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Differentiating irreversible and reversible electroporation zones: a correlation library between electroacoustic tomography and histopathology

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ABSTRACT

Pulsed electrical ablation has become a powerful tool in biomedicine, with applications in cancer treatment, atrial fibrillation therapy, and opening the blood-brain barrier (BBB) for drug delivery. A key limitation, however, is that the electric field distribution is invisible in soft tissue, making it difficult to monitor and guide treatment in real time. To overcome this challenge, we have developed electroacoustic tomography (EAT)—a novel imaging modality that captures ultrasound signals generated by pulsed electric fields to visualize energy deposition within tissue. EAT offers the potential to distinguish between irreversible electroporation (IRE) and reversible electroporation (RE) regions during therapy. To establish a robust correlation between EAT and tissue response, we constructed a comprehensive ablation library using potato models, which are low-cost, relatively convenient, and free of ethical issues. We systematically varied treatment voltage and performed EAT imaging during each ablation. Following treatment, samples were stained with triphenyltetrazolium chloride (TTC), and photos were taken after 5–6 hours to assess tissue viability. EAT reconstructions were then compared with histopathological staining to assess spatial agreement and the ability to differentiate IRE from RE zones. Our results demonstrate that EAT reliably visualizes the electric field distribution for IRE and correlates well with post-treatment tissue response, whereas RE visualization-correlation is satisfactory only for low-voltage parameters. This study lays the foundation for real-time treatment monitoring and personalized control of pulsed electroporation therapies.

Keywords: Electroacoustic Tomography, Electroporation, pulsed ablation, Irreversible Electroporation, and Reversible Electroporation.

1. INTRODUCTION

Pulsed electric field (PEF) ablation induces nanoscale pores that disrupt the plasma membrane of cells [1–4]. When the membrane can be repaired, the ablation is termed reversible electroporation (RE) and can be leveraged for gene transfection and the delivery of chemotherapeutics [1–3]. In contrast, irreversible electroporation (IRE) occurs when the electric field exceeds a critical biological threshold, impairing membrane repair mechanisms and ultimately leading to cell death [1–4]. IRE is used in oncology and cardiology for localized, nonthermal ablation that preserves surrounding anatomical structures [3–8]. Nanosecond pulsed electric field (nsPEF) ablation has been shown to minimize thermal effects, enhance cellular membrane destabilization, and modulate oxidative stress, making nsPEF a promising direction for clinical ablation [9–15].

Current clinical standards for monitoring PEF treatments include magnetic resonance imaging (MRI), computed tomography (CT), and ultrasound (US) [3, 4]. MRI and CT provide high-resolution images for treatment planning, whereas US provides real-time guidance for electroporation equipment within the body [3, 4]. Further, electroanatomic mapping systems for cardiac ablation have been used to provide physiologic guidance during ablation [16]. However, these modalities cannot directly visualize electric field deposition or provide real-time dosimetric information. To address this limitation, our group proposed and demonstrated electroacoustic tomography (EAT) as an alternative modality, documenting its capabilities for real-time monitoring and electric-field visualization [17–20].

Although previous studies have established the dosimetric potential of EAT and demonstrated its utility in ablation monitoring, quantitative histopathological validation remains lacking [17–20]. Such histopathological characterization is essential for the clinical translation of novel ablation monitoring techniques. Therefore, in this study, we aim to

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quantitatively characterize the IRE and RE ablation zones following nsPEF treatment using histopathological staining. Histopathological correlation studies for electroporation devices are traditionally performed in living organisms, which is costly, time-consuming, and requires Institutional Animal Care and Use Committee (IACUC) approval. Plant-based models, particularly potatoes, are not affected by the aforementioned limitations, have consistently produced results comparable to those of in vivo electroporation testing, and are widely used for PEF evaluation [21–26]. Building on previous work, we employ 2,3,5-triphenyltetrazolium chloride (TTC) staining to assess nsPEF ablation in potato models [21–26].

To investigate the fidelity of EAT, we test a range of nsPEF ablation parameters in a potato model. The nsPEF parameters include voltage, pulse number, and pulse width; all experiments use nanosecond pulse widths, and the repetition frequency is held constant. Potatoes are thinly sliced before treatment to approximate a two-dimensional slice in the EAT reconstruction and are stained with TTC following nsPEF exposure. EAT data are acquired during treatment for each slice. To quantify the characterization of RE and IRE regions, we used the Structural Similarity Index Measure (SSIM) between a binary map of the TTC staining and the corresponding EAT-derived RE/IRE regions. Higher SSIM values indicate better characterization of the RE/IRE regions in terms of shape and size after post-processing alignment. In this work, we demonstrate that EAT provides high-fidelity characterization ($\text{SSIM} \geq 0.9$) of the IRE region and lower-fidelity characterization of the RE region.

2. METHODOLOGY

Yukon Gold potatoes, purchased from various vendors, were used for all experiments. Each potato was manually cut into 2–4 mm-thick slices, then shaped into 2-inch-diameter circles using a cookie cutter. To ensure freshness, potatoes were processed immediately before treatment. To account for variability, three potato slices were treated under identical conditions for each parameter combination (e.g., three slices at 1 kV, 100 pulses, 60 ns, 50 Hz). The repetition frequency was fixed at 50 Hz for all experiments. Potatoes were treated across a wide range of ablation parameters: voltages from 1 kV to 4 kV in 0.5 kV increments, pulse numbers of 100, 200, and 400, and pulse widths of 60 ns, 100 ns, and 160 ns.

The voltage, pulse width, and pulse number ranges were selected based on prior nsPEF studies in both biomedical and clinical settings [9–15]. nsPEF voltages are typically reported in kV/cm and, depending on the application, range from 0.5 to 200 kV/cm [9–15]. For cardiac and cancer tumor ablation, fields of 1–50 kV/cm are common. In our experiments, we investigated electric field strengths of 0.67, 1.00, 1.33, 1.67, 2.00, 2.33, and 2.67 kV/cm. All of these values lie within the typical range of 1 to 50 kV/cm. Pulse width and pulse number also vary by application. In biomedical research, pulse widths of 1–600 ns and pulse numbers of 1–500 pulses are commonly reported [9–15]. Guided by these prior studies, we examined pulse widths of 60, 100, and 160 ns and pulse numbers of 100, 200, and 400.

Two nanoknife electrodes, with 1.5 cm of exposed tip, were positioned 1.5 cm apart. The electrodes pierced the centers of the potato disks, perpendicular to the disks' surfaces. After piercing, each slice was lowered into a plastic tub filled with deionized water and positioned approximately 10.5 cm above a 256-element matrix transducer (nondestructive testing (NDT) probe, Doppler Co. Limited, Guangzhou, China). The matrix transducer, with a center frequency of 1 MHz, was used to acquire all EAT experimental data.

Once the potato was fully submerged, the 10 kV generator delivered nsPEF according to the specified treatment parameters (e.g., 2 kV, 200 pulses, 60 ns, 50 Hz). The generator delivered monophasic pulses for all experiments. Both the generator and the data acquisition system (DAQ) (MoleculUS, PhotoSound Technologies, Houston, Texas, USA) were initialized via control commands from a computer application. The DAQ operated at a 40 MHz sampling rate with 12-bit depth and was controlled by the MoleculeUS software (version 1.2.1). The matrix transducer was connected to the DAQ and triggered to capture data simultaneously. When the generator was triggered, it output a high-voltage (HV) trigger to a delay generator (DG535, Stanford Research Systems, USA), which converted it into a transistor-transistor logic (TTL) signal. This TTL signal triggered the DAQ to begin data capture, ensuring synchronization between the generator and DAQ. Once the DAQ buffer was full, data acquisition ceased, and the data were offloaded and stored on the computer. A schematic of the equipment setup is shown in Fig. 1.

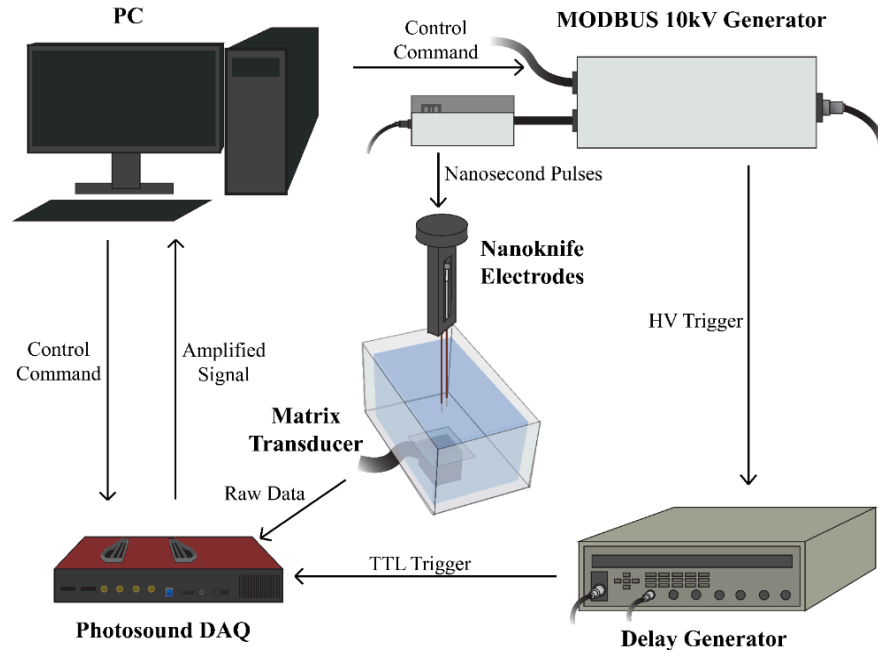


Figure 1. Equipment Set-up Diagram. The PC serves as the primary external controller for both the pulse generator and the photosound DAQ. First, the PC initializes the DAQ buffer via a control command. It then sends a command to the pulse generator to begin pulse delivery. In response, the generator produces a high-voltage (HV) trigger that is routed to a delay generator. The delay generator converts this HV trigger into a TTL-level trigger, which signals the DAQ to start data acquisition. While the DAQ is capturing, the pulse generator delivers pulses through the electrodes, and the resulting broadband ultrasound signals are detected by the matrix transducer. The transducer outputs the raw data to the DAQ until the buffer is full, at which point the DAQ stops acquiring. The amplified data are then transferred to the PC for further processing and analysis.

The acoustic data collected from the DAQ were stored on the computer and converted into .mat files using MATLAB. The raw data were then processed and filtered to enhance the signal-to-noise ratio (SNR) and to remove head-wave artifacts. SNR enhancement was achieved by applying high-pass, low-pass, or band-pass filters as appropriate, followed by envelope application. All filtering and envelope functions were implemented using MATLAB's Signal Processing Toolbox. The filtered data were reconstructed using a filtered back-projection algorithm, yielding a three-dimensional EAT reconstruction. The maximum intensity projection of the EAT volume was calculated in MATLAB and used for subsequent analysis.

Immediately after nsPEF ablation, the potato is removed from the water tank and patted dry with a paper towel. The slice is then placed into a jar filled with TTC solution. The recipe for this solution is 5 grams of powdered TTC and 495 grams of phosphate-buffered saline (PBS), yielding a total of 500 grams of TTC solution (1% concentration). The stain was kept refrigerated at 37°F to 40°F (3°C to 4°C) using a mini-refrigerator. The jar was sealed once all three potato slices completed nsPEF treatment. The incubation protocol for all data sets was to let the jar rest at room temperature (25°C or 77°F) for 5 to 6 hours. After the resting period, the potatoes were removed from the staining solution, patted dry with a paper towel, and photographed using an iPhone camera and a lightbox.

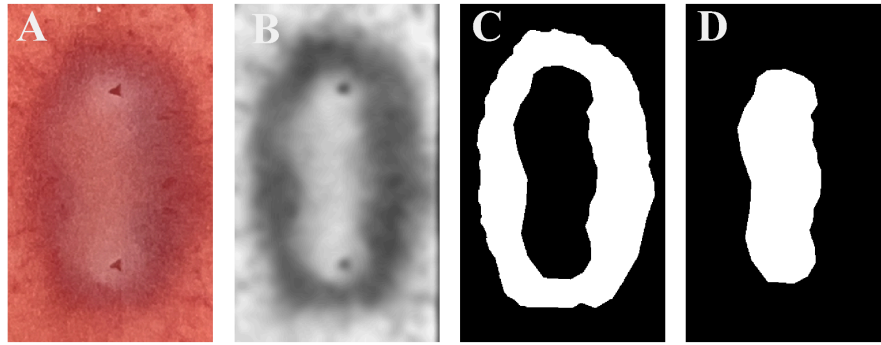


Figure 2. The RE and IRE Ablation Zones. A) Cropped potato photograph centered on the electrode puncture marks. The white–pink region corresponds to the IRE zone, and the surrounding dark red ring corresponds to the RE zone. B) Red color channel after enhancement and processing, improving isolation of the RE zone and making it darker and more distinct. C) RE binary image generated from the enhanced image using LAAB thresholding and manual refinement to isolate the RE zone. D) IRE binary image derived from the RE binary image by selecting the area enclosed by the RE zone.

TTC is a colorless compound that visualizes metabolic activity in biological tissue[25]. In metabolically active cells, dehydrogenases facilitate cellular respiration and donate electrons to TTC during redox reactions[25]. TTC accepts these electrons, becoming reduced to TPF (1,3,5-triphenylformazan), a red, insoluble precipitate that remains in the tissue[25]. This process creates a clear visual distinction between metabolically active and inactive regions (white indicating nonactive regions and red indicating active regions). In the potato slices, areas of IRE range from light pink to white, and the non-ablated area is light red, as shown in Fig.2A. Moreover, previous studies have concluded that the dark red region surrounding the IRE zone is the RE area[21–26]. This phenomenon is thought to stem from poration, enhancing the intracellular uptake of TTC, resulting in elevated concentrations of TTC at the sites of reversible poration [21–26].

To isolate the RE and IRE area, the potato photographs were converted to RGB and processed using MATLAB. The images were cropped to center around the puncture marks left from the nanoknife electrodes. Subsequently, the red channel was extracted from the RGB image. To reduce noise from background texture, both a Weiner and a median filter were applied. To reduce noise caused by uneven lighting, contrast-limited adaptive histogram equalization was employed [27]. The processed image is shown in Fig. 2B. Lastly, to isolate the RE and IRE regions, a combination of local adaptive automatic binarization (LAAB) and manual ROI selection was utilized (Fig. 2C-D)[28]. The resulting binary image was used to quantify the RE and IRE areas in the staining results (Fig. 3C-D). The RE and IRE areas for EAT reconstruction were manually isolated in MATLAB using global thresholding of the maximum-intensity projection. The criteria for IRE and RE thresholds were based on assumed electric field strength and cross-sample comparison.

The SSIM between EAT and staining results was computed using the SSIM function in the MATLAB Image Processing Toolbox. Images were manually aligned (scaling, rotation, and translation) before SSIM was calculated. Scaling was performed as the reconstruction slice is smaller than the processed photograph.

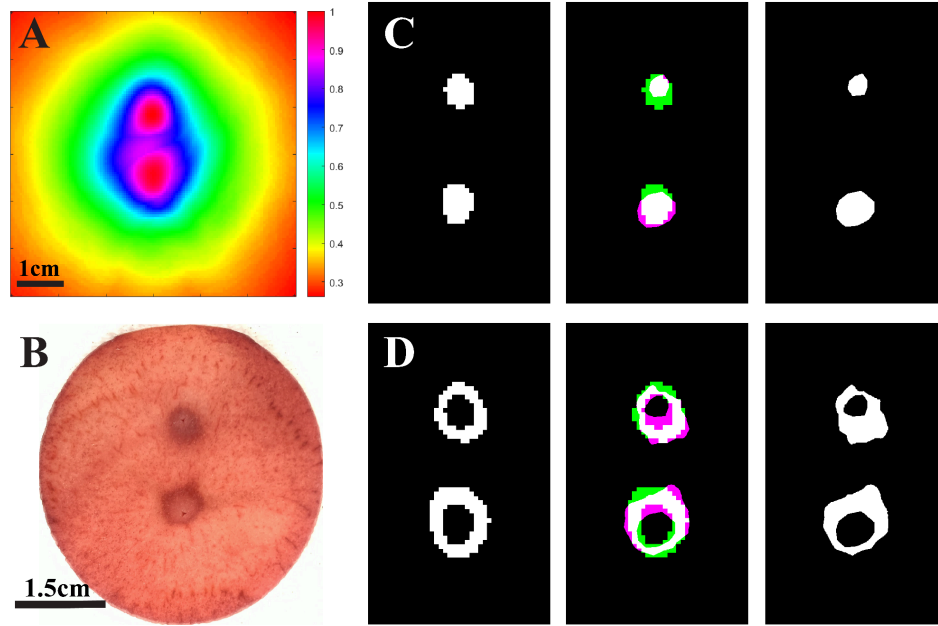


Figure 3. Sample RE and IRE region isolation and comparison. A) Maximum-intensity projection of the 3D EAT reconstruction. Intensities are normalized and displayed using an HSV colormap. Scale bar: 1 cm. B) Uncropped potato staining photograph for experiment parameters 2.5 kV, 100 ns, 400 pulses, 50 Hz. The red region corresponds to the RE zone, and the pinkish-white region corresponds to the IRE zone. Scale bar: 1.5 cm. C) Binary images of the IRE zone. From left to right: IRE region extracted from staining, overlay of staining and EAT IRE regions, and IRE region extracted from EAT. In the overlay, pink indicates staining, green indicates EAT, and white indicates overlap. D) Binary images of the RE zone. From left to right: RE region extracted from staining, overlay of staining and EAT RE regions, and RE region extracted from EAT. In the overlay, pink indicates staining, green indicates EAT, and white indicates overlap.

3. RESULTS AND DISCUSSION

Across all experiment sets, one of the three potatoes was selected as the representative for the parameter setting. This potato was selected because it produced the best staining results and SNR compared with the other two potatoes within the same set (i.e., of the three potatoes for 2.5 kV, 100 pulses, and 60 ns parameter settings, one was selected to represent 2.5 kV, 100 pulses, and 60 n). After selection, the total number of slices was 63. Of the 63 slices, only 52 demonstrated IRE staining, and all demonstrated RE staining. The SSIM values for the IRE and RE zones are shown in Table 1.

Our analysis defined "good characterization" as achieving an SSIM of 0.9 or greater. Based on this criterion, EAT imaging for IRE showed a significantly higher success rate than for RE. Of the 52 cases with an IRE ablation area, 49 (94.2%) achieved an SSIM score of 0.9 or higher. In contrast, only 23 of the 63 cases with an RE ablation area (36.5%) met this threshold. This suggests that IRE is over 2.5 times more likely to result in good characterization than RE.

A comparison of their statistical profiles further supports these findings. The mean SSIM for IRE was 0.946, which is comfortably above the 0.9 threshold, whereas the mean for RE was 0.880, falling below the criterion. Furthermore, the IRE standard deviation was very low at 0.025, indicating high consistency. The standard deviation for RE was nearly double at 0.047, pointing to significantly greater variability in its outcomes. Assuming a normal distribution of the data, the 0.9 threshold is approximately 1.84 standard deviations below the mean for IRE, placing the vast majority of cases well above the target. For RE, however, the threshold is 0.43 standard deviations above the mean. This suggests that only 33–34% of RE cases would meet the criterion, a figure that closely aligns with our observed rate of 36.5%.

In conclusion, the combination of a higher mean SSIM, lower variability, and a mean value exceeding the established threshold strongly indicates that the ablation zone is more reliably and consistently characterized following IRE than following RE. The variability in the RE characterization was further analyzed by assessing the impact of voltage levels (Table 2).

Table 1. SSIM Results For All Experiments. The parameter settings for each experiment are given in the voltage, pulse width, and pulse number columns. A blank entry in the IRE SSIM column indicates that no IRE region was observed in the staining results. The abbreviations are as follows: V, applied voltage; pw, pulse width; pn, pulse number; SSIM, structural similarity index measure; IRE, irreversible electroporation; RE, reversible electroporation.

nsPEF parameters			Similarity metrics		nsPEF parameters			Similarity metrics		nsPEF parameters			Similarity metrics	
V (kV)	pn	pw (ns)	IRE SSIM	RE SSIM	V (kV)	pn	pw (ns)	IRE SSIM	RE SSIM	V (kV)	pn	pw (ns)	IRE SSIM	RE SSIM
4000			0.884	0.736	4000			0.953	0.851	4000			0.907	0.827
3500			0.906	0.803	3500			0.926	0.826	3500			0.895	0.819
3000			0.950	0.872	3000			0.963	0.842	3000			0.931	0.838
2500	100		0.951	0.867	2500	100		0.971	0.902	2500	100		0.965	0.879
2000			0.965	0.908	2000			0.961	0.909	2000			0.915	0.901
1500			0.974	0.928	1500				0.946	1500				0.916
1000			0.993	0.960	1000				0.960	1000				0.982
4000			0.942	0.838	4000			0.938	0.850	4000			0.943	0.806
3500			0.958	0.862	3500			0.940	0.855	3500			0.946	0.830
3000			0.940	0.841	3000			0.919	0.856	3000			0.904	0.891
2500	200	160	0.947	0.866	2500	200	100	0.978	0.840	2500	200	60	0.892	0.841
2000			0.951	0.844	2000			0.929	0.849	2000			0.980	0.900
1500			0.950	0.876	1500			0.990	0.931	1500			0.991	0.931
1000				0.921	1000				0.951	1000				0.959
4000			0.955	0.871	4000			0.933	0.851	4000			0.941	0.871
3500			0.955	0.848	3500			0.936	0.870	3500			0.938	0.875
3000			0.945	0.873	3000			0.938	0.881	3000			0.980	0.905
2500	400		0.928	0.833	2500	400		0.975	0.936	2500	400		0.942	0.874
2000			0.957	0.914	2000			0.954	0.931	2000			0.961	0.898
1500			0.940	0.883	1500			0.947	0.900	1500				0.796
1000				0.918	1000				0.935	1000				0.938

Table 2. SSIM Mean and Standard Deviation for SSIM Data. Table 2 illustrates the statistical profiles of the IRE and RE SSIM for the complete set and the two subsets (low-voltage and high-voltage). The abbreviation kV and STD stand for voltage (measured in kilovolts) and standard deviation, respectively.

IRE SSIM Analysis			RE SSIM Analysis		
Set	Mean	STD	Set	Mean	STD
All kV	0.946	0.025	All kV	0.880	0.047
≤ 2.5 kV	0.956	0.024	≤ 2.5kV	0.903	0.042
> 2.5kV	0.936	0.022	> 2.5kV	0.848	0.033

The potato data set was split into two groups: low-voltage (≤ 2.5 kV) and high-voltage (> 2.5 kV). In the low-voltage group, among the 25 cases with IRE ablation area, 24 (96.0%) achieved an SSIM of 0.9 or greater (Table 1). Similarly, among the 36 cases with RE ablation area, 23 (63.9%) achieved an SSIM of 0.9 or greater (Table 1). In high-voltage cases, of the 27 cases with IRE ablation area, 22 (81.5%) achieved an SSIM greater than or equal to 0.9 (Table 1). Similarly, among the 27 cases with RE, only one (3.70%) achieved an SSIM of 0.9 or higher (Table 1). Thus, low-voltage IRE and RE are 1.1 and 17.3 times more likely, respectively, to achieve good characterization than their high-voltage counterparts.

A comparison of low-voltage and high-voltage statistical profiles further backs this conclusion. The means for low-voltage and high-voltage IRE are 0.956 and 0.936, respectively, and fall above the 0.9 threshold (Table 2). In contrast, the means for low-voltage and high-voltage RE are 0.902 and 0.848, respectively, with only low-voltage meeting the threshold requirement (Table 2). Furthermore, the standard deviations for low-voltage (0.024) and high-voltage (0.022) IRE are low, indicating high consistency for both sets (Table 2). The standard deviations for low-voltage (0.042) and high-voltage (0.033) RE are much higher, with low-voltage showing slightly more variation than high-voltage (Table 2).

Overall, these results demonstrate that EAT can reliably characterize IRE across the full range of voltages tested. EAT performs exceptionally well at low voltage and remains highly effective at high voltage. In contrast, RE characterization is highly sensitive to high voltages. While RE provides a viable, though less consistent, characterization approach at low voltage, it performs poorly at high voltage. These findings suggest that high voltages may generate increased noise or signal interference, potentially distorting the EAT signal. Because the amplitude of the generated acoustic signal is proportional to the energy deposited in the tissue, it is plausible that noise or interference at high voltages preferentially obscures the lower-amplitude signal associated with RE than the stronger-amplitude signal associated with IRE[12].

4. CONCLUSION

In summary, EAT imaging provides robust and reliable characterization of IRE ablation zones across the full range of voltages tested, with consistently high SSIM values, low variability, and a large majority of cases exceeding the predefined quality threshold ($\text{SSIM} \geq 0.9$). In contrast, RE ablation zones are characterized less reliably overall and are particularly susceptible to degradation at higher voltages, where successful characterization becomes rare. The strong performance of EAT for IRE, together with the markedly reduced reliability for RE at high voltage, suggests that voltage-dependent noise or signal interference may preferentially obscure the lower-amplitude acoustic signals associated with RE, while the higher-amplitude IRE signals remain more robust. Collectively, these findings indicate that EAT is a dependable modality for delineating IRE ablation zones, whereas its utility for RE is more limited and strongly constrained by the applied voltage.

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CONFLICT OF INTEREST

Liangzhong Xiang has a financial interest in SoundDose, Inc., which, however, did not support this work. The remaining authors declare no conflict of interest.

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