvement is quantified by comparing the strains calculated using PIHI tracking against the strains calculated from both 6.6 MHz and 3.3 MHz fundamental tracking. For large homogeneous targets $>100 \text{ mm}^2$, precise measurements can be made independently of tracking frequency; however, for small homogeneous targets, the SNR will play a role in determining the precision of the estimate. In homogeneous oil-gelatin phantoms with clutter, PIHI tracking provides an average SNR improvement of 52.1% and 213% as compared to 6.6 MHz and 3.3 MHz tracking. In liposuction phantoms with clutter, the contrast-to-noise ratio is degraded by 35.1% for PIHI as compared to 43.7%, and 62.4% for 6.6 MHz and 3.3 MHz tracking respectively. This degradation in CNR is accompanied by an increase in false positives and decrease in true positives that parallels the trend in CNR. These changes to image quality were reflected in *ex vivo* CEA data which showed that PIHI tracking reduces false positives, identifies true positive lipid regions, and maintains the CNR between lipid rich regions and the background. Pulse inversion-based tracking can be used to improve thermal (temporal) strain estimates with applications in identification of the lipid rich cores of atherosclerotic plaques as well as quantifying hepatic steatosis.

Acknowledgments

This work was supported by NIH grants 5R01HL098320 (PI: Kim). Student training was supported by NIH training grant 5T32HL076124 (PI: Shroff).

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Figure 1.

Schematic for TSI pulse sequence using PIHI, B-mode image showing thermocouple positioning, and a photographic image of the copper mesh. The overall TSI sequence, as well as individual pulse sequences for imaging and heating are shown in a). The duration of the heating portion of the sequence is fixed at 2 seconds and a variable length “wait period” is used to control the heating duty cycle. For temperature measurements, the tip of the thermocouple was placed at 25 mm axially and 0 mm laterally as shown in b). The red dashed lines designate the region used to calculate the SNR. A single layer of copper mesh is shown in c).

Figure 2.

Results for the homogeneous gelatin (0% castor oil) phantom. In a), λ_{exp} as a function of the measured temperature change is shown for three different TSI tracking modes. In b), the SNR in the heated region as a function of measured temperature changes is calculated. The “*” designates the temperatures over which PIHI tracking provides better SNR than both 6.6 MHz and 3.3 MHz tracking ($p < 0.05$).

Figure 3.

Results for the mixed gelatin-castor oil phantoms. In a), λ_{exp} as a function of % castor oil is shown for three different TSI tracking modes with zero layers of clutter. The same data are shown with two layers of clutter in b). In c), the SNR in the heated region as a function of % castor oil is calculated. The “*” designates the temperatures over which PIHI tracking provides better SNR than both 6.6 MHz and 3.3 MHz tracking with no clutter (solid line) and with two layers of clutter (dotted line).

Figure 4.

A set of spatially matched images of TSI using 3.3 MHz, 6.6 MHz, and PIHI tracking with zero and two layers of clutter in the liposuction phantom. a), b), and c) show the B-mode reference images used for tracking. The dynamic range is in dB. Gelatin and liposuction layers are labeled in b). TSI images with no clutter are shown in d), e), and f). TSI images with 2 layers of clutter are shown in g), h), and i). In d) – i), the scale bar indicates % strain. Black dashed lines designate the -6 dB width of the heating beam.

Figure 5.

The CNR, true positive area, and false positive area for liposuction phantom for varying degrees of clutter. In a), the CNR for 3.3 MHz, 6.6 MHz, and PIHI TSI tracking modes are calculated for zero, two, and four layers of clutter. In b), the true and false positive percent area within the lipid and gelatin regions, respectively, are shown for the same conditions.

Figure 6.

A set of spatially matched images of a CEA sample with TSI using 3.3 MHz, 6.6 MHz, and PIHI tracking with no clutter and with three layers of clutter. a), b), and c) show the B-mode reference images used for tracking. The scale bar indicates the dynamic range in dB. The heated region for TSI is overlaid on the reference B-mode image in e), f), and g). Spatially matched images tracked in the presence of three layers of clutter are shown in i), j), and k). The scale bar for TSI images is the dynamic range in % strain. A gross image of the matching Oil red O histology is shown in d) and red staining indicates the presence of lipids. Red and blue dashed boxes designate lipid rich and lipid-poor regions and are magnified in h) and l) respectively.

Table 1

Differences in TSI Tracking Mode Performance for Liposuction Phantom

	Pair Compared	CNR (%)	True Positive (%)	False Positive (%)	
PIHI	6.6 MHz	NS	2.78*	3.65*	No clutter
PIHI	3.3 MHz	63.9**	NS	15.4***	
6.6 MHz	3.3 MHz	52.2*	2.76*	11.8**	
PIHI (0X)	PIHI (2X)	NS	3.84*	2.38*	2X clutter
6.6 MHz (0X)	6.6 MHz (2X)	NS	4.63**	NS	
3.3 MHz (0X)	3.3 MHz (2X)	NS	4.28*	NS	
PIHI (0X)	PIHI (4X)	35.1*	13.0*	11.1***	4X clutter
6.6 MHz (0X)	6.6 MHz (4X)	43.7**	16.6*	15.3***	
3.3 MHz (0X)	3.3 MHz (4X)	62.4*	15.3***	17.1***	

Tracking modes are compared against each other in the absence of clutter. They are also compared against themselves in the presence of two and four layers of clutter. For CNR, the percent difference is tabulated. For true and false positives, the absolute difference expressed as the percent area is tabulated.

* $p < 0.05$,
 ** $p < 0.01$,

*** $p < 0.001$, NS = Not significant