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Quantitative Ultrasound for Monitoring High-Intensity Focused Ultrasound Treatment *In Vivo*

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Abstract

The success of any minimally invasive treatment procedure can be enhanced significantly if combined with a robust noninvasive imaging modality that can monitor therapy in real time. Quantitative ultrasound (QUS) imaging has been widely investigated for monitoring various treatment responses such as chemotherapy, radiation, and thermal therapy. Previously, we demonstrated the feasibility of using spectral-based QUS parameters to monitor high-intensity focused ultrasound (HIFU) treatment of *in situ* tumors in euthanized rats [*Ultrasonic Imaging* 36(4), 239–255, 2014]. In the present study, we examined the use of spectral-based QUS parameters to monitor HIFU treatment of *in vivo* rat mammary adenocarcinoma tumors (MAT) where significant tissue motion was present. HIFU was applied to tumors in rats using a single-element transducer. During the off part of the HIFU duty cycle, ultrasound backscatter was recorded from the tumors using a linear array co-aligned with the HIFU focus. A total of 10 rats were treated with HIFU in this study with an additional sham-treated rat. Spectral parameters from the backscatter coefficient, i.e., effective scatterer diameter (*ESD*) and effective acoustic concentration (*EAC*), were estimated. The changes of each parameter during treatment were compared with a temperature profile recorded by a fine-needle thermocouple inserted into the tumor a few millimeters behind the focus of the HIFU transducer. The mean *ESD* changed from 121 ± 6 to 81 ± 8 μm (p -value = 0.0002), and the *EAC* changed from 33 ± 2 to 46 ± 3 dB/cm^3 (p -value = 0.0002) during HIFU exposure as the temperature increased on average from $38.7 \pm 1.0^\circ\text{C}$ to $64.2 \pm 2.7^\circ\text{C}$. The changes in *ESD* and *EAC* were linearly correlated with the changes in tissue temperature during the treatment. When HIFU was turned off, the *ESD* increased from 81 ± 8 to

$121 \pm 7 \mu\text{m}$ and the EAC dropped from 46 ± 3 to $36 \pm 2 \text{ dB/cm}^3$ as the temperature decreased from $64.2 \pm 2.7^\circ \text{C}$ to $45 \pm 2.7^\circ \text{C}$. QUS was demonstrated *in vivo* to track temperature elevations caused by HIFU exposure.

Index Terms

Backscatter coefficient (BSC); focused ultrasound; quantitative ultrasound (QUS) imaging; ultrasound-based therapy

I. Introduction

High-intensity focused ultrasound (HIFU) is a noninvasive thermal therapy approach for treating tissue for various diseases including cancer. HIFU treatment can heat or ablate tissue in the focal region of the transducer without damaging the surrounding healthy tissue. Thermal therapy remains an important cancer therapy option because it not only kills the cancer cells but can also activate anticancer immunity in the body [1].

HIFU is a noninvasive procedure with various advantages compared to invasive procedures, such as less blood loss, decreased pain and need for postoperative medications, quick patient recovery, and reduced scarring. HIFU is currently being tested and, in some cases, adopted for clinical therapy [2]. Several investigators have reviewed clinical studies involving HIFU to treat diseases [2], [3]. Currently, HIFU therapy has been approved in the United States for the treatment of uterine fibroids and bone metastases [4], [5]. HIFU is being investigated for treatment of various diseases such as the cancer of the prostate, breast, liver, and to treat Parkinson's disease.

The recent success of HIFU treatment largely depends on the availability of a robust imaging and monitoring system [6]. Various imaging modalities such as ultrasound, X-ray CT, and MRI have been investigated for guiding and monitoring HIFU treatment. Currently, magnetic resonance thermal imaging (MRTI) is considered as the best-suited imaging modality for guiding HIFU (MRgHIFU). An accuracy of 1°C and update time of 1 s has been reported for MRI [7]. The spatial resolution of MRI thermometry imaging is around 2 mm in stationary tissue. Proton-resonance frequency approaches to MRI have achieved near-real time (i.e., 10 Hz) imaging [8]–[10]. However, these techniques require predictable motion patterns in order to register image frames. Finally, MRI is expensive and requires an MRI compatible HIFU system for the treatment. Moreover, MRI cannot currently provide a real-time monitoring capability and may inaccurately estimate tissue temperature with motion artifacts or low signal-to-noise ratio (SNR). Finally, the accessibility of MRgHIFU is limited in North America.

Significant research has been conducted to develop robust, portable, accessible, and low-cost imaging methods for monitoring HIFU treatments [11]–[14]. Foremost among these are ultrasound imaging techniques, which have been widely investigated for monitoring ablation techniques including HIFU treatments. Ultrasound imaging is low cost, uses nonionizing radiation, has real-time capabilities, and is portable with the potential to increase accessibility [15]. Quantitative ultrasound (QUS) imaging techniques involving estimation

of changes in sound speed, backscatter, attenuation, stiffness, and strain in tissue have been investigated to monitor thermal therapies. Changes in sound speed have been correlated to temperature changes during HIFU exposure [16], [17]. Some investigators have explored spectral-based ultrasound techniques to monitor the temperature changes in tissues treated with HIFU [18]–[20]. Changes in the backscatter coefficient (BSC) have been observed in coagulated tissue formed by HIFU exposure when compared with normal tissue [21]. Ultrasound attenuation and backscatter have been used to detect lesion formation during HIFU exposure [22]. However, all of these techniques have yet to be adopted clinically and issues with tissue motion or nonlinear changes in the parameter space versus temperature may limit their adoptability. However, it must be noted that recent advances in adaptive filter design for motion compensation and high frame rate imaging have alleviated some of the sensitivity to tissue motion of frame-to-frame correlation-based methods for estimating temperature in tissues [23]–[25].

As an alternative, we have been developing QUS imaging techniques that can be used for both assessment [26]–[28] and monitoring of HIFU therapy [29]–[31]. In experiments using biological phantoms and fresh liver samples in a saline bath, changes in spectral-based QUS parameters [i.e., the effective scatterer diameter (*ESD*) and effective acoustic concentration (*EAC*)] were correlated to temperature elevations in tissues [30], [32]. The accumulation of research findings by several groups clearly indicates that heating of tissues, such as that occurs with HIFU exposure, induces changes in tissue microstructure. Because ultrasound scattering depends on the properties of the propagating media, HIFU can be monitored by analyzing the backscattered signal during exposure. In our previous study, we examined spectral-based QUS parameters for their ability to track temperature elevations in tumors in euthanized rats induced by HIFU with no tissue motion or perfusion [29]. In the present study, we are extending our previous work by using spectral-based QUS parameters (i.e., the *ESD* and *EAC*) to monitor *in vivo* HIFU treatment in a rat mammary adenocarcinoma where significant tissue motion is present. The changes in the various spectral-based QUS parameters were monitored during the HIFU exposure and compared with the temperature profile recorded from a fine needle thermocouple inserted into the tumor behind the focus of the HIFU transducer. The shape and the amplitude of the frequency-dependent BSC were analyzed to monitor *in vivo* HIFU exposure.

II. Methods

A. Animal Model and Handling

The experimental protocol was approved by the Institutional Animal Care and Use Committee (IACUC), University of Illinois, Urbana-Champaign and satisfied all University and NIH rules for the humane use of laboratory animals. All the animals ($N = 11$) were provided food *ad libitum*. Mammary adenocarcinoma tumors (MAT B III) cells [American Type Culture Collection (ATCC), Manassas, VA, USA] were cultured and used for growing mammary tumors in rats. The cells were cultured in ATCC-formulated McCoy's 5a Medium Modified along with 10% fetal bovine serum (HyClone Laboratories, Logan, UT, USA) and 1% penicillin-streptomycin (HyClone Laboratories, Logan, UT, USA). Approximately, 500 cells were suspended in saline (100 μL) and injected subcutaneously into the fat pad of a rat

to grow a tumor. Specifically, female 10–21 week-old Fischer 344 rats (Harlan Laboratories, Inc., Indianapolis, IN, USA) were used in the experiments. At the time of injection of the MAT cells, the weight of the rats was recorded. When a tumor reached a size of 8–9 mm in diameter, the ultrasound experiment was conducted. During a HIFU exposure, the rat was anesthetized using isoflurane gas through a mask covering the mouth and nose of the rat. The hair over the tumor was shaved and depilated to maximize sound transmission. The rats were placed on a specially designed holder and partially submerged in a tank of degassed water maintained at 37° C such that the tumor was under water.

B. Ultrasound Method

Ten of the rat tumors were treated with HIFU and one rat tumor was sham treated. The sham-treated rat was used to quantify the effects of breathing on QUS estimates without HIFU. All experiments were conducted *in vivo*. The HIFU system consisted of a 1-MHz (f 1.1) transducer with aperture diameter of 4.7 cm. An arbitrary waveform generator (HP 33120a, Agilent Technologies, Santa Clara, CA, USA) was used to generate a long duty cycle tone burst. The tone burst was amplified by an A150-55-dB power amplifier (ENI, Rochester, NY, USA). The –6 dB beamwidth and –6 dB depth of field of the HIFU transducer was characterized using a needle hydrophone (Precision Acoustics, Dorchester, U.K.) in degassed water at 37° C. The –6 dB beamwidth and depth of field were 1.8 mm and 5.2 cm, respectively. During the off cycle of the duty cycle, an L14-5/38 imaging probe attached to a SonixRP ultrasound imaging system (Ultrasonix, Richmond, BC, Canada) recorded a single ultrasonic imaging frame. The SonixRP system provided the raw radio frequency (RF) signals at a sampling frequency of 40 MHz. The imaging array had a center frequency of 6 MHz as estimated from recorded signals in pulse-echo mode from a planar reflector. An analysis bandwidth of 4–9 MHz was used in the subsequent spectral analysis.

A custom holder was machined to align the focus of the HIFU transducer with the imaging plane of the imaging array. Once the HIFU transducer and imaging array were placed in the holder, no further adjustment was needed. Fig. 1 illustrates the experimental configuration and the custom holder that was machined. To continuously estimate temperature in the tumor, a needle thermocouple (HYP1-30-1/2-T-G-60-SMPWM, Omega Engineering, Inc., Stamford, CT, USA) was inserted into the tumor behind the focus of the HIFU source. The thermocouple was placed behind the focus of the HIFU source for two reasons: 1) this placed the thermocouple out of the way of the region undergoing RF analysis and 2) to reduce the presence of viscous heating artifacts [33]. The tumor is identified by the red dot in Fig. 1(a). The needle thermocouple had a diameter of 0.3 mm and a length of 15 mm. The output of the thermocouple was connected to a temperature reader (NI USB-TC01, National Instruments Corporation, Austin, TX, USA). The reader was connected to a computer and the temperature was recorded to hard drive once every second. In order to maintain the temperature of the degassed water bath, in which the rat was partially submerged, at 37° C a heating element was placed in the bath and controlled by an automated temperature controller. A computer-controlled micropositioning system (Parker Hannifin Corporation, Rohnert Park, CA, USA) was used during the experiment to move the HIFU transducer and imaging probe.

HIFU was used to elevate the temperature in the tumor. A spatial peak temporal average intensity (I_{SPTA}) of 502 W/cm^2 was used in the HIFU exposures with 75% duty cycle and 30 Hz pulse repetition frequency. The intensity of 502 W/cm^2 was used based on our previous studies, where visible tissue thermal damage was observed from histopathology analysis [43]. The I_{SPTA} was estimated from pressure measurements at the focus of the HIFU source in degassed water using a needle hydrophone (Precision Acoustics, Dorchester, U.K.). RF imaging frames of the tumor were collected by the SonixRP system during the therapy by synchronizing the acquisition of RF imaging frames with the off portion of the HIFU exposure duty cycle [see Fig. 1(c)]. At the end of each HIFU tone burst, the SonixRP was triggered on to record a single RF imaging frame. The acquisition of the RF data was required to calculate the BSC from the tumor. The tumors were exposed for a duration of 60 s.

C. Processing Procedures

B-mode images of a tumor intersecting the HIFU focus were constructed from the RF data acquired by the SonixRP. A region of interest (ROI) was manually drawn in the B-mode image corresponding to the tumor area. The ROI was used to localize the tumor region for the spectral analysis of the backscattered RF echoes. The pulse length (PL) was measured as 0.37 mm. Data blocks of size $7 \text{ PL} \times 7 \text{ PL}$ were constructed within the ROI. The data blocks in the ROI had an 80% overlap between adjacent data blocks both axially and laterally. Bias and variance of QUS estimates are related to the time-bandwidth product of the range gate length (axial data block size) [34]. Therefore, the data block size was quoted in terms of the number of PLs. A data block $7 \text{ pulse lengths} \times 7 \text{ pulse lengths}$ was chosen to provide good QUS estimates in terms of low bias and low variance versus data block size. An 80% overlap of data blocks provides the ability to enlarge the data blocks while reducing the pixel sizes of the QUS image maps.

Fig. 2 shows an image depicting the selection of the data blocks within the ROI. First, the ROI, corresponding to the tumor boundary, was outlined as depicted by the red line in Fig. 2. Next, data blocks were created inside the ROI boundary using a custom MATLAB (MathWorks, Natick, MA, USA) code to automate the selection of the data blocks. The BSC was estimated for each scan line in a data block and averaged to provide the BSC estimate for the data block. Calibration of the BSC was accomplished using the reference phantom technique and a well-characterized phantom [35]. Specifically, the BSC was calculated from the averaged power spectrum from the windowed scan lines from a data block. This averaged power spectrum was divided by an averaged power spectrum calculated from a data block at the same depth in the well-characterized reference phantom. To estimate the BSC, the averaged power spectrum from the sample data block was divided by the averaged spectrum from the reference phantom data block and corrected for attenuation differences between the sample and phantom. ESD and EAC were then estimated from the BSC for each data block using a spherical Gaussian model [36].

In a separate set of experiments, the insertion loss technique was used to estimate attenuation from untreated excised tumors ($n = 4$) [37]. In these experiments, the tumor from a rat was excised and any overlaying skin removed prior to scanning. An attenuation slope

was estimated for each of the excised tumor samples and averaged. The average attenuation slope of 0.7 ± 0.1 dB/cm/MHz was used to compensate the power spectra for losses in the RF signals due to frequency-dependent attenuation.

The average attenuation slope was used to compensate the power spectrum for frequency-dependent losses for each recorded RF frame in the *in vivo* HIFU experiments. Because we used a single attenuation value throughout the *in vivo* experiments, differences in the attenuation due to biological variability between rat tumors, temperature elevations, or from irreversible tissue changes were not used in the compensation of the BSC. However, the size of the tumors was less than 1 cm; therefore, differences in attenuation from the average attenuation would not result in large errors in BSC estimates over the frequency ranges used in the analysis. Based on results from our previous *in situ* study, it was assumed that attenuation effects would be small for the current study [29].

A spherical Gaussian scattering model and rapid estimator was used to extract *ESD* and *EAC* estimates from the BSC for each data block in a tumor [36], [38]. The change in *ESD* and *EAC* for each data block was estimated with respect to its initial value before the HIFU exposure. For example, the change in *EAC* after 5 s is given by

$$\Delta EAC = \frac{EAC(5\text{ s}) - EAC(0\text{ s})}{EAC(0\text{ s})} \quad (1)$$

where *EAC*(0 s) and *EAC*(5 s) refer to *EAC* at the beginning of the exposure just before HIFU exposure and 5 s after the start of the HIFU exposure, respectively. The changes in the *ESD* estimates versus exposure time were similarly calculated.

The changes in *ESD* and *EAC* were assessed through a Kruskal–Wallis one-way analysis of variance by ranks when comparing the estimates at three different time points. Statistically significant differences were quantified through *p*-values. Case I corresponded to a time point just before exposure. Case II corresponded to a time point just before the last HIFU tone burst was delivered (i.e., the peak temperature). Finally, Case III corresponded to a time point after the tissue was cooled to post exposure. Based on continuous thermocouple readings from the tumor, a return to 37° C typically occurred 5–10 min after the HIFU was turned off. In addition, linear curve fitting was performed between the recorded temperature (*x*-variable) and *ESD* or *EAC* (*y*-variable). Specifically, the relationship between the *ESD* and *EAC* parameters and temperature during the exposure was quantified both for the heating period (HIFU on) and during the cooling period (HIFU off).

III. Results

Fig. 3 shows representative B-mode images and QUS images enhanced by *ESD* and *EAC* of a rat tumor at different time points during HIFU exposure. The temperature profile recorded by the thermocouple and the changes in the *ESD* and *EAC* as a function of time are shown in Fig. 3 (right). The *ESD* and *EAC* curves were generated by averaging the QUS parameters over the whole ROI and calculating the percent difference between the QUS parameter at

each time point and the QUS parameter average at baseline, i.e., before HIFU was turned on. Examination of the images reveals that significant motion occurred during the HIFU exposure due to breathing. Because the rat is a small animal and the tumor was on the abdominal wall, large motion out of plane both laterally and vertically was observed. The jagged nature of the QUS parameters as the temperature increased is hypothesized to be due in part to the motion of the tumor during exposure. In all animals, the *EAC* was observed to increase with temperature and decrease once the temperature began to decrease.

The changes in QUS parameters due to the breathing cycle of the rat alone were quantified using the same experimental setup shown in Fig. 1 with no HIFU as a sham experiment. Changes in *ESD* and *EAC* were less than 5% for a period of 120 s as shown in Fig. 4. In the sham exposure, no trends in the QUS parameters were observed; however, small fluctuations were observed in the QUS parameters during the sham exposure. These variations are mainly due to motion of the tumor during imaging and would partially account for some of the observed fluctuations observed in the QUS images created during and after HIFU exposure.

For all the animals, the *ESD* and *EAC* parameters were correlated to temperature changes during the HIFU treatment. The increases in temperature during the exposure and the decreases in temperature during the cool down period were correlated to the changes in *ESD* and *EAC* parameters. A linear fit between changes in QUS parameters (*ESD* and *EAC*) and temperature recorded by the thermocouple for the heating and cooling period for each experiment were performed. Example linear fits between the QUS parameters and tissue temperature during heating and cooling periods are shown in Figs. 5 and 6 for two tumors (labeled R1 and R2). The *ESD* decreased with increasing temperature and increased with decreasing temperature, indicating an inverse relationship with changes in tissue temperature. On the other hand, the *EAC* increased with increasing temperature and decreased with decreasing temperature. The slope, intercept, and the R^2 of the linear fit are tabulated in Table I for all the tumors used in the current study. The slopes of the linear fit between the temperature and the *ESD* during the heating and cooling periods were -1.22 ± 0.16 and -1.21 ± 0.26 , respectively. The slopes of the linear fit between the temperature and *EAC* during the heating and cooling periods were 1.48 ± 0.26 and 1.51 ± 0.27 , respectively. The goodness-of-fit between the changes in *ESD* and temperature was measured using R^2 values which were 0.90 ± 0.05 and 0.75 ± 0.05 for the heating and cooling periods, respectively. Similarly, the R^2 between changes in *EAC* and temperature were 0.92 ± 0.04 and 0.85 ± 0.03 for the heating and cooling periods, respectively.

Statistical analysis was conducted to investigate the significance of the estimated *ESD* and *EAC* at different time periods during the HIFU treatment (Cases I, II, and III). The mean *ESD* and *EAC* values before HIFU exposure (Case I) were $121 \pm 6 \mu\text{m}$ and $33 \pm 2 \text{ dB/cm}^3$, respectively. Just before the last HIFU tone burst was delivered to the tissue (Case II), the *ESD* and *EAC* values were $81 \pm 8 \mu\text{m}$ and $46 \pm 3 \text{ dB/cm}^3$, respectively. After the tissue cooled down to body temperature of approximately 37°C as recorded by the thermocouple (Case III), the *ESD* and *EAC* values were $120 \pm 7 \mu\text{m}$ and $36 \pm 2 \text{ dB/cm}^3$, respectively. Statistically significant differences were observed between Case I and Case II in the average *ESD* (p -value = 0.0002) and *EAC* (p -value = 0.0002) estimates. No statistically significant

differences were observed between Cases I and III (p -value = 0.5956 and p -value = 0.0833 for *ESD* and *EAC*, respectively). The changes in the *ESD* and *EAC* parameters significantly changed with increases in tissue temperature during HIFU treatment and then returned to pretreatment values after cooling off.

IV. Discussion

QUS imaging was used to monitor HIFU treatment of rat tumors *in vivo*. A needle thermocouple was positioned in the tumor and behind the focus of the HIFU source to measure temperature in the tumor tissue. An ultrasound array and SonixRP imaging system were used to guide the placement of the needle thermocouple and also to acquire raw RF signals for QUS imaging. The changes in the QUS parameters were correlated with the changes in the temperature during the HIFU exposures. Because the thermocouple was placed behind the focus and at the back of the tumor, it was not possible to associate specific values of *ESD* or *EAC* with a specific temperature. More than likely, higher temperature elevations were occurring in the actual regions being analyzed with QUS than were recorded by the thermocouple.

The results demonstrated good correlation between the spectral-based QUS parameters and the changes in tissue temperature during the HIFU exposure and also during the cool-down period after the HIFU was turned off. It was observed that tissue motion due to the breathing cycle had small but noticeable influences on the changes in the spectral-based QUS parameters. The results from the sham-exposure experiments exhibited small changes of around 5% in spectral-based QUS parameters as time elapsed suggesting that the QUS imaging technique is robust against tissue motion effects. These small fluctuations in the sham exposure were comparable in size to fluctuations observed in the *in vivo* HIFU-exposure experiments. Similar fluctuations were not observed in the *in situ* experiments in dead rats [29]. Small fluctuations were observed in the results due to motion, which can be further minimized by averaging RF signals from several frames, i.e., averaging over slow time. Averaging of frames in slow time could further alleviate the artifacts observed in QUS thermometry over time. This would provide a tradeoff between reduced estimate variance and frame rate.

While the QUS parameters were affected by tissue motion, the underlying trends, i.e., that the QUS parameters changed with temperature, were still observable. The estimates of the spectral-based QUS parameters do not depend on the previous frame of ultrasound to deduce their properties like techniques to estimate temperature elevations from changes in sound speed [18]. The scattering properties (i.e., *ESD* and *EAC*) of the tissue microstructure change during temperature elevation and these are absolute properties of the underlying medium, which can be deduced from analysis of the RF-backscattered signals from any individual frame.

The changes in the *ESD* and *EAC* provided good correlation with changes in tissue temperature both during the heating period while the HIFU was turned on and during the cooling period after the HIFU was turned off as observed quantitatively from the slopes of the linear fit between the spectral-based QUS parameters and the changes in temperature.

The R^2 values shown in Table I suggest that the spectral-based QUS parameters closely tracked the changes in temperature recorded by the thermocouple. A linear fit was initially chosen due to the lack of availability of a better model. However, during the heating period, the R^2 values for all rats were above 0.81 and most of these were in the 0.90 and above range. This suggests that assuming a linear fit allowed 81% or more of the observed changes in ESD and EAC to be explained by the temperature changes. Typically, at the end of the cool-down period, the tissue temperature decreased to the normal body temperature.

While we hypothesize that the changes in the spectral-based QUS parameters was due mainly to the changes in temperature, we cannot rule out mechanical effects from HIFU on the spectral-based QUS estimates versus time. Mechanical effects due to boiling or cavitation could also contribute to the changes in ESD and EAC observed. Additional study will need to be conducted to quantify the contributions of mechanical effects to the observed changes in the spectral-based QUS parameters.

In the tumors, the EAC increased with increasing temperature and decreased with decreasing temperature. In experiments on tumors in dead rats, the EAC was observed to increase with increasing temperature, while the ESD trend with increasing temperature was less clear [29]. The reasons for this lack of a trend in the ESD from tumors in dead rats are presently not understood. However, it is worth reporting that studies using HIFU to treat liver samples have also exhibited a correlation between these parameters and tissue temperature, but with some differences [39]. In liver, the EAC decreased with increasing temperature from HIFU and then increased with decreasing temperature. Similarly, using QUS to monitor microwave ablation of liver samples demonstrated similar behavior for EAC as was observed for HIFU therapy of liver samples [40]. Therefore, the changes observed in the spectral-based QUS parameters versus temperature elevations appear to be tissue-dependent. Understanding the mechanisms behind this tissue-dependent observation is an ongoing investigation.

Finally, previous studies have suggested that the BSC and, therefore, ESD and EAC estimates are relatively insensitive to irreversible changes in tissue properties brought on solely by thermal effects (i.e., no mechanical effects) [41]–[43]. This is promising in the respect that as the tissues undergo thermal coagulation, the BSC changes are still due to changes in temperature, unlike sound speed and attenuation which change their trends versus temperature after thermal coagulation. On the other hand, using the BSC to detect acute thermal coagulation does not appear to be promising.

V. Conclusion

QUS techniques were studied for their potential to track temperature in rat MAT tumors *in vivo* during HIFU treatment. The EAC parameter increased with increasing temperature and decreased with decreasing temperature in all rat experiments. The ESD was observed to decrease with increasing temperature and then increase with decreasing temperature. The results suggest that spectral-based QUS parameters can monitor temperature elevations due to HIFU therapy *in vivo* and, furthermore, can be robust against tissue motion.

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Fig. 1.
 (a) Experimental configuration. (b) Custom holder made for the HIFU and imaging array. (c) Illustration of HIFU and imaging sequences.

Fig. 2.
Illustration of data block selection from a tumor (tumor outlined in red).

Fig. 3.

(Top) B-mode images at different time points. (Middle) Series of ESD images of tumor during treatment with HIFU and corresponding temperature/ ESD curves versus time. (Bottom) Series of EAC images of tumor during treatment with HIFU and corresponding temperature/ EAC curves versus time. Blue circles correspond to the location of the HIFU focus.

Fig. 4.

Changes in (a) ΔESD and (b) ΔEAC versus time from rat tumor with no HIFU.

Fig. 5. Best fit line of temperature versus ΔESD for (a) $R1$ during heating; (b) $R2$ during heating; (c) $R1$ during cooling; and (d) $R2$ during cooling phase of the HIFU experiment.

Fig. 6. Best fit line of temperature versus ΔEAC for (a) *R1* during heating; (b) *R2* during heating; (c) *R1* during cooling; and (d) *R2* during cooling phase of the HIFU experiment.

TABLE I
Parameters of the Linear Fits of Temperature Recorded by Thermocouple Versus ESD (%) and EAC (%)

Rat no.	ESD (%)						EAC (%)					
	Heating (HIFU ON)			Cooling (HIFU OFF)			Heating (HIFU ON)			Cooling (HIFU OFF)		
	Slope (1/°C)	Intercept (%)	R^2	Slope (1/°C)	Intercept (%)	R^2	Slope (1/°C)	Intercept (%)	R^2	Slope (1/°C)	Intercept (%)	R^2
R1	-1.29	47.90	0.93	-1.70	80.61	0.64	1.96	-71.66	0.91	1.79	-77.42	0.78
R2	-1.15	42.55	0.88	-1.18	51.79	0.71	1.86	-66.87	0.87	1.56	-57.08	0.87
R3	-0.85	32.68	0.81	-0.81	37.14	0.74	1.35	-52.07	0.95	1.79	-81.71	0.87
R4	-1.49	64.21	0.82	-0.96	44.59	0.79	1.67	-68.58	0.87	1.49	-60.26	0.81
R5	-1.12	44.33	0.97	-0.85	34.62	0.76	1.28	-52.51	0.91	1.92	-88.97	0.82
R6	-1.14	39.65	0.95	-1.28	54.26	0.70	1.07	-38.67	0.96	0.98	-27.83	0.86
R7	-1.23	50.50	0.89	-1.27	58.11	0.78	1.43	-44.25	0.88	1.43	-50.85	0.85
R8	-1.34	49.43	0.90	-1.36	44.47	0.82	1.31	-25.45	0.93	1.28	-63.7	0.87
R9	-1.26	39.06	0.90	-1.38	66.74	0.75	1.49	-42.84	0.99	1.28	-53.5	0.86
R10	-1.30	42.45	0.96	-1.35	50.01	0.78	1.39	-52.19	0.94	1.45	-55.23	0.89