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Quantitative evaluation of internal marks made using MRgFUS as seen on MRI, CT, US, and digital color images – a pilot study

Elizabeth Hipp^a, Xiaobing Fan^a, Ari Partanen^{b,c}, James Vosicky^a, Charles A. Pelizzari^d, Christopher M. Straus^a, Sham Sokka^c, and Gregory S. Karczmar^{a,*}

^aDepartment of Radiology, University of Chicago, Chicago IL 60637, USA ^bDepartment of Physics, University of Helsinki, Helsinki, Finland ^cPhilips Healthcare, Cleveland, OH, USA

^dDepartment of Radiation Oncology, University of Chicago, Chicago, IL 60637, USA

Abstract

This pilot study compared the detectability of internal thermal marks produced with MRI-guided focused ultrasound (MRgFUS) on MRI, computed tomography (CT), ultrasonography (US), and color images from digital scanning. Internal marks made using MRgFUS could potentially guide surgical, biopsy or radiotherapy procedures. New Zealand White rabbits (n = 6) thigh muscle were marked using a Philips MRgFUS system. Before and after sonications, rabbits were imaged using T₁- and T₂-weighted MRI. Then rabbits were sacrificed and imaging was performed using CT and US. After surgical excision specimens were scanned for color conspicuity analysis. Images were read by a radiologist and quantitative analysis of signal intensity was calculated for marks and normal muscle. Of a total of 19 excised marks, approximately 79%, 63%, and 62% were visible on MRI, CT, and US, respectively. The average maximum temperature elevation in the marks during MRgFUS was 39.7 ± 10.1 °C, and average dose diameter (i.e., the diameter of the area that achieved a thermal dose greater than 240 cumulative equivalent minutes at 43 °C) of the mark at the focal plane was 7.3 ± 2.1 mm. On MRI the average normalized signal intensities were significantly higher in marks compared to normal muscle (p < 0.05). On CT, the marked regions were approximately 10 HU lower than normal muscle (p < 0.05). The results demonstrate that MRgFUS can be used to create internal marks that are visible on MRI, CT and US.

Keywords

high intensity focused ultrasound; magnetic resonance imaging; computed tomography; rabbit

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*Address correspondence to: Gregory Karczmar, Ph.D., Professor, Department of Radiology, MC2026, University of Chicago, 5841 S. Maryland Ave., Chicago, IL, USA 60637, Phone: 773-702-0214, gskarczm@uchicago.edu.

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Introduction

Localized marking techniques are often used to guide radiotherapy in cancer treatments. Radiotherapy positioning can be checked prior to treatment using computed tomography (CT), but only if there are identifiable features in the images, such as bony anatomy, biopsy clips or other implants [1]. The utility of bony anatomy is limited when the targeted regions are highly deformable (e.g. in the breast), or can be moved due to changes in nearby anatomy (e.g. bladder filling impacting prostate position). Internal implants using gold or tantalum seeds for guiding radiotherapy have shown promising results, but come with limitations because they require invasive surgical placement [2, 3] and may move after placement. Therefore, an internal fiducial, placed non-invasively under MRI guidance, would be beneficial for patients undergoing radiotherapy.

In addition to guiding radiotherapy, localized marking techniques are also used to guide surgical procedures. Current preoperative localization marking techniques include guide wire locators, radioactive injections and charcoal suspensions. Notably, all these techniques are invasive. Wire localization is performed under image guidance to mark a lesion so that greater accuracy can be achieved during lumpectomy. Radioactive occult lesion localization involves injecting technetium (Tc-99m) labeled serum albumin particles into the center of the lesion and then using a gamma camera probe to detect the lesion for removal [4]. Mathieu et al. proposed using a charcoal suspension for tumor marking and demonstrated its use in both breast and colon cancer [5]. The various marking techniques for breast cancer have risen out of a need to map deformable anatomical regions during cancer treatment. These previous results suggest that magnetic resonance guided focused ultrasound (MRgFUS) could play an important role in guiding surgery, and because it is non-invasive and potentially more accurate – could have advantages relative to existing methods.

MRgFUS provides excellent anatomic resolution for treatment planning and online feedback of temperature elevation during the treatment. It is commonly used for ablative procedures [6-9], but can also be used for internal marking [8]. Schmitz et al. demonstrated the utility of high intensity focused ultrasound ‘tattoos’ in an *ex vivo* model as a guide for surgical excision [10], as compared to guidewire locators. Yang et al. [11] evaluated the appearance of thermal lesions under CT and ultrasound in rabbit liver. MRI detection of marks made using other methods such as RF ablation [12] and laser-ablation [13] have also been demonstrated.

In this research, we use a multi-modality approach to detect marks made with MRgFUS *in vivo* in rabbit thigh muscles and quantitatively evaluate thermal lesions observed on MRI, CT, US, and on a digitized photograph. The purpose of this study was to assess these internal thermal marks for their conspicuity on multiple imaging modalities, to demonstrate their potential utility for guiding radiation therapy, biopsy or surgery in the future.

Materials and methods

Animals

All procedures were carried out with approval from and in accordance with our Institutional Animal Care and Use Committee. A total of six New Zealand White rabbits were used in this study. Animals were anaesthetized prior to experimental studies and anesthesia was maintained during imaging with 1.5-3% isoflurane. The respiration rate and heart rate were monitored at all times using standard MR-compatible devices. Both rabbit thighs were clipped to remove as much hair as possible and a depilatory cream (Avon Products Inc, New York, NY, USA) was applied to remove the remaining fine undercoat. The thigh to be marked was submerged in an in-house built degassed water bath (diameter = 18 cm), that sat within the acoustic window of focused ultrasound (FUS) tabletop (diameter = 25 cm) and was coupled to the membrane using degassed water.

MR guided FUS

FUS marking experiments were performed using an integrated clinical MRgFUS platform (Sonalleve V1 1.5T, Philips Medical Systems, Vantaa, Finland). The Sonalleve system includes a 256-element phased array transducer that can be operated at 1.2 or 1.45 MHz [14]. Prior to sonication, T₂-weighted (T2W) images for localization and planning were acquired using a 3D Turbo Spin Echo (TSE) pulse sequence (TR/TE = 1000/110 ms, echo train length = 50, field of view (FOV) = 180×180×132 mm, acquisition matrix = 148×140×115, reconstructed in-plane resolution = 1.2×1.13 mm, slice thickness = 1.15 mm, number of excitations (NEX) = 2). Saturation bands were applied to suppress the water signal from the water bath. The target area for marking was selected within a uniform muscle region to avoid bony anatomy in the path of ultrasound propagation. A 4 mm diameter short-axis ellipsoid spot was placed using the FUS planning software. The 4 mm sonication was achieved using a geometric focal spot size ~2 mm, plus electronic steering for volumetric ablation operating at a frequency of 1.2 MHz [14].

For each rabbit, two to five sonications were planned. FUS sonications were performed in two parts. A previous study [15] demonstrated that pulsed FUS treated muscle tissue increases signal intensity in T2W MR images. Therefore, first we applied pulsed sonication to enhance the visibility of marks on MRI. A single-point 100-second pulsed sonication (80 watt, 100 cycles, 50 ms pulse interval, 0.17% duty cycle, frequency 1.2 MHz) was delivered to produce mechanical force in the target region. This pulsed sonication did not cause temperature elevation, nor was it observed to cause hemorrhage. Immediately following this pulsed sonication, a 60-80 watt continuous wave volumetric sonication was applied to the same location for 30 seconds. Six seconds prior to the sonication, a dynamic temperature monitoring sequence began, continued for the duration of the sonication, and continued to record temperature data for 60 seconds after the sonication ended. Dynamic temperature monitoring based on changes in proton resonance frequency [16] was performed using 2D fast field echo echo-planar imaging (EPI) (TR/TE = 38/20 ms, flip angle = 19.5°, EPI factor = 11, FOV = 200×200 mm, acquisition matrix = 100×100, reconstructed in-plane resolution = 1.25 mm, number of slices = 6, slice thickness = 7 mm, temporal resolution = 2.9 seconds/dynamic). Cumulative thermal doses were calculated based on the temperature data [17, 18].

To avoid near field overheating, a minimum of 30 seconds wait time was used before marking the next spot, which was always at least 4 mm away from any other marks.

Marks observed by MRI, CT and US

To visualize the FUS marks on MRI, the same T2W imaging used in planning was repeated after all sonications. In addition, a fluid attenuated inversion recovery (FLAIR) sequence was acquired before and after marking (TR/TE = 11000/140 ms, inversion time = 2800 ms, flip angle = 90°, echo train length = 53, FOV = 230×230 mm, acquisition matrix = 256×200, in-plane resolution = 0.9×1.15 mm, slice thickness = 5 mm, NEX = 2).

Immediately after the MRI experiments, the rabbit was sacrificed. Sacrificed rabbits were then imaged using a clinical CT (Philips Brilliance Big Bore, Philips Healthcare, Cleveland, OH, USA) at 120 kVp, 395 mAs, contiguous 1 mm slices, typically 0.45 mm in-plane pixel size. Ultrasound imaging was performed using clinical ultrasound equipment (Acuson Sequioa, 15L8w-S, Siemens, Medical Systems, Mountain View, CA, USA). Five of the six rabbit subjects were available for US studies (due to scheduling conflicts one rabbit did not receive US).

Excision and visualization

Once all imaging was completed, dissection of the leg was performed to locate all successfully sonicated marks. Marks were excised with a margin of 5 to 10 mm normal muscle tissue and scanned digitally (Visioneer OneTouch 9420 USB, Visioneer, Pleasanton, CA, USA) to evaluate visual conspicuity.

For this study, conspicuity was defined using the International Commission on Illumination (CIE) $L^*u^*v^*$ color space (CIELUV) criteria, established for distinguishing color [19]. Briefly, red/green/blue color values can be converted to the LUV colorspace, and the value

E can be calculated describing quantitatively how well a sample color matches a reference color. A value of 0 is a perfect match, while anything less than 6 can only be distinguished by the naked eye if two colors are side by side. Previous work demonstrates that a CIELUV E value of 12 can be detected in less than five seconds for a target object of the sample color on a solid reference background [20].

Image analysis

Data were processed and analyzed using either IDL (Exelis VIS, Inc., Boulder, CO, USA) or OsiriX (Pixmeo, Geneva, Switzerland). The marks seen on MRI and CT were located and contoured by a radiologist with 15 years experience. Based on a previous study [21], the FUS marks at muscle tissue are expected to be oval in shape with a bright rim on T2W MRI. In addition, the heated tissues are observed as a hypodense area on CT images [22]. These properties were used to guide delineation of the contours of the regions-of-interest (ROIs). The ROIs over the marked and healthy muscle tissue were manually drawn on MRI and CT. To facilitate comparison across different experiments, MRI was normalized by the signal in the water bath. Then the average signal intensity over the ROIs was calculated to quantitatively evaluate the marks compared to normal muscle. In addition, the diameter of the area that achieved a thermal dose greater than 240 cumulative equivalent minutes at 43

°C (240CEM43) was calculated and the maximum temperature was recorded for the focal plane based on temperature monitoring MRI.

Due to modest soft tissue contrast on CT, we processed the CT by subtracting it from the intensity of normal muscle to improve contrast as follows:

$$P_{CT} = |C_T - U|, \quad (1)$$

where, C_T is the original CT image, U is a uniform matrix with the same dimensions as the original CT image with each value calculated from average of normal muscle region of CT. We refer to this P_{CT} matrix as ‘processed CT’ in the remaining text.

Statistical analysis

A Wilcoxon signed rank test was performed to compare the signal intensity averaged over ROIs to determine if marks vs. healthy tissue were statistically significantly different. Each individual mark is considered as an independent data point, rather than averaging over all marks for a given rabbit. Results are considered significant for $p\text{-value} < 0.05$.

Results

Figure 1 shows a typical example of the same FUS mark (white thin arrow) on FLAIR MRI (A), on CT (B), and (C) on processed CT. On FLAIR images, the marks were observed to be circumscribed areas of high intensity with a brighter rim. On the T2W images (not shown here), the marks presented as ovoid regions of increased signal, consistent with edema. On CT the marks presented as more radiolucent than the surrounding healthy tissue. In processed CT images, the marks appeared bright compared to the original CT. However, the processing method did not result in detection of additional marks.

The edema-like characteristics of the FUS mark (white thin arrow) can also be seen on ultrasonography as shown in Figure 2. However, due to the nature of ultrasound as an interactive, real-time modality, it is difficult to demonstrate reliable correspondence between MRI or CT with US.

The marks at the time of necropsy in the rabbit’s thigh are shown in Fig. 3A with the skin and subcutaneous fat removed. The white color of the marks is consistent with thermal coagulation tissue damage. After excision of the marks, it was clear on visual inspection that one was contained within one single muscle bundle (Fig. 3B), and the other permeated several adjacent muscle bundles (Fig. 3C).

The mark’s color contrast is described by the value E , which represents the difference in color between the mark and the background muscle tissue on visual inspection. The average E value comparing the mark to the background muscle tissue is 11.4 ± 6.7 , which indicates that on average, the marks can be detected in less than 5 seconds.

Table 1 reports the number of marks that were successfully seen at excision, and detected by an experienced radiologist on MRI, CT and US. For all 21 FUS marks, the average maximum temperature elevation was 39.7 ± 10 °C above baseline, and the average thermal

dose diameter at the focal plane was calculated for thermal dose greater than 240CEM43 was 7.3 ± 2.1 mm. There was a strong linear correlation ($r = 0.89$, $p < 0.001$) between the maximum temperature and the dose diameter at focal plane. Of a total of 19 excised marks, approximately 79%, 63%, and 62% of them were visible on MRI, CT, and US, respectively. Two marks were not found at excision; one did not reach a critical thermal dose level (Case 1), and one was missed on excision (Case 2) due to inexperience.

Finally, to quantitatively evaluate those marks seen on MRI and CT, the average signal intensity over the mark and normal muscle ROIs were compared. The average normalized signal for the marks was about three times higher than muscle ($p = 0.0007$) for T2W imaging (Fig. 4A), and about 1.5 times higher than muscle ($p = 0.0007$) for FLAIR (Fig. 4B). The average signal in the mark was about 10 HU smaller than muscle ($p = 0.005$) for CT (Fig. 5A), and about 10 HU larger than muscle ($p = 0.004$) for the processed CT (Fig. 5B). There was no correlation between the average signal intensity over the mark for either MRI or CT with the dose diameter measured at focal plane. The average longest dimension of marks measured on MRI and CT in the direction of beam propagation was 1.61 ± 0.53 cm and 1.21 ± 0.34 cm, respectively. These measurements provide only a rough estimate of mark dimensions, because the boundary of marks is not well defined on MRI and CT. The size reported is smaller on CT likely due to poorer visualization of the marks on CT images compared to MRI.

Discussion

In this study, approximately 60% of the FUS marks were visible on all the modalities investigated, but more marks were detected on MRI. The percentage of visible marks observed in this study was low compared to other methods such as laser ablation [13]. This may be due to the fact that the ultrasound ablation was completely non-invasive and therefore did not cause as much denaturation and structural change as the more direct ablation methods. However, this is a promising result, suggesting that with further development and optimization this marking technique could be used to guide surgical and radiotherapy procedures. For example, FUS marks could be used to locate the boundary of tumor and guide breast conservation surgery or liver resection. In addition, FUS marks could be used as fiducials within the prostate to guide radiation therapy for prostate cancer.

Of the imaging modalities explored, MRI showed the most consistency in visualizing the FUS marks, as well as the largest difference in signal intensity compared to surrounding normal muscle tissue. Some marks contained a rim enhancement, suggesting that tissue changes at the edge the sonication region were different from those on the interior of the marked region. Also, the initial 100 s pulsed sonication likely causes structural changes due to mechanical pressures interacting with the tissue without heating, prior to the continuous wave sonications. Previous studies in animal models [23] have shown that when pulsed sonication is used at a relatively low duty cycle, heat generation is limited and non-thermal mechanisms come into play. These non-thermal processes may have enhanced the effect of the subsequent continuous wave volumetric sonication. However, the role of non-thermal effects was not specifically addressed in this pilot study; this will require further investigation in the future.

Our results demonstrated that there is about 10 HU difference between a FUS mark and normal muscle (65 vs 75 HU). This supports the theory that the marked tissue region contains increased fluid content, which is similar to the results previously visualized rabbit liver ablations using contrast-enhanced CT [11]. The relatively small difference in HU between a mark and normal muscle suggests a very small increase in tissue electron density due to edema [24]. The heterogeneity of rabbit thighs may have been an additional source of variability, because the radio density of different muscle bundles may differ by 10-15 HU; this could have obscured some marks. In the future, improvements are needed for the method used to enhance FUS marks on CT images so that more marks will be visible, especially on daily cone beam imaging for radiotherapy applications. Computer-aided diagnostic methods could be used to more reliably detect FUS marks on cone beam CT based on prior knowledge concerning their shape and texture.

Although US also showed the FUS marks, this was not with the same reliability or conspicuity as MRI and CT. The presentation of marks was more difficult to visualize on the US images. However, the result is promising, since the US images were read retrospectively and not at the time of acquisition. The FUS marks were seen at excision as ovoid white spots. They may be contained within one muscle bundle, or permeating multiple adjacent muscle bundles. The visual conspicuity analysis showed that on average the marks could be detected in less than 5 seconds. This is useful for procedures that involve surgical approaches.

There are several limitations of this study:

- a. More work is required to increase reproducibility and conspicuity as some marks were not visualized on all modalities. Optimal FUS marking parameters should be investigated by systematically varying a combination of sonication time and acoustic power.
- b. The CT and US images were acquired in recently sacrificed rabbits. It is desirable to image live rabbits after marking, in case post mortem processes impact the marks.
- c. The durability of the marks has not been evaluated, and pathology has not been performed. Pathological analysis would be useful to provide insight into cellular and morphologic changes within the tissue.
- d. Finally, only one radiologist inspected all images and manually traced the marks. In the future, multiple readers should be used and computer aided diagnosis may be investigated to assist in evaluation of marks.

While many of the marks were detectable with a conventional diagnostic CT scanner, fewer would be detected by cone beam CT (CBCT) attached to radiotherapy devices, since scatter on CBCT masks low contrast objects. Therefore, further changes in electron density in marks relative to background will be required to produce a change in contrast that is reliably detected on CBCT. The conspicuity of marks on CT could be increased by fixing contrast agents in the marks using focused ultrasound. This would be similar to the previous use of

focused ultrasound to burst contrast-agent-filled micro-bubbles to release fluorescent dye that can be optically detected and imaged [25].

FUS produced marks may change in shape and/or size over the period of radiation treatment. Therefore, a study of FUS marks' durability using survived study subjects is warranted. If the marks persist but are deformed over time, a new MRI scan before each radiation treatment could be performed to re-define the spatial relationship between the marks and the target area. Moreover, since MRgFUS is noninvasive and does not involve ionizing radiation, these marks could be re-encoded if necessary.

Conclusions

In summary, MRgFUS marking is a promising approach that addresses an important clinical concern regarding tumor marking and localization. In the research discussed here, a multi-modality approach was used to demonstrate that these marks can be detected on MRI, CT, US, and on digital scans. These internal marks have potential clinical applications. They could provide internal fiducials that are detectable on CT. This would assist in treatment planning and allow real-time adjustments during radiotherapy. They could also be visible to surgeons intra-operatively to guide surgery and biopsy.

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Figure 1.

An example of a FUS mark (long arrow) shown on the MRI (A), CT (B) and processed CT (C). The white chevron arrow marks the femur. The displayed FOV for each image is 11 cm $\times$ 5 cm.

Figure 2.

An example of the mark (long arrow) on ultrasound, with the wand below the image. The white chevron arrow marks the femur. The mark can be seen here to permeate two separate adjacent muscle bundles. The displayed FOV for this image is 5 cm $\times$ 4 cm.

Figure 3. The marks *in situ* at excision (A) after the skin and subcutaneous fat have been removed, (B) sliced in half during excision, and (C) in three separate muscle bundles. Please note that three contiguous muscle bundles were not cut apart but merely fall apart when no longer attached in their standard anatomical arrangement.

Figure 4.

Box plots of the signal intensity (normalized to water) of the marks compared to the muscle as seen on both the TSE T2W (left) and inversion recovery (right) MR images. Included here are only tissue changes considered 'marks' – i.e. possessing clear boundaries that were traceable by a radiologist. The plus signs (+) indicate mean, and the asterisks (*) indicate the upper and lower limits of the data.

Figure 5.

Box plots of the signal intensity (HU) of the marks compared to the muscle as seen on both the original (left) and processed (right) CT images. Only those tissue changes with well delineated boundaries were considered circumscribed marks and are included here. The plus signs (+) indicate mean, and the asterisks (*) indicate the upper and lower limits of the data.

Table 1

Number of FUS marks that were seen on each modality.

	Number of marks	Number excised	Number seen on MRI	Number seen on CT	Number seen on US
<i>Case 1</i>	4	3	3	3	2
<i>Case 2</i>	3	2	2	2	2
<i>Case 3</i>	5	5	4	4	3
<i>Case 4</i>	4	4	2	2	1
<i>Case 5</i>	3	3	2	0	-
<i>Case 6</i>	2	2	2	2	2
<i>Totals</i>	21	19	15	13	10