

TEL AVIV UNIVERSITY

The Iby and Aladar Fleischman Faculty of Engineering

The Zandman-Slaner School of Graduate Studies

**Temperature monitoring during cryoablation with
dense ultrasonic speed-of-sound shift imaging**

A thesis submitted toward the degree of
Master of Science in Biomedical Engineering
by

Gaya Lamm

This research was carried out in the School of Biomedical Engineering
Under the supervision of Prof. Tali Ilovitsh

August 2026

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I would like to express my sincere gratitude to my supervisor, Prof. Tali Ilovitsh, for her invaluable guidance, support, and mentorship throughout this work. I deeply appreciate the time, effort, and thought she dedicated to this research, as well as the opportunity to pursue my academic interests under her supervision. Her insight, encouragement, and confidence in me were instrumental in shaping both this project and my development as a researcher.

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Abstract

Cryoablation is a minimally invasive technique for the treatment of pathological tissues, in which extreme cooling induces irreversible cellular damage. Accurate temperature monitoring is essential to ensure complete ablation while preserving surrounding healthy tissue; however, existing methods are limited by invasiveness, lack of spatial resolution, or high cost. In this work, a dense speed-of-sound shift imaging framework is proposed for noninvasive, spatially resolved temperature monitoring during cryoablation using conventional ultrasound.

The method estimates local speed-of-sound changes from ultrasound data by tracking temperature-induced pixel displacements via Farnebäck optical flow and solving a regularized inverse problem to reconstruct slowness maps. Cryoablation experiments were conducted in tissue-mimicking agar phantoms and ex-vivo turkey breast using a custom-built liquid nitrogen cryoprobe, with invasive thermometry serving as reference.

Results demonstrate a strong relationship between temperature decrease (ΔT) and slowness changes. In agar, this dependence was linear ($-20.70 \text{ ns}\cdot\text{m}^{-1}\cdot^\circ\text{C}^{-1}$; $R^2=0.988$), while in turkey breast it followed an exponential trend ($34.04 \times \exp(0.075(-\Delta T)-1) \text{ ns}\cdot\text{m}^{-1}\cdot^\circ\text{C}^{-1}$; $R^2=0.950$). The method enabled tracking of temperature changes over a wide range, down to $-39.4 \pm 5.6^\circ\text{C}$. Quantitative evaluation showed an RMSE of 1.7°C in agar and 4.2°C in turkey breast. Bland-Altman analysis indicated good agreement with thermocouple measurements, with mean biases of 1.48°C in agar and -2.91°C in turkey, and 95% limits of agreement of -3.37°C to $+6.34^\circ\text{C}$ in agar and -8.11°C to $+2.28^\circ\text{C}$ in turkey.

These results demonstrate the feasibility of ultrasound-based, noninvasive temperature monitoring during cryoablation. The proposed approach provides a practical, real-time alternative to existing techniques, with potential for clinical translation following further validation in in-vivo settings.

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Abbreviations and symbols

US.....	Ultrasound
A-line.....	Amplitude line
B-mode.....	Brightness mode
DSI	Dense speed-of-sound shift imaging
IR.....	Infrared
MRI	Magnetic resonance imaging
SoS	Speed of sound
RF.....	Radiofrequency
B[Pa]	Bulk modulus
$\rho \left[\frac{\text{kg}}{\text{m}^3} \right]$	Density
$c \left[\frac{\text{m}}{\text{sec}} \right]$	Speed of sound
t[sec]	Time
z[mm]	Axial depth
f_0 [Hz]	Fundamental frequency
d[mm]	Thickness of piezoelectric element
T[°C].....	Temperature
δT [°C].....	Temperature change
$\delta \tau$ [sec]	Round-trip time
$\beta \left[\frac{\text{mm}}{^\circ\text{C}} \right]$	Thermal expansion coefficient
M	Integration matrix
Δd [mm].....	Pixel shift
$\Delta s \left[\frac{\text{sec}}{\text{mm}} \right]$	Slowness deviation
$\Delta \hat{s} \left[\frac{\text{sec}}{\text{mm}} \right]$	Estimated slowness deviation
$\alpha \left[\frac{\text{ns}}{\text{m}^\circ\text{C}} \right]$	Slowness-Temperature relationship parameter
Γ	Regularization parameter

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List of publications

- Lamm, G., Grutman, T., Bismuth, M., & Ilovitsh, T. (2026). Cryoablation temperature monitoring with dense ultrasonic speed-of-sound shift imaging. *Physics in Medicine & Biology*, 71(10), 105008. <https://doi.org/10.1088/1361-6560/AE6AF9>

Conference Presentations

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- G. Lamm, T. Grutman, M. Bismuth, and T. Ilovitsh. Cryoablation Temperature Monitoring with Dense Ultrasonic Speed-of-Sound Shift Imaging, International Society for Therapeutic Ultrasound (ISTU) 2026, Trondheim, Norway. Poster presentation.
- G. Lamm, T. Grutman, M. Bismuth, and T. Ilovitsh. Cryoablation Temperature Monitoring with Dense Ultrasonic Speed-of-Sound Shift Imaging, International Society for Therapeutic Ultrasound (ISTU) 2025, Banff, Alberta, Canada. Poster presentation.
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1 Introduction

Cryoablation is a minimally invasive procedure used to destroy pathological tissues in a target area by exposing them to extremely low temperatures. The controlled freezing process induces irreversible cellular damage through mechanisms such as intracellular and extracellular ice formation, membrane disruption, and ultimately apoptosis or necrosis. The extent of injury depends on factors including cooling rate, minimum temperature (typically -20°C to -40°C), and exposure duration [1]-[4]. Clinically, cryoablation is widely applied in the treatment of renal tumors [5], [6], liver metastases [7], breast cancer [8], [9], and cardiac arrhythmias [10]. As with other thermal therapies, accurate monitoring of the treated region is essential to ensure complete destruction of the target tissue while minimizing damage to surrounding healthy structures.

During cryoablation, a spatial thermal gradient develops around the cryoprobe, with temperature increasing as a function of distance from the probe [1], [11]-[13]. This gradient defines the extent of the frozen region and the surrounding tissue response, making temperature monitoring critical for confirming that lethal thresholds are achieved throughout the target volume [11], [14]. However, existing monitoring methods present significant limitations. Invasive sensors such as thermocouples, fiber optic sensors [15], [16] and thermistors [17] provide only localized measurements and cannot capture the full spatial temperature distribution. Non-contact approaches such as infrared (IR) thermometry [18], [19] are restricted to surface measurements and are therefore unsuitable for assessing deeper tissue.

Magnetic resonance imaging (MRI) thermometry is widely regarded as the gold standard for non-invasive, spatially resolved temperature mapping in thermal ablation applications [20]-[22]. Nevertheless, its application in cryoablation remains limited. While MRI is commonly used to guide probe placement and visualize ice-ball formation [23]-[25], accurate temperature mapping at cryogenic conditions is still under development. Furthermore, MRI systems are costly and require specialized infrastructure, limiting their accessibility in routine clinical practice.

Ultrasound (US) offers an attractive alternative due to its real-time imaging capability, low cost, and wide availability [26]-[28]. It is widely utilized for guiding and monitoring thermal ablation procedures [29]-[32], including cryoablation, although its role is still evolving. During freezing, the formation of ice produces a hyperechoic region with posterior acoustic shadowing, which serves as a qualitative indicator of the frozen zone [33]-[35]. However,

conventional B-mode US imaging does not provide quantitative temperature information, and clinically relevant temperature thresholds cannot be distinguished. As a result, treatment assessment remains largely qualitative.

To address these limitations, researchers have explored advanced US techniques. US elastography, for instance, measures tissue stiffness and has been investigated for monitoring the frozen zone post-ablation [36]. One promising approach involves estimating the speed of sound (SoS), which is sensitive to temperature-dependent changes in tissue properties [37]-[41]. Previous studies have demonstrated that SoS varies significantly during freezing, particularly in water-rich materials where phase transitions dominate acoustic behavior. In biological tissues, however, the relationship is more complex due to variations in composition, especially lipid content [42]. Existing SoS measurement techniques for freezing processes are typically based on time-of-flight methods and require specialized experimental setups [42], often providing only bulk or point measurements under controlled conditions. Consequently, they are not well suited for real-time, spatially resolved monitoring during clinical cryoablation.

In this work, a dense speed-of-sound shift imaging (DSI) framework is proposed for estimating temperature changes during cryoablation. The method analyzes raw US radiofrequency data to detect pixel displacements associated with temperature-induced variations in acoustic properties. Displacements are estimated using the Farnebäck optical flow algorithm [43] and incorporated into a regularized inverse problem to reconstruct spatial maps of SoS shifts. These shifts are directly related to temperature changes and enable quantitative temperature estimation. The approach was evaluated in both tissue-mimicking phantoms and *ex vivo* tissue, with simultaneous thermocouple measurements providing ground-truth validation. By establishing the relationship between temperature and SoS-derived slowness changes, this approach enables non-invasive, spatially resolved monitoring of cryoablation using conventional ultrasound systems, offering a practical alternative to existing techniques.

2 Research Objectives

This work aims to develop and evaluate non-invasive, US-based framework for spatially resolved temperature estimation during cryoablation. Specifically, this study aims to utilize temperature-dependent changes in the SoS in order to infer thermal distributions within tissue, addressing the limitations of existing monitoring techniques. To achieve this goal, a DSI approach is proposed and adapted for cryogenic conditions. The framework is designed to

extract temperature-related information from raw US data by analyzing image-derived displacements and linking them to underlying acoustic property changes. Our specific aims:

Aim 1: Develop and implement a dense SoS-shift imaging framework for cryoablation monitoring. In this aim, we adapt an existing US-based DSI algorithm [29], originally developed for thermal ablation, to the context of cryoablation. This includes processing raw RF US data to reconstruct B-mode images, estimating pixel displacements using the Farnebäck [43] optical flow algorithm, and formulating a regularized inverse problem to recover spatial maps of SoS shifts associated with temperature variations.

Aim 2: Establish the quantitative relationship between temperature and SoS-derived slowness changes during cryoablation. In this aim, controlled cryoablation experiments are conducted in tissue-mimicking phantoms and ex-vivo biological tissue. Simultaneous thermocouple measurements are used to provide ground-truth temperature data during US acquisitions. These measurements enable the characterization of the relationship between temperature decrease (from 20°C to approximately -40°C) and corresponding SoS shifts, including the investigation of material-specific behavior and functional dependencies.

Aim 3: Evaluate the feasibility of DSI for spatially resolved temperature estimation during cryoablation. In this aim, the ability of the proposed framework to estimate temperature distributions is assessed by comparing SoS-based estimations with ground-truth measurements. The performance of the method is analyzed in terms of its capability to capture spatial temperature gradients, distinguish freezing regions, and quantitatively estimate temperature distributions, including the estimation of minimum temperatures, in comparison with ground-truth thermocouple measurements.

3 Theoretical Background

3.1 US physics

Sound is a form of mechanical energy that propagates through a material medium via coordinated oscillations of its constituent particles. In diagnostic US, acoustic waves are typically generated within the frequency range of 1-20 MHz. The propagation characteristics of these waves are governed by the physical properties of the medium, primarily its density (ρ)

and stiffness, often expressed by the bulk modulus (B). These properties determine the speed of sound, given by

$$c = \sqrt{\frac{B}{\rho}} \quad (1)$$

which plays a central role in US imaging and signal interpretation [44].

In biological tissues and fluids, US waves propagate predominantly as longitudinal waves, in which particle motion occurs parallel to the direction of wave propagation [43], [44]. In contrast, solid materials such as bone can support both longitudinal and transverse (shear) waves, the latter involving particle motion perpendicular to the direction of propagation (Fig. 1) [46]. The nature of wave propagation is also influenced by the structural properties of the medium. In isotropic materials, acoustic properties are uniform in all directions, whereas anisotropic materials, such as cortical bone, exhibit direction-dependent behavior, leading to variations in sound speed based on the propagation orientation [44].

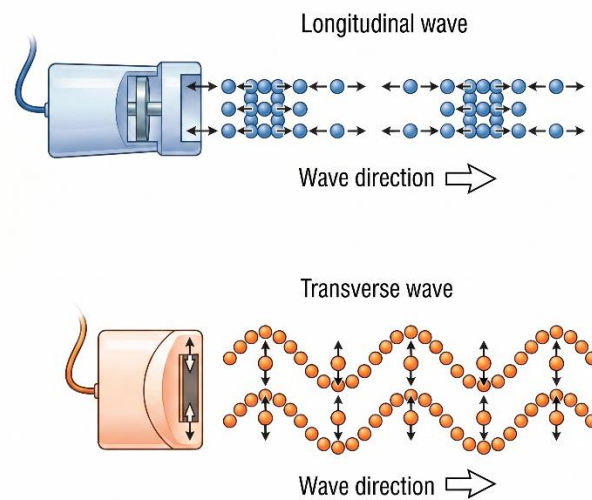


Figure 1. Longitudinal and transverse wave motion. Longitudinal waves have particle vibrations parallel to the direction of the wave propagation. Transverse waves have vibrations perpendicular to the direction of the wave propagation.

US waves are typically generated and detected using piezoelectric materials, which possess the unique ability to convert electrical energy into mechanical vibrations and vice versa. When an electrical pulse is applied across a piezoelectric crystal, the piezoelectric effect induces mechanical strain, causing the material to vibrate and emit acoustic waves into the surrounding medium (Fig. 2) [44]. Conversely, incoming US waves produce mechanical

deformation in the material, generating an electrical signal that can be detected. The piezoelectric crystal is typically designed to resonate at a specific fundamental frequency, f_0 , such that it is primarily determined by the thickness of the piezoelectric element:

$$f_0 = \frac{c}{2d} \quad (2)$$

where d is the thickness of the element [44]. In modern systems, these elements are subdivided into arrays of small, independently controlled units, enabling the generation of complex acoustic fields through coordinated excitation. This array configuration allows for advanced beamforming, steering, and focusing capabilities, thereby enhancing imaging performance over a larger field of view.

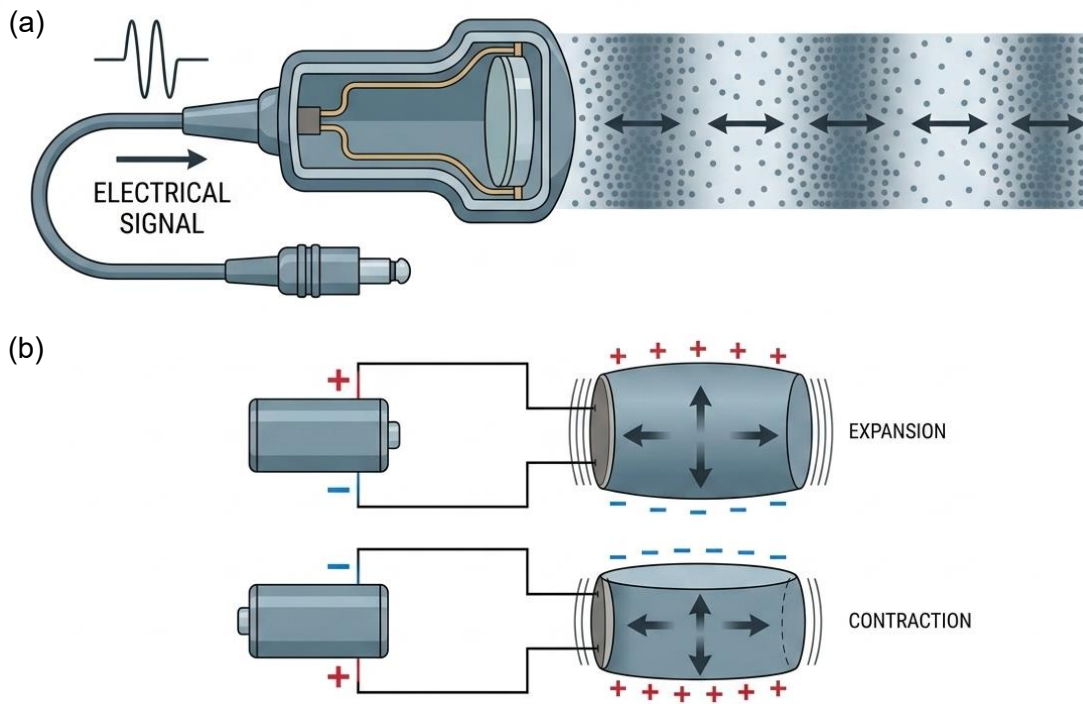


Figure 2. Piezoelectric transducer and the piezoelectric effect. (a) When a sound wave is incident on the transducer, it produces an electrical signal. When an electrical signal is applied to the transducer, it vibrates, producing sound waves. (b) An electrical signal is applied to the piezoelectric crystal and causes it to vibrate.

3.2 US imaging

Modern US imaging systems are based on the pulse-echo principle, in which short acoustic pulses are transmitted into tissue and the returning echoes are detected and processed to form an image [44]. The round-trip time of an echo is the total time elapsed from the moment an US pulse is transmitted until its reflection (echo) is received back at the transducer, expressed by [46]:

$$t = \frac{2z}{c} \quad (3)$$

where z is the distance from the transducer and c is the speed of sound. In biomedical US applications, the acoustic properties of various tissues are well characterized and assumed constant in each tissue type [47]. For example, in soft tissue the speed-of-sound is assumed to be 1540 m/s.

In conventional systems, this process is achieved using transducer arrays composed of multiple piezoelectric elements, enabling electronic beamforming. During transmission, a subset of elements is activated to generate an acoustic wavefront, which can be shaped and controlled through the application of time delays [44]. By introducing linear delays across the array, the beam can be electronically steered at different angles, while quadratic delays enable focusing at a specific depth by ensuring constructive interference of the emitted wavelets at a focal point. On reception, the returning echoes are detected by the transducer elements, and dynamic receive focusing is applied by continuously adjusting time delays to maintain optimal focus throughout the imaging depth (Fig. 3) [28].

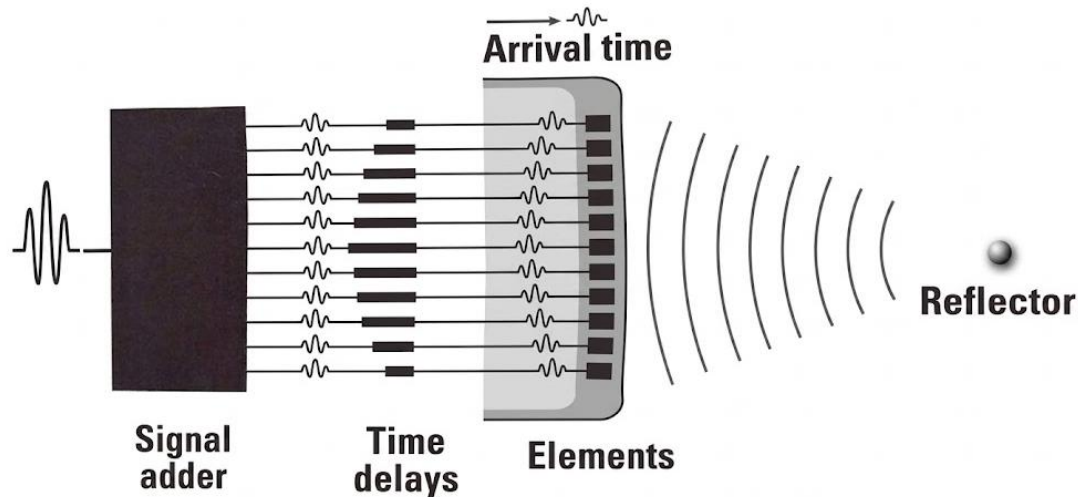


Figure 3. Focusing during echo reception. The geometric location of the reflector results in different distances from the reflector to each individual element, such that echo signals arrive at slightly different times. Focusing is done by applying different time delays to each signal, so the signals will be in phase when summed. Figure adapted from [44].

The detected signals are digitized as a function of time to produce one-dimensional signals known as A-lines, which represent echo amplitude along a single beam direction. By sequentially transmitting and receiving across the transducer aperture, multiple A-lines are acquired and spatially combined to reconstruct a two-dimensional B-mode US image of the field of view. However, the transmit delays required for conventional beam focusing exhibit a parabolic dependence on the element position, resulting in relatively long delay profiles that limit the achievable frame rate. When imaging over a full field of view using multiple steered and focused beams, the need to sequentially transmit hundreds of such beams significantly reduces the overall acquisition speed. To overcome this limitation, coherent plane wave compounding can be employed. In this approach, linear delays are applied across the transducer elements to generate tilted plane waves, which are typically transmitted using the full aperture simultaneously. Although individual plane waves are unfocused and therefore provide lower instantaneous image quality, their short transmit duration enables the rapid acquisition of multiple plane wave transmissions from different angles. These acquisitions are subsequently coherently compounded, improving image quality and contrast while maintaining a substantially higher frame rate compared to conventional focused imaging.

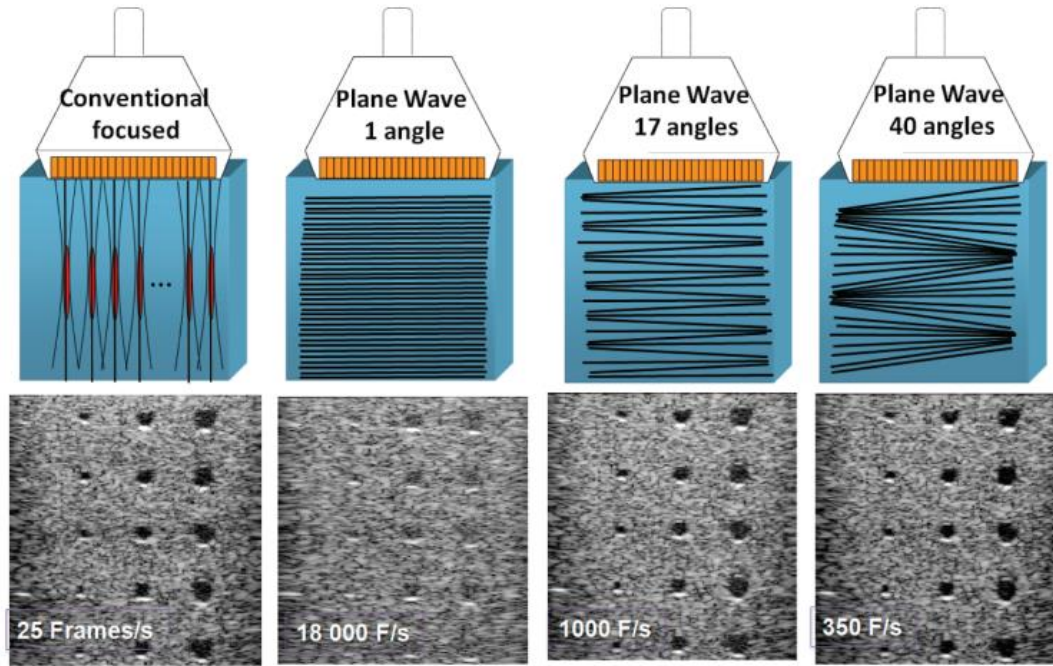


Figure 4. Schematic illustration of coherent plane wave compounding compared to conventional B-mode imaging. In plane wave compounding, linear delays are applied across the transducer to generate angled plane wave transmissions using the full aperture. Although individual plane waves are unfocused, their rapid acquisition enables multiple transmissions from different angles, which are coherently combined to improve image quality. Figure reprinted from [48].

3.3 Cryoablation

Cryoablation is a minimally invasive procedure used to destroy pathological tissues in a target area by exposing them to extremely low temperatures. Clinically, cryoablation is widely used to treat various conditions [49], including renal tumors [5], [6], liver metastases [7], breast cancer [8], [9], and cardiac arrhythmias [10]. This technique involves controlled freezing in a defined region, offering a less invasive alternative to surgical resection with reduced associated morbidity [1], [4], [11]. This targeted freezing process leads to irreversible cellular damage and tissue necrosis through two primary mechanisms: immediate direct cellular injury and delayed vascular-mediated injury, which are further supplemented by programmed cell death (apoptosis) and a potential immunological response [1], [4].

3.3.1 Direct Cellular Injury

Direct cellular damage is primarily governed by the cooling rate, which determines whether ice forms inside or outside the cell [1], [3]. At slow cooling rates, ice crystals form

first in the extracellular spaces. This process withdraws free water from the environment, creating a hyperosmotic state that draws water out of the cells via osmosis [1], [3], [4]. The resulting cell dehydration, shrinkage, and high solute concentrations lead to the damage of cytoplasmic enzymes and the destabilization of cellular membranes. On the other hand, when cooling is rapid (typically $>20\text{-}50^{\circ}\text{C}/\text{min}$) the process is more destructive. Water does not have enough time to leave the cell and instead freezes into intracellular ice crystals. These crystals are considered universally lethal, as they physically disrupt the plasma membrane and vital internal organelles, such as the mitochondria and endoplasmic reticulum [1], [4], [11].

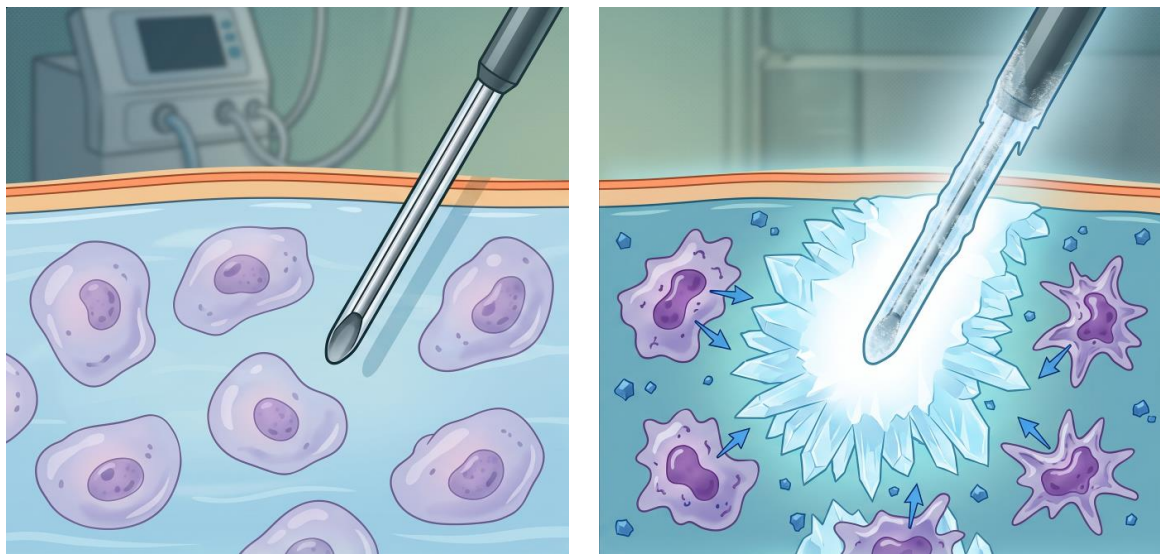


Figure 5. Illustration of direct cellular injury caused by a slow cooling rate. Ice crystals form in the extracellular space and withdraw water from the cells via osmosis. The result is cell dehydration and damage to cellular structures.

3.3.2 Delayed vascular-mediated injury

Delayed injury involves the progressive failure of microcirculation. Freezing causes immediate endothelial damage to the lining of blood vessels, such that upon thawing, this damage leads to increased vascular permeability, edema, platelet aggregation, and microthrombus formation [1], [3], [4], [11]. This eventually results in circulatory stasis and ischemia, depriving remaining cells of oxygen and nutrients and leading to uniform necrosis within the target area.

3.3.3 Thawing and Recrystallization

The thawing phase is a prime destructive factor in cryoablation. During slow thawing, a phenomenon known as recrystallization occurs, where small ice crystals fuse to form larger, more destructive ones. These large crystals exert mechanical shearing forces on compromised cell membranes. Furthermore, as extracellular ice melts, the environment becomes hypotonic, causing water to rush back into the damaged cells, which often results in osmotic rupture [1], [3], [4], [11].

3.3.3 Programmed Cell Death (Apoptosis)

Cells that are not immediately killed by mechanical ice disruption may later die via apoptosis [1], [4], [11]. This is most significant at the periphery (freeze margin) of the ablation zone, where temperatures are sub-lethal (typically between 0°C and -20°C). Apoptosis is regulated by mitochondrial dysfunction, involving the release of cytochrome c and a shift in the ratio of pro-apoptotic (Bax) and anti-apoptotic (Bcl-2) proteins.

3.3.4 Immunological Response

Cryoablation can trigger an immunologic response as tumor-specific antigens are released from the ablated tissue. This response may include the production of antitumor antibodies and the induction of cytotoxic T-cells, which have the potential to target and kill malignant cells that were not in the immediate vicinity of the freezing [3], [4], [11].

3.4 Temperature monitoring

As a minimally invasive technique, accurately defining and monitoring the treated region is critical for effective and safe treatment. During cryoablation, a thermal gradient is established around the cryoprobe, such that with increasing distance the achieved minimum temperature increases [1], [11]. This decrease in cooling efficiency has been demonstrated in previous studies [12], [13]. This spatial variation in temperature influences the shape of the frozen region (encapsulated by the freeze margin) and the surrounding hyperemic tissue, both of which must be considered to ensure complete destruction of pathological cells while preserving healthy structures [11], [14]. Therefore, temperature monitoring is essential to confirm that a lethal temperature threshold is reached within the target area, and to avoid excessive freezing of adjacent tissue.

3.4.1 Existing methods for temperature monitoring

A common method for temperature monitoring during cryoablation includes the use of thermocouples. In research settings, invasive temperature sensors such as fiber optic sensors [15], [16] and thermistors [17] have been used for monitoring temperature and freezing depth, though their clinical adaptation remains limited. These sensors are inherently invasive and provide only localized, point-based temperature measurements rather than a full spatial temperature map of the treated region, which restricts their ability to capture thermal gradients or heterogeneity across the treated region. Infrared (IR) thermometry has also been explored in research as a non-contact method to estimate surface temperatures during cryoablation [18], [19]. However, IR thermometry is limited to superficial measurements and cannot capture temperature dynamics within deeper tissue, since IR radiation does not penetrate beneath the surface.

MRI thermometry is widely regarded as the gold standard for non-invasive, spatially resolved temperature mapping in thermal ablation applications [20]-[22]. However, its role in cryoablation procedures remains limited. An experimental study by Overduin et al. (2016) [50] has investigated the feasibility of MRI for temperature spatial mapping during cryosurgery in ex-vivo porcine muscle specimens, reaching -40°C in the process. In a more recent study, Gutt et al. (2026) [51] developed a similar model in ex-vivo bovine liver samples using a FLORET approach, reaching a minimal temperature of -20°C . Nevertheless, currently MRI is mainly used in cryoablation to guide cryoprobe placement and to monitor ice-ball formation during image-guided cryoablation, helping ensure adequate tumor coverage [23]-[25]. Despite its advantage as a reliable tool for visualizing the ablated tissue and its surrounding region without invasive contact, MRI thermometry remains less established in the context of cryoablation, and introduces a high cost and requires specialized infrastructure, thus limiting availability.

US is an especially appealing technology for temperature monitoring, as it is noninvasive, cost effective and capable of imaging deep tissues in real time [26]-[28]. US imaging operates by transmitting acoustic waves into tissue and detecting the echoes that return from interfaces with different acoustic impedances, allowing the reconstruction of structural information based on the amplitude and timing of these reflections. It is widely utilized for guiding and monitoring thermal ablation procedures due to its real-time feedback, portability, and wide availability [29]-[32]. US is also used for guiding and monitoring cryoablation procedures, although its role is still evolving. During cryoablation, the formation of ice leads to a hyperechoic region with posterior acoustic shadowing, which can obscure the visualization of deeper tissue

structures. Despite this limitation, the appearance of the hyperechoic zone serves as an indirect indicator of the frozen region [33]-[35]. However, this information is qualitative rather than quantitative; US imaging cannot reveal the actual temperature within the ice ball or in the surrounding tissue, and lethal isotherms such as -20°C or -40°C cannot be distinguished from visual appearance alone. As a result, clinicians performing cryoablation must rely solely on the B-mode image without access to spatially resolved thermal information, limiting their ability to assess treatment adequacy or avoid unintended cooling of adjacent structures. To enhance the assessment of thermal and structural changes during cryoablation, researchers have explored advanced US techniques. US elastography, for instance, measures tissue stiffness and has been investigated for monitoring the frozen zone post-ablation [36].

3.4.2 Speed-of-Sound approach

Another promising approach involves estimating the local SoS, which is sensitive to temperature-dependent changes in tissue properties. SoS measurements were utilized in various applications such as estimating ice-content [37], [38], assessing thaw cycles [39], and identifying pre-thaw stages in frozen foods [40] as well as temperature estimation in orange juice [41]. A comprehensive review by Liang et al. [42] examined acoustic properties measured in materials down to -50°C , including water, aqueous solutions, lipids and biological tissues. A pronounced change in SoS was observed (i.e., longitudinal velocity) in water, aqueous solutions and biological tissues when the samples underwent freezing, whereas lipids and oils exhibited a more gradual but still distinct shift. Contrary to the well-studied linear relationship of US image strain and temperature change in heating applications of biological tissue [29], [52], it has been shown that tissue composition significantly affects SoS behavior during freezing, particularly the water-to-lipid ratio in biological tissues [42]. In water-rich samples, the SoS change is dominated by the phase transition of water, while in biological tissues, additional factors such as fat distribution and microstructural freezing dynamics cause smaller SoS change [42]. Consequently, it is expected that the relationship will be more complex in ex-vivo tissues containing lipids, compared to water-dominated samples. Studies reviewed by Liang et al. [42] relied on pulse-echo or through-transmission configurations combined with sample-insertion or multiple-reflection methods, all of which estimate SoS through time-of-flight measurements. Importantly, these approaches require two transducers or a reflector, provide single-point or bulk measurements, and are generally performed in controlled laboratory setups, with freezing induced by air-cooling or plate-based conduction rather than

clinically relevant cryoprobes. As a result, prior SoS-based methods are not directly applicable to spatially resolved, real-time temperature monitoring during in-situ cryoablation.

3.4.3 Proposed method

The above findings establish a foundation for employing SoS shifts as indicators of underlying acoustic property changes in tissue during cryoablation. In particular, the strong dependence of SoS on temperature enables the estimation of local temperature variations based on measured shifts in sound speed. Here, we propose a DSI algorithm for SoS shift estimation, which was implemented in an experimental platform designed to enable controlled cryoablation in different samples. Simultaneous imaging and recording of ground-truth temperature was conducted with a thermocouple monitoring temperature during acquisitions of raw RF data. Our algorithm transforms the raw data into B-mode US images and analyzes them for pixel displacements induced by temperature variations between 20°C and -40°C, using the Farnebäck optical flow algorithm [43]. A regularized inverse problem framework was subsequently established to relate these displacements to SoS shifts. These shifts correspond to temperature decreases and can be utilized for estimating temperature (Figure 6). The algorithm implemented in this work is derived from a previously established framework for thermal ablation monitoring and has been adapted to the cryoablation setting [29]. Using this framework, the relationship between cryogenic temperature decrease and slowness deviations of the speed of sound was characterized in both tissue-mimicking phantoms and ex vivo tissue samples. Establishing this quantitative relationship between temperature and SoS, including material-specific behavior and functional dependence, provides a basis for inferring temperature profiles from image-based slowness estimation during cryoablation. This approach enables non-invasive, spatially resolved assessment of temperature using conventional ultrasound systems, while reducing reliance on implantable sensors or high-cost imaging modalities.

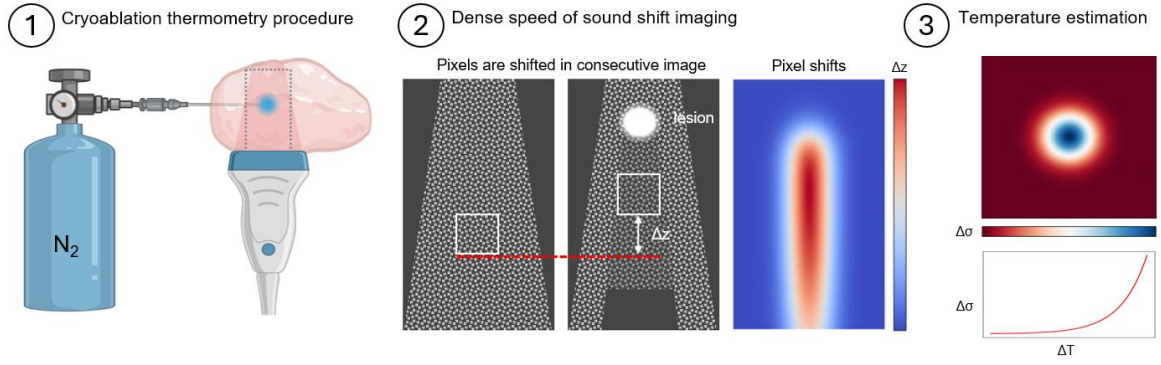


Figure 6: Cryoablation thermometry illustration. (1) Cryoablation is performed on a sample using a liquid nitrogen tank connected to a cryoprobe, which injects liquid nitrogen to locally cool the sample. An US imaging transducer is positioned perpendicular to the sample and records RF data during the cryoablation procedure. (2) RF data are converted into B-mode images and pixel displacements within the region affected by the growing cryoablation lesion are tracked using optical flow. (3) Pixel displacements between consecutive frames are used to compute the local slowness deviation within the imaged region. A calibration between slowness and temperature is established using simultaneous thermocouple measurements.

3.5. DSI Background

The mathematical relationship between the predicted echo-shift or change in round-trip time $\delta\tau$, and the temperature change δT along the wave propagation path, was described in our previous work [29]. Briefly, the relationship between $\delta\tau$ to the local shift in the sound speed is given by:

$$\delta\tau = 2 \oint \frac{1+\beta(z)\delta T}{c(z,T)} - \frac{1}{c(z,T_0)} dz \quad (4)$$

where the difference in round-trip time $\delta\tau$ is the cumulative sum of the inverse of changes in local speed of sound $c(z,T)$ due to temperature change δT from the initial temperature T_0 in tissue with a thermal expansion coefficient of $\beta(z)$ along the propagation path. In our method, the approach is linearization of the problem, such that Equation (4) can be vectorized. The integral is represented as a matrix, denoted as M below, and the following equation is obtained:

$$\Delta \underline{d} = M \Delta \underline{s} \quad (5)$$

where the pixel shift $\Delta \underline{d}$ is observed in a plane-wave B-mode image due to an echo-shift of $\delta \tau$. $\Delta \underline{s}$ represents the change in sound slowness, described by:

$$\Delta \underline{s} = \frac{1+\beta(z)\delta T}{c(z,T)} - \frac{1}{c(z,T_0)} \quad (6)$$

Once the integral operand $\Delta \underline{s}$ is isolated, it is equivalent to the derivative of (4) such that

$$\Delta \underline{s} = \alpha \times \delta T \quad (7)$$

where α is a medium-specific parameter describing the relationship of speed of sound with temperature, as well as a unit conversion parameter from strain to temperature. Importantly, in this study α may be a function of temperature change, ΔT , rather than a constant coefficient, such that the relationship between $\Delta \underline{s}$ and ΔT is described by $\alpha(\Delta T)$ and may not be linear. This is due to the varying acoustic properties in low temperatures that are dependent on tissue composition, as described by [42]. α is extracted from a curve of Δs , which is calculated by the algorithm, vs. ΔT , which is measured as ground-truth. This calibration is common to thermal measurements from acoustic strain images [53], [54], [55], [56].

To calculate the change in sound slowness $\Delta \underline{s}$, the following inverse problem is formulated:

$$M^{-1} \Delta \underline{d} = \Delta \underline{s} \quad (8)$$

The pixel shift $\Delta \underline{d}$ is measured from acquired B-mode images. The optimal $\Delta \underline{s}$ which satisfies the data is calculated via a Tikhonov pseudo-inverse. The spatial gradient was regularized using L2 regularization of the local gradient. Finally, the change in sound slowness $\Delta \underline{s}$ is estimated by solving the following least squares problem, using $M^\dagger$ as a pseudo-inverse matrix for the solution [29], [57]

$$\Delta \hat{\underline{s}} = \underset{\Delta \underline{s}}{\operatorname{argmin}} |\Delta \underline{d} - M \Delta \underline{s}|^2 + \Gamma |\nabla(\Delta \underline{s})|^2 \quad (9)$$

US plane wave B-mode images are acquired, and the pixel shift in between consecutive images is computed via optical flow to produce the measured vector $\Delta \underline{d}$. The slowness deviation reconstruction method applied in this work is based on a previously developed optical-flow-based framework designed for thermal ablation, in which temperature increases

are associated with an increase in the speed of sound [29]. Thus, the direction of apparent speckle motion in B-mode images corresponded to positive speed of sound shifts. In contrast, during cryoablation, tissue cooling results in a decrease in SoS, which causes the speckle pattern to shift in the opposite direction. To maintain consistency with the inverse model and ensure correct speed of sound shift estimation, the sign of the computed pixel displacements field was inverted prior to solving the reconstruction problem. Then, the measurements vector Δd was inverted as an inverse problem to receive the estimator for change in slowness, $\Delta \hat{s}$. Finally, the change in slowness can be utilized for temperature estimation.

4. Materials and Methods

4.1 Cryoablation procedure and setup

Two types of samples were used. The first was a tissue mimicking phantom. The phantom was made by heating 4.5 grams of agarose powder (214010, Becton, Dickinson and Company, MD, USA) in 300 ml deionized water in the microwave in pulses until boiling. The mixture was left in room temperature for 10 minutes and stirred with a magnetic stirrer for another 10 minutes. 1.5 grams of silicone carbide (357391, Sigma Aldrich, MO, USA) were added to the mixture as acoustic scatterers and stirred for 30 minutes. The mixture was poured into a mold to congeal. The result was a phantom with a shallow sunken circular area as a target for nitrogen contact.

The attenuation and speed of sound values for the phantom were measured in a water tank. A 3-cm thick phantom was placed between an L12-5 transducer operating at a center frequency of 7.5 MHz, and a 0.5 mm needle hydrophone (NH0500, Precision Acoustics, Dorchester, UK) that conducted the measurement. The attenuation coefficient was calculated by [58]:

$$\alpha = \frac{20}{\ln(10)} \frac{\ln\left(\frac{A_s}{A_a}\right)}{\Delta x \cdot f} \quad (10)$$

where A_s is the reference peak-to-peak voltage measured by the hydrophone measurement, A_a is the peak-to-peak voltage with the addition of the phantom between the transmitter and the hydrophone, Δx is the phantom thickness and f is the frequency. The phantom attenuation was found to be $0.20 \text{ dB} \cdot \text{cm}^{-1} \cdot \text{MHz}^{-1}$.

The speed of sound was measured in the same setup, where the oscilloscope was used to determine time-of-flight of the US wave with and without the agar phantom in the path. The speed of sound in the phantom, c_p , can be calculated by [59]:

$$c_p = \frac{c_w}{1 + \Delta t \frac{c_w}{\Delta x}} \quad (11)$$

where c_w is the speed of sound in water (e.g. $1490 \text{ m}\cdot\text{s}^{-1}$), Δx is the sample thickness, and Δt is the measured time shift. The speed of sound measured for our phantom was $1489 \text{ m}\cdot\text{s}^{-1}$.

The nitrogen was gently sprayed on the circular target area from above, while the imaging transducer, positioned perpendicularly and 4-5 mm under the sunken area plane, imaged the area adjacent to the sprayed target area. This area's temperature decreased due to the ice-ball that was formed around the target area, as presented in Figure 7(c). The procedure was repeated in six samples of this type.

The second set of samples were fresh ex-vivo turkey breast samples, obtained from a local butcher shop. Samples were cut from the thickest part of a turkey breast in a single piece that could fill the field of view and contain the cryoprobe depth. Each sample was placed in a 3D printed cartridge, and the custom cryoprobe was inserted into it to a depth of 4-5 mm. To minimize reflections, 6 mm rubber was placed around the samples and coupled with US gel. The experimental setup is presented in Figure 7(a). As a ground truth, in both phantoms and turkey breasts, a T-type thermocouple connected to a thermometer (TL253, Kamtop, China) was placed within the frozen region, as close as possible to the cryoprobe, at $2.75 \pm 0.42 \text{ mm}$ laterally from the cryoprobe surface, where temperatures would reach -40°C after cooling [60], [61]. This spatial registration was performed via simultaneous visualization of the cryoprobe and the thermocouple in real-time B-mode imaging, and the distance between the bright hyperechoic regions centers was measured using the on-screen lateral axis. After confirming their relative positioning, both instruments were retracted slightly (1 mm) out of the imaging plane to prevent persistent acoustic reflection artifacts during acquisition. In addition, the distance between the insertion points at the tissue surface was additionally measured using a ruler. These two measurements yield an uncertainty of $\pm 0.7 \text{ mm}$, accounting for the accuracy of the B-mode visualization, considering the discretization of the lateral axis and the diffraction-limited lateral resolution of the transducer [62], and the ruler measurement at the tissue surface. The temperature measured with the thermocouple was recorded at the same time points of each data acquisition. The procedure was repeated in five samples of this type.

Direct injection of liquid nitrogen into the sample can lead to rapid pressure buildup and consequential movements of the tissue. This is undesirable for reproducible thermal measurements based on US imaging. Therefore, it is required that the freezing system confine the liquid nitrogen in the probe and would not allow it to be released into the tissue. Clinical cryotherapy systems, such as ISOLIS Cryoprobe System (Varian Medical Systems, Palo Alto, USA) or ProSense (IceCure Medical, Caesarea, Israel) rely on the Joule-Thompson effect (Roebuck et al., 1942), where gas expansion inside the probe lowers its temperature, allowing heat to be conducted from surrounding tissue. In these systems, the gas remains within the probe and is cycled back for repeated expansion and cooling, which requires specialized cryotherapy systems [4], [9]. Inspired by this approach, we designed a custom liquid nitrogen cryoprobe connected to a 350 ml stainless steel liquid nitrogen sprayer can (Mini cryo-can liquid nitrogen sprayer, Rana Traders, India) (Figure 7(a)). A custom freeze-head was fabricated to interface with the tank's valve, allowing for the controlled flow of nitrogen through the probe, cooling its surface. The probe, shown in Figure 7(b), has a sharp hollow body to penetrate the sample, while permitting gas flow, such that the hollow body cools down to cryogenic temperatures while inside the sample. The nitrogen gas exits through the last section of the probe, outside of the sample, to avoid tissue displacement due to gas pressure. This cooling mechanism enabled effective and localized tissue freezing at the contact site, minimizing sample motion and imaging artifacts during US acquisition.

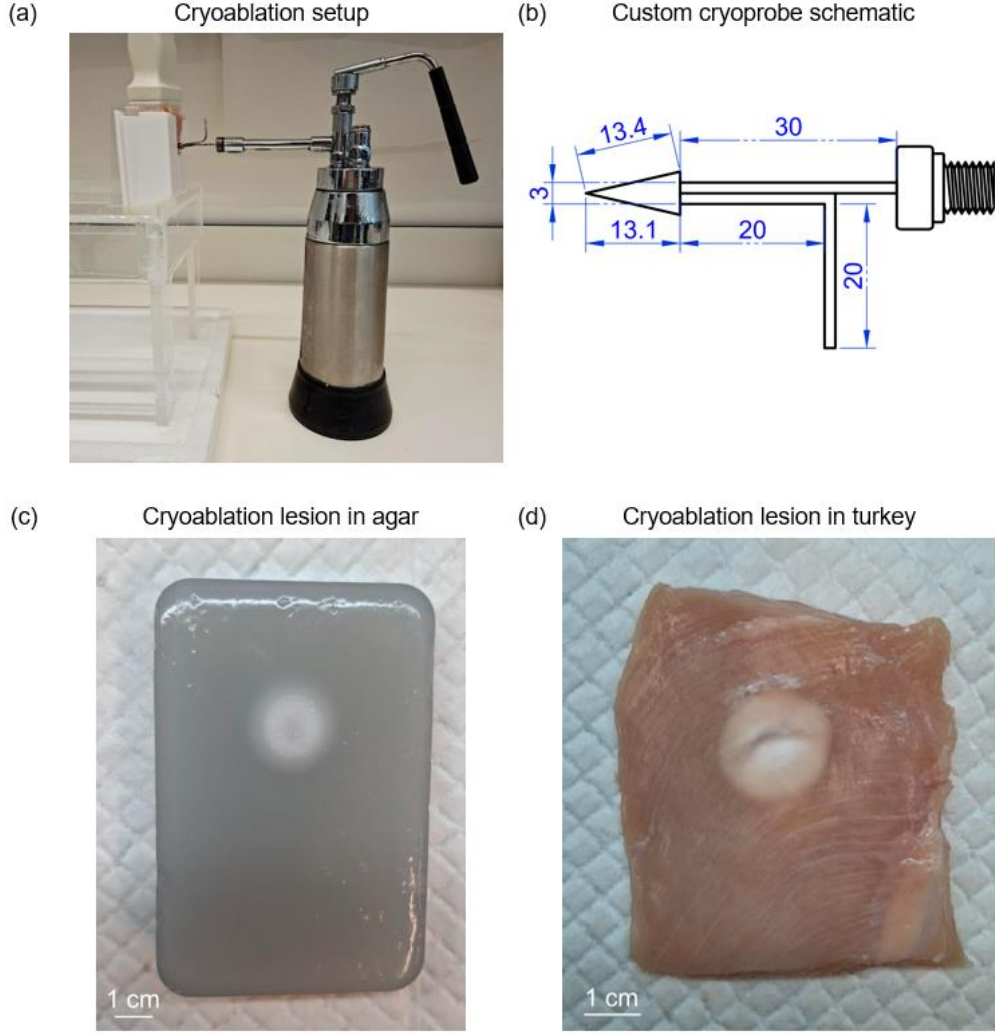


Figure 7. Experimental setup and samples used for cryoablation experiments. (a) Experimental setup, showing the US imaging transducer positioned perpendicular to the cryoprobe, cryoprobe connected to a liquid nitrogen tank, and 3D printed white box as a sample holder, during cryoablation. (b) Schematic illustration of the custom-designed cryoprobe, consisting of a sharp copper tip connected to a stainless-steel pipe, enabling controlled surface cooling without direct nitrogen injection into the sample. Units are mm. (c) Tissue-mimicking agar phantom after local cryoablation, exhibiting the frozen area formed at the target surface exposed to the cryoprobe cooling. (d) Ex-vivo turkey breast sample following local cryoablation using the cryoprobe, showing the frozen region at the contact site.

4.2 Ultrasound imaging

Imaging of the sample was performed using an L12-5 50 mm linear transducer, with a center frequency of 7.5 MHz (ATL Philips, WA, USA), positioned perpendicular to the cryoprobe and coupled with US gel. A programmable US system (Vantage 256, Verasonics Inc., WA, USA) was used for imaging of a 60 mm $\times$ 40 mm field of view. A plane steering

acquisition protocol was used to acquire nine plane waves per image. Three main steering angles were selected at 5° intervals $[-5^\circ, 0^\circ, 5^\circ]$, each composed of three steering angles selected at 1.5° intervals (for example, $[3.5^\circ, 5^\circ, 6.5^\circ]$). Raw RF data was acquired automatically every 3 seconds, for a duration of 2 minutes, while the sample underwent local cryoablation. The data was stored for offline processing.

4.3 Post processing and speed of sound shift calculation

RF data was beamformed and interpolated onto a 600×390 pixel² grid, such that the axial z direction represents 60 mm and the lateral x direction represents 40 mm. Each image was then Hilbert transformed, and the 1.5° angled plane waves sub-sets were coherently compounded: after performing a Hilbert transform on all the sub-images, the transforms of each sub-angle in a set $[x-1.5^\circ, x^\circ, x+1.5^\circ]$ were summed such that it resulted in a single steering angle of x° [57]. Each image acquisition provided three main steering angles.

The dense optic flow was calculated between consecutive images at each of the main steering angles using Farneback's method [43] and was inverted to maintain consistency with the inverse model. The three calculated pixel-shift images acquired at each time interval were down-sampled $\times 5$ in the lateral direction and $\times 10$ in the axial direction, then stacked to create the data vector. The data vector Δd was multiplied by the matrix $M^\dagger$, and the regularization parameters affecting smoothness were selected as $\Gamma_x=10$ and $\Gamma_z = \Gamma_{xz} = 1$. Then, the image was up-sampled back to the original image dimensions and summed. To derive the relationship between slowness deviation vs. temperature change, six agar-phantom samples and five turkey-breast samples were used, one time each, in a cryoablation experiment. Notably, although the raw RF-data was acquired in intervals of 3 seconds, the temperature of the sample in each experiment was recorded in intervals of 9 seconds, and the corresponding slowness-deviation values calculated by DSI were taken for calibration. The data points for each sample were grouped by their temperature change (ΔT) value proximity, and the mean and standard deviation were calculated for each data point, both for ΔT and for their corresponding slowness deviation ($\Delta \sigma$) mean value. Agreement between DSI-derived temperature estimates and reference thermocouple measurements was evaluated using Bland-Altman analysis [63] on a validation dataset. For each paired measurement, the mean temperature and the difference between methods were calculated, and the mean bias and 95% limits of agreement (± 1.96 SD) were determined. DSI was implemented in python, and the pixel displacements were calculated via the OpenCV library [64]. The inverse problem solution (Eq. 8) was implemented as a sparse matrix multiplication using scipy [65].

5. Results

The results have been published in Physics in Medicine & Biology in May 2026 [66].

5.1 Tissue mimicking phantom results

Initial experiments were conducted in a tissue-mimicking phantom. A thermocouple was inserted prior into the phantom in proximity to the center of the target area in the sample to a depth of 1 cm. The phantom underwent local cryoablation in a target area (methods), and an ice-ball was created around the contact site. In each repetition, the duration of the freezing treatment ranged from 1.5 to 2 minutes until the temperature decreased by over 50°C from the initial temperature ($11.30 \pm 1.13^{\circ}\text{C}$). Consecutive US images were acquired automatically every 3 seconds, and the values for calibration were taken at intervals of 9 seconds, corresponding to thermocouple simultaneous measurements of the temperature. The temperature change is the subtraction of the temperature recorded at the time point from the initial temperature. Cooling was performed until a large ice-ball was created and the hyperechoic ice-ball edge was visible in the B-mode frame (Figure 8(a)), meaning the ice-ball grew and entered the transducer's imaging plane, resulting in a dark region below the hyperechoic edge. Pixel shifts between consecutive US B-mode images were calculated (Figure 8(b)) and used to generate the map of slowness deviation at each point of the imaged region (Figure 8(c)).

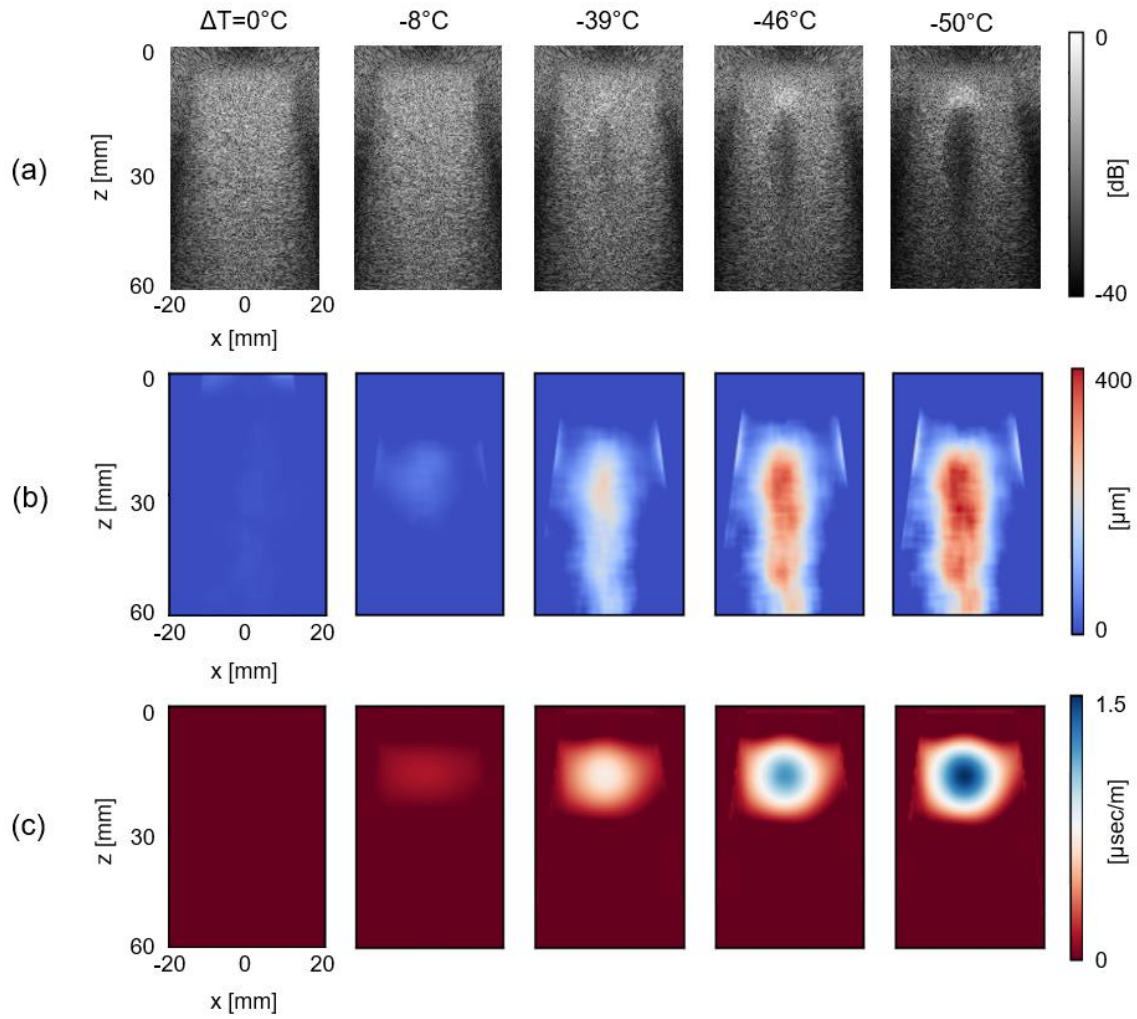


Figure 8. Effect of temperature decrease on the slowness deviation calculated by DSI in a tissue-mimicking sample. Cryoablation treatment was performed, and the temperature change was measured at each timepoint $\Delta T = 0^\circ\text{C}$, -8°C , -39°C , -46°C , -50°C . (a) B-mode images of the treated region at each timepoint. (b) Pixel shifts in the respective B-mode images, calculated by the algorithm. (c) Sound slowness calculated by the algorithm from the pixel shifts in (b). Axes are shown only for the first timepoint in (a)-(c) and are common to all timepoints. Colorbar is common to all subfigures in (a)-(c).

Slowness deviation was assessed as a function of temperature reduction (Figure 9(a)). The maximum slowness deviation measured within each imaged region was used to construct the slowness deviation-temperature curve. Since the largest temperature decrease was expected to occur in the area nearest to the cryoprobe and thermocouple, this region was assumed to exhibit the highest slowness deviation. A linear fit was then applied to the collected data using linear regression yielding $R^2 = 0.988$, $\text{RMSE} = 1.7^\circ\text{C}$ which describes the relationship between temperature change and slowness deviation with a slope of $\alpha_a = -20.70 \text{ } \mu\text{s/mC}^\circ$ in agar phantom. In addition, the areas in the image where the temperature was below 0°C based on

the calibration, were calculated in each image and are presented as a function of temperature (Figure 9(b)), starting from $121.0 \pm 60.0 \text{ mm}^2$ and gradually reaching $312.0 \pm 85.4 \text{ mm}^2$ as the recorded temperature decreased. The ice-ball diameter was measured immediately (within <1 minute following termination of freezing) following freezing using B-mode imaging. The empirically measured diameter was $2.2 \pm 0.075 \text{ cm}$, slightly larger than the diameter of the frozen region estimated from DSI ($1.99 \pm 1.04 \text{ cm}$). This enables the estimation of the frozen region around the cryoprobe, complementary to the maximal temperature change estimation. Agreement between DSI-estimated and thermocouple-measured temperature changes was further evaluated using Bland-Altman analysis (Figure 9(c), N=30 paired measurements obtained from two independent samples). The analysis demonstrated a small mean bias of 1.48°C , and the 95% limits of agreement ranged from -3.37°C to $+6.34^\circ\text{C}$, with most measurements falling within these limits.

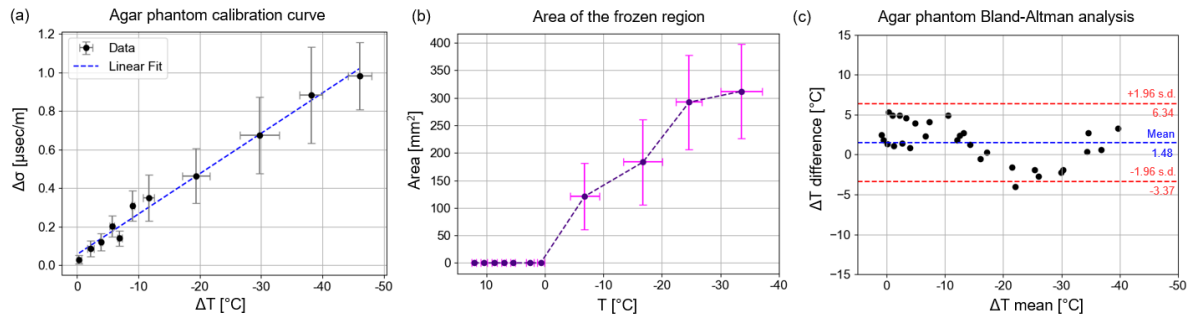


Figure 9. Analysis of temperature-slowness relationship and cold-region estimation during cryoablation in agar. (a) Calibration curve relating temperature change (ΔT) to slowness deviation ($\Delta\sigma$) during cryoablation in agar phantoms. The maximum slowness deviation measured in each imaged region was plotted against the corresponding temperature change recorded by the thermocouple. Data are presented as mean $\pm$ standard deviation in both axes (N = 6). A linear fit was applied using linear regression, yielding $R^2 = 0.988$, RMSE= 1.7°C . (b) Area in reconstructed slowness deviation image where $T < 0^\circ\text{C}$ as function of the temperature T recorded by the thermocouple. Data are presented as mean $\pm$ standard deviation in both axes (N = 6). (c) Bland-Altman analysis comparing DSI-estimated and thermocouple-measured ΔT values in a validation dataset of agar phantoms (N = 30). The blue dashed line represents the mean bias (1.48°C), and the red dashed lines indicate the 95% limits of agreement (-3.37°C to $+6.34^\circ\text{C}$).

5.2 Ex-vivo turkey breast sample

A similar procedure was performed on an ex vivo turkey breast sample to validate the method in biological tissue. In this case, the freezing was achieved using the custom-made

liquid nitrogen cryoprobe described earlier, which was inserted approximately 1 cm into the tissue. The thermocouple was positioned near the cryoprobe tip within the freezing zone to record local temperature changes. Cryoablation was applied for 1.5-2 minutes, producing a temperature drop of over 50°C from the initial temperature ($15.30 \pm 3.60^{\circ}\text{C}$) and forming a visible ice ball around the probe. US B-mode images (Figure 10(a)) were continuously acquired at 3-second intervals throughout the procedure, and the values for calibration were taken at intervals of 9 seconds, corresponding to thermocouple simultaneous measurements of the temperature. The resulting pixel shifts between consecutive B-mode frames were calculated (Figure 10(b)) and converted into slowness deviation maps (Figure 10(c)), following the same analysis pipeline used for the phantom experiments.

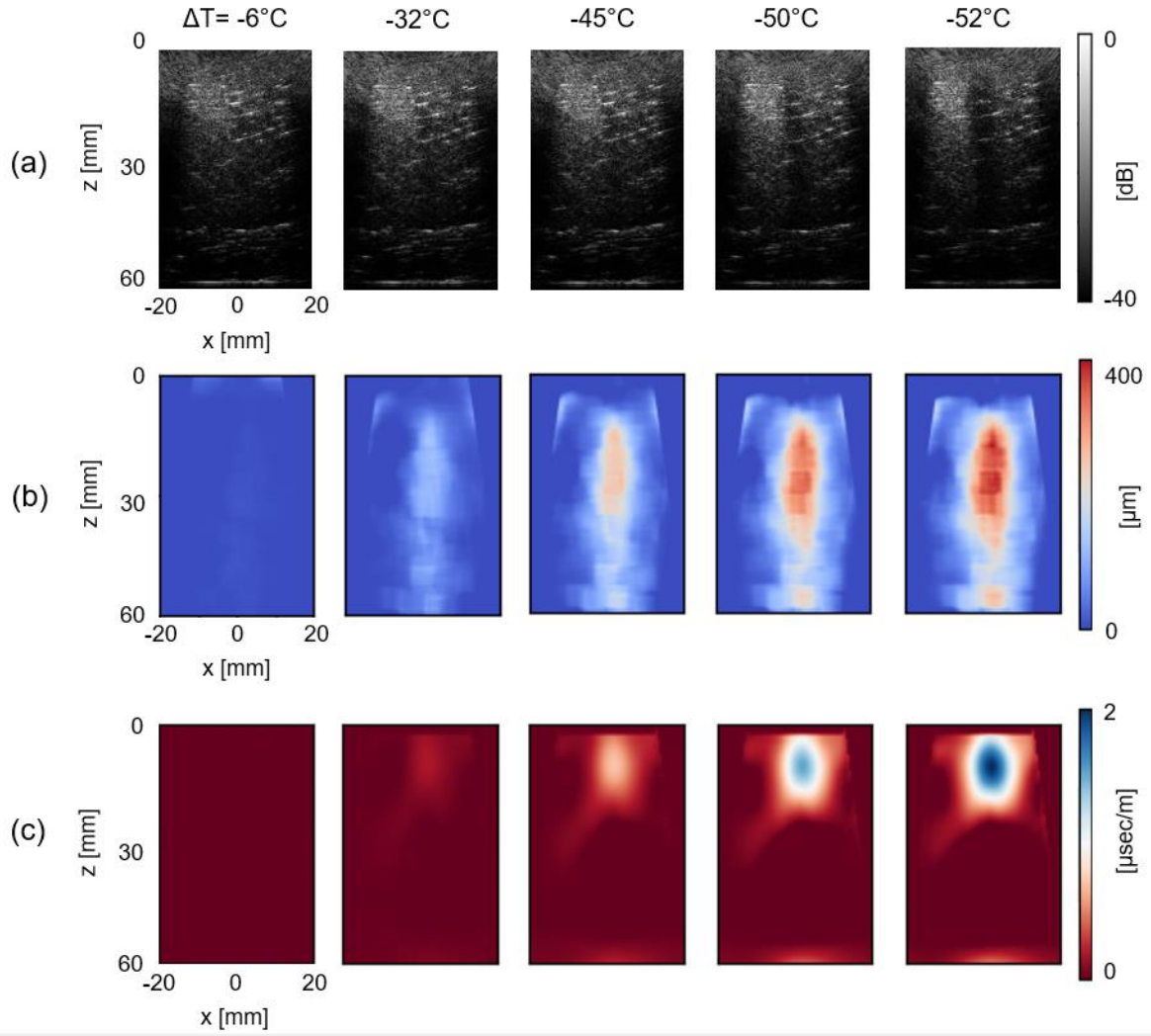


Figure 10. Effect of temperature decrease on the slowness deviation calculated by DSI in an ex-vivo turkey breast sample. Cryoablation treatment was performed, and the temperature change was measured at each timepoint $\Delta T = -6^\circ\text{C}$, -32°C , -45°C , -50°C , -52°C . (a) B-mode images of the treated region at each timepoint. (b) Pixel shifts in the respective B-mode images, calculated by the algorithm. (c) Sound slowness calculated by the algorithm from the pixel shifts in (b). Axes are shown only for the first timepoint in (a)-(c) and are common to all timepoints. Colorbar is common to all subfigures in (a)-(c).

The resulting data were analyzed following the same procedure as in the phantom experiment. The maximum slowness deviation from each region was correlated with temperature change. Since the data displayed an exponential relationship between slowness deviation and temperature change, the data was logarithmically transformed to obtain a linear relationship. Then, a linear fit was calculated using linear regression, yielding $\ln(\Delta\sigma) = 0.075 \times (-\Delta T) - 3.38$, $R^2 = 0.950$, $\text{RMSE} = 4.0^\circ\text{C}$. The resulting coefficients of the linear fit of the transformed data were taken as the coefficients of the exponential fit of the data, such that it is described by $\Delta\sigma = 34.04 \times (\exp(0.075 \times (-\Delta T)) - 1) \text{ } \mu\text{s/m}^\circ\text{C}$ (Figure 11(a)). The area where $T < 0^\circ\text{C}$ was also calculated and is presented as a function of temperature (Figure 6(b)), showing an

estimation of the frozen region, growing from $67.10 \pm 13.90 \text{ mm}^2$ and reaching $504.90 \pm 127.20 \text{ mm}^2$ at the end of the cryoablation process. The empirically measured ice-ball diameter ($2.0 \pm 0.047 \text{ cm}$) while the diameter of the frozen region estimated from DSI was $1.71 \pm 0.038 \text{ cm}$. Agreement between DSI-estimated and thermocouple-measured temperature changes was further evaluated using Bland-Altman analysis (Figure 11(c), N=40 paired measurements obtained from two independent samples). The mean bias was -2.91°C , and the 95% limits of agreement ranged from -8.11°C to $+2.28^\circ\text{C}$, with most measurements falling within these limits.

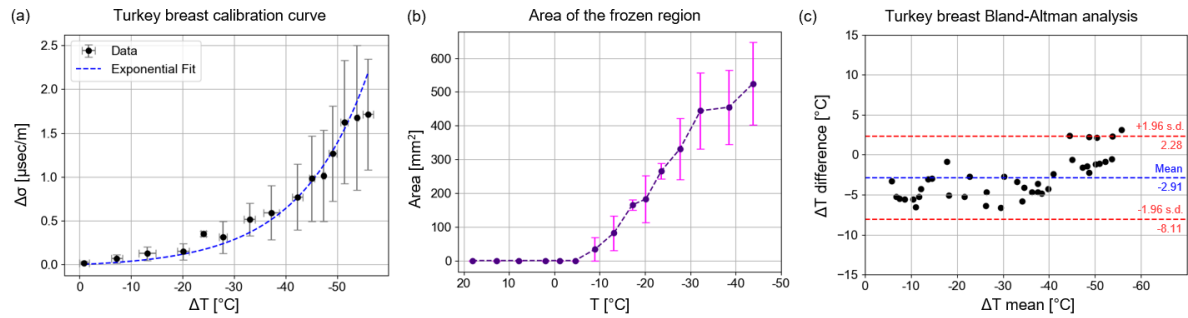


Figure 11. Analysis of temperature-slowness relationship and cold-region estimation during cryoablation in turkey breast. (a) Calibration curve relating temperature change (ΔT) to slowness deviation ($\Delta\sigma$) during cryoablation in turkey breast. The maximum slowness deviation measured in each imaged region was plotted against the corresponding temperature change recorded by the thermocouple. Data are presented as mean $\pm$ standard deviation in both axes (N = 5). A linear fit was applied to the transformed data using linear regression, yielding $R^2 = 0.95$, RMSE= 4.0°C . (b) Area in reconstructed slowness deviation image where $T < 0^\circ\text{C}$ as function of the temperature T recorded by the thermocouple. Data are presented as mean $\pm$ standard deviation in both axes (N = 5). (c) Bland-Altman analysis comparing DSI-estimated and thermocouple-measured ΔT values in a validation dataset of turkey breast samples (N = 40). The blue dashed line represents the mean bias (-2.91°C), and the red dashed lines indicate the 95% limits of agreement (-8.11°C to $+2.28^\circ\text{C}$).

6. Discussion

In this study, we investigated the feasibility of using SoS shifts to monitor temperature distributions during cryoablation, motivated by the known sensitivity of SoS to temperature-dependent changes in biological tissues. Conventional SoS measurement approaches typically rely on two transducers or a transducer-reflector configuration and provide SoS or time-of-flight measurements on a single-point basis [42]. Our method enables spatially resolved estimation of SoS shifts across the entire imaging region using a single transducer. This allows

for real-time visualization of the cooling process and temperature gradients during cryoablation. Compared with thermal strain imaging, which estimates SoS changes indirectly through echo time shifts and often relies on cross-correlation between pre- and post-heating frames, our previously developed algorithm, DSI, [29], directly computes the slowness deviation field from pixel displacements using optical flow. Previously we showed that DSI offers several key advantages over thermal strain imaging, including improved spatial coherence, greater robustness across both simulated and ex vivo data, and significantly faster computation by eliminating the correlation search step. These improvements make our algorithm well suited for real-time monitoring applications. Combined with a simple, low-cost cryogenic platform and an US imaging system, this approach provides a practical and scalable framework for spatially resolved temperature mapping during cryoablation.

Our method was implemented during cryoablation and calculated the slowness-deviation caused by temperature changes in the sample. We measured slowness deviations both in a tissue-mimicking phantom and in an ex vivo sample, at depths extending several millimeters below the surface adjacent to the cryoprobe contact point. These measurements revealed a consistent pattern of increased slowness deviation with decreasing temperature. While in tissue-mimicking agar phantom, a water-rich sample, the data exhibited a linear relationship between temperature change and slowness deviation, with a coefficient of $\alpha_a = -20.70 \text{ ns/m}^\circ\text{C}$ (Figure 9(a)), in turkey breast sample, a biological tissue containing fat and other microstructures, the relationship was exponential, described by $\alpha_t = 34.04 \times \exp(0.075 \times (-\Delta T) - 1) \text{ ns/m}^\circ\text{C}$ (Figure 11(a)). This indicates that the acoustic response to cooling, represented here by the α parameter, is material-specific and depends on both composition and structural properties such as water and lipid content, as expected [42]. Therefore, α should be experimentally determined for each tissue type to ensure accurate temperature estimation during cryoablation. Notably, the DSI method is based on tracking relative pixel displacements over time, arising from local changes in SoS during freezing with respect to the initial state of the same sample, and any constant offset caused by a different baseline SoS value of a sample is not expected to contribute to the displacement calculation.

Complementary to the temperature change estimation, it is also possible to estimate the frozen region around the cryoprobe using DSI. In agar phantom, the frozen region where the temperature was lower than 0°C was initially $\times 1.8$ larger than in turkey breast, but the final size of the frozen region was $\times 1.62$ larger in turkey breast compared to the agar phantom (Figure 9(b), Figure 11(b)). This difference could be the result of several parameters: the maximal temperature decrease achieved in each sample type (decrease of $46.1 \pm 2.0^\circ\text{C}$ from

initial temperature $11.30 \pm 1.13^{\circ}\text{C}$ in agar vs. decrease of $56.10 \pm 1.0^{\circ}\text{C}$ from initial temperature $15.30 \pm 3.60^{\circ}\text{C}$ in turkey breast), which directly affects the temperature distribution in the surrounding tissue during the freezing process, and resulting in a larger frozen surrounding area for a greater decrease in temperature; the size of the contact area with the cryogenic temperature origin, resulting in a larger frozen area when the contact site size is larger, reached several mm in turkey breast, while in agar phantom ranged from 0.5-1 cm; and the sample composition, which is more aqueous in agar, while richer in lipids and microstructures in turkey breast, affecting the rate of the ice ball growth and the temperature distribution [67]. The ice-ball diameter was measured immediately after the experiment (within <1 minute following termination of freezing) for both the agar phantom and the ex-vivo turkey breast samples. The diameter was measured via B-mode visualization of the full ice ball diameter with the transducer in the plane where the ice ball appeared the largest, and its diameter was taken via the on-screen lateral axis. The empirically measured ice-ball diameter was 2.2 ± 0.075 cm in agar and 2.0 ± 0.047 cm in turkey breast samples. In contrast, the region visualized using the DSI reconstruction appeared smaller (1.71 ± 0.038 cm in agar and 1.32 ± 0.035 cm in turkey breast). This difference arises possibly because the ice ball strongly reflects and attenuates the US beam. As the ice grows, it increasingly blocks the acoustic propagation path, preventing imaging directly through the frozen region. Consequently, US measurements were obtained in regions where the beam can still partially propagate near the ice boundary, rather than across the full ice-ball cross-section. To account for this effect, the DSI measurements are calibrated using the thermocouple temperature readings, allowing the reconstructed temperature field to be referenced to the known freezing point. This calibration compensates for the measurement shift caused by the acoustic shadowing of the ice ball.

Although the ice ball itself appears as a hypoechoic region in B-mode US and attenuates the acoustic signal, preventing direct imaging beyond its boundary, our approach capitalizes on the measurable region adjacent to the ice ball. The B-mode image does not capture the pixel shifts beyond the hyperechoic boundary due to the strong attenuation and scattering at the frozen interface. However, by calibrating the slowness deviation in the adjacent region to temperature measurements, we were able to estimate the temperature profile indirectly inside the ice ball and distinguish between the frozen region and surrounding cooled but unfrozen tissue. This concept aligns with prior studies that profiled and modeled isotherms around cryoprobes, consistently showing that critical lethal isotherms such as -20°C and -40°C reside within the frozen volume [12], [68], [69]. Thus, estimating the temperature in the surrounding tissue offers a noninvasive pathway for inferring thermal conditions within the ice ball itself.

During the cryoablation process, several physical mechanisms occur in parallel, and all contribute to the measurable US signal changes. First, the reduction in temperature within the cryoablated region alters the local SoS. Second, the phase transition of water during freezing leads to volumetric expansion within the frozen region. As previously reported [70], [71], this expansion generates local mechanical compression in the adjacent unfrozen tissue near the freezing front. This mechanical interaction modifies the local mechanical state and density of the surrounding tissue, which in turn also affects the local SoS. Therefore, the measured pixel shifts reflect the combined effects. Nevertheless, the observed slowness deviations remained consistent with the expected acoustic response to cooling, suggesting that the dominant contributor is indeed temperature change: as discussed by Liang et al. (2024) [42], the temperature dependence of SoS differs between water-based media and lipid-rich biological tissues, and corresponds to phase transition of water, such that in pure water these changes are sharp, while in tissues with higher lipid concentration the transition is moderate. Since SoS change is inverse to slowness deviation, small or moderate changes in SoS can manifest as amplified slowness deviations, and vice versa. In our experiments, the agar phantom (water-based with solutes) demonstrated an approximately linear relationship between slowness deviation and temperature change, i.e. a more moderate relationship, whereas the ex-vivo turkey tissue, which contains a higher lipid concentration, exhibited an exponential dependence of slowness deviation and temperature change.

A key strength of our approach is its compatibility with standard US equipment and the use of a single imaging transducer. Although our algorithm was designed for real-time implementation, as it does not require complex calculations and has a runtime of 3 frames per second [29], the current experiments were performed ex-vivo and processed offline. Previous studies have demonstrated that models calibrated in ex vivo tissues can be translated to in-vivo conditions [72], [73]. Accordingly, the relationship between slowness deviation and temperature could first be characterized ex-vivo and then compared to in-vivo measurements. If the relationships are consistent, ex-vivo calibration parameters may be directly applied. Otherwise, a one-time in vivo calibration can be performed for the relevant tissue type using reference temperature measurements, for example via thermocouple insertion [13], [74]. Importantly, in clinical cryoablation the target organ is typically known in advance, and its acoustic and compositional properties generally fall within well-characterized ranges. For example, the speed of sound has been measured and documented for various tissues [47]. This suggests that calibration could be performed a priori for major tissue categories, extending current practice and reducing the need for repeated in-vivo calibration in each procedure. In

addition, future work will evaluate the proposed method using a commercial clinical cryoablation system, which will allow assessment of the algorithm under clinically relevant operating conditions.

A limitation of the present study is the sample size used for validation, with $N = 6$ in the tissue-mimicking phantom and $N=5$ in ex-vivo turkey breast. While this cohort size is sufficient for a proof-of-concept study, it may limit the generalizability of the quantitative findings, particularly given the variability observed in the measured slowness deviations and temperature mappings (Figures 9 and 11). Future studies incorporating larger sample sizes, a wider range of tissue types, and potentially in-vivo models will be valuable for further evaluating the robustness of DSI and establishing more comprehensive calibration parameters.

In the transition to in-vivo experiments, several factors including perfusion and physiological motion, may influence the results. Blood flow can reduce cooling rates near vessels [75], [76], thereby modifying the spatial progression and the size of the ice front [77]. However, perfusion represents a heat-transfer mechanism that alters the rate and distribution of cooling rather than the fundamental relationship between temperature and acoustic properties. As long as tissue cooling is induced by the cryoprobe and local temperature is directly measured by thermocouples, the DSI-derived slowness deviation can be calibrated to the actual in-vivo temperature regardless of whether cooling is slowed by perfusion. Therefore, once calibrated, the DSI-derived temperature estimation can be used to assess the underlying tissue temperature. Physiological motion, such as respiratory motion and cardiac pulsation, can be accommodated by integrating motion compensation algorithms into the processing pipeline when required. Multiple approaches for motion detection and correction in US imaging have been reported, including reference-frame alignment methods [78], deep-learning-based motion correction tools [79], and rejection of frames with out-of-plane motion [80]. Notably, bulk physiological motion is typically global, whereas freezing-induced displacements are spatially localized around the cryoprobe. By estimating and compensating for global motion components prior to DSI analysis, a relatively stable B-mode sequence can be obtained, enabling reliable pixel-tracking based estimation during in vivo use. Since DSI operates directly on beamformed B-mode images, it can be readily implemented with a wide range of transducers, including linear, curvilinear, and phased-array probes, by accounting for the image geometry and lateral sampling. Plane-wave acquisitions are particularly suitable, as they provide the high frame rates needed for robust optical-flow estimation.

The present experiments were conducted at depths adjacent to the cryoprobe contact point. However, DSI itself is not inherently limited to superficial targets and can be applied to deeper

organs, provided that adequate US imaging is achievable. The transducer used in this study supports imaging depths of up to 15 cm, enabling visualization of targets within the clinically relevant 5-10 cm range, as well as the pixel displacement trail extending several centimeters beyond the cooling region that is utilized by the DSI algorithm. However, lower-frequency transducers may be required for deeper targets to ensure sufficient acoustic penetration. Such adjustments introduce a trade-off between penetration and spatial resolution. Lower frequencies generally produce larger pixels in the reconstructed image, meaning that each pixel represents a larger physical region of tissue. Because DSI relies on optical-flow, which tracks pixel displacements, larger pixel sizes may reduce sensitivity to subtle slowness changes compared with higher-frequency imaging. Nevertheless, the DSI method can be compatible with probe types commonly used for deep abdominal imaging (e.g., curvilinear or phased-array transducers), to support deep target imaging, as long as reliable displacement tracking is maintained.

The sensitivity of the algorithm depends primarily on the temporal spacing between consecutive B-mode frames. Because the method uses optical flow to estimate the pixel-wise motion between successive US images, shorter frame intervals improve sensitivity by capturing smaller and smoother displacements. This enhances the precision of the optical flow calculation and, consequently, the accuracy of the derived slowness deviation. Importantly, while the temperature was acquired at 9-second intervals to generate the calibration curves, the B-mode images themselves were acquired every 3 seconds, and the B-mode images corresponding to the temperature measurements were used for DSI calibration. This 9-second interval used for temperature recording was selected to ensure reliable synchronization with the thermocouple-based ground-truth temperature measurements: at shorter intervals, the thermocouple exhibited a delayed response relative to the US imaging, often producing repeated temperature readings across consecutive frames despite ongoing cooling. Using a 9-second interval therefore allowed sufficient time for the thermocouple measurements to reflect the temperature changes observed in the US images, improving the reliability of the calibration. As the DSI algorithm itself does not impose a constraint on frame rate, shorter acquisition intervals would be expected to further improve performance by reducing inter-frame displacement and enhancing the accuracy of the optical-flow estimation. A shorter B-mode images acquisition interval could be achieved through improved data transmission between the US system and the computer, as well as optimization of the acquisition and storage code.

In the present implementation, DSI detected slowness deviations corresponding to temperature variations ranging from sub-degree changes to several degrees: based on DSI

calibration, the smallest detectable inter-frame temperature changes were 0.50 °C in the agar phantom and 0.60 °C in the turkey breast samples. These values were determined from consecutive B-mode frames acquired at 3-second intervals during the early phase of cooling, while at later stages of cooling, the temperature differences between frames increased as the samples continued to cool. In the context of cryoablation, where tissue temperatures typically decrease from physiological levels (~37°C) down to -40°C or lower, such sensitivity is sufficient to monitor both the progression of freezing in the target zone and to ensure that surrounding tissues are not inadvertently overcooled. In this study, the lowest temperature achieved *ex vivo* was $-39.4 \pm 5.6^{\circ}\text{C}$, and the corresponding slowness deviation was successfully detected by the algorithm. The observed temperature decrease was strongly correlated with both the cooling duration and the growth of the ice ball surrounding the probe. However, as the ice ball enlarges, it creates a hyperechoic region that limits US penetration, imposing a practical constraint on the minimal temperature that can be measured. The formation and evolution of the ice ball during cryoablation depend on the probe's cooling rate and the thermal properties of the surrounding tissue [2], [68]. A commercial cryoprobe, capable of reaching cryogenic temperatures within a shorter period, would likely reduce excessive ice-ball formation and enable detection of even lower temperatures when combined with a shorter frame acquisition interval.

MRI thermometry, while widely used for thermal ablation applications [20], [21], [22], has not yet been widely adopted for cryoablation procedures. Several studies have investigated the feasibility of MRI for temperature spatial mapping during cryosurgery. Overduin et al. (2016) [50] calibrated a model relating MR-signal to temperature, reaching -40°C in *ex-vivo* porcine muscle specimens, with a spatial resolution of 1.63 mm³ isotropic voxel size and a temporal resolution exceeding 1 minute per volume. Using fiber-optic probes as a reference standard, they reported a mean bias of -1.2°C with upper and lower limits of agreement of +4.1°C and -6.5°C. More recently, Gutt et al. (2026) [51] developed a similar model in *ex-vivo* bovine liver samples using a FLORET approach, reaching a minimal temperature of -20°C. The temporal resolution was 18 seconds per volume, and the spatial resolution was 1.60 mm³ isotropic voxel size. An RMSE of 1.33°C was reported, along with a mean bias of 0.08°C and limits of agreement of -2.08°C and 2.23°C. In comparison, our DSI approach operates on B-mode US images acquired with a 7.5 MHz linear array. For linear array transducers similar to the one used in this study, phantom measurements report axial resolution in the range of 0.25-1.0 mm and lateral resolution of 0.5-3.0 mm at depth range of 3-6.5 cm for linear array transducer with frequencies in the range of 4-18 MHz [62]. The temporal resolution in the

calibration process was 9 seconds, however DSI is sensitive to displacements obtained in shorter time intervals. For ex-vivo turkey breast samples used in this study, the RMSE is 4.0°C, with a mean bias of -2.91°C. Importantly, DSI relies solely on a standard US system and a single imaging transducer, making it a cost-effective, widely accessible alternative to MRI-based monitoring, with potential for real-time, bedside implementation without the need for specialized interventional MRI suites.

In summary, the proposed algorithm enables spatially resolved, noninvasive temperature monitoring during cryoablation, capturing slowness deviations across the imaging region and providing indirect estimates of frozen tissue temperatures. The method performed robustly in both phantoms and ex-vivo samples and can be adapted for real-time use with various US transducers. Calibration of the α parameter is needed for different tissues when implementing this method for cryoablation procedures. Future work should extend this approach to in-vivo settings and optimize acquisition parameters to enhance sensitivity, supporting DSI as a practical tool for precise and safe cryoablation monitoring.

7. Conclusions

In this work, a DSI framework was developed for non-invasive, spatially resolved temperature monitoring during cryoablation. By analyzing US B-mode image sequences, the method estimates slowness deviations associated with temperature changes and enables quantitative assessment of thermal distributions. Validation in tissue-mimicking phantoms and ex-vivo tissue demonstrated a consistent relationship between temperature decrease and slowness deviation, with material-specific behavior. The approach allowed visualization of temperature gradients, estimation of minimum temperatures, and indirect assessment of the frozen region, even in the presence of acoustic shadowing. Its compatibility with standard US systems and a single transducer highlights its potential as a practical and cost-effective solution. While demonstrated ex-vivo, the method shows promise for in-vivo application following appropriate calibration and further optimization.

8. References

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תקציר

הקפאה טיפולית היא שיטה לטיפול ברקמות פתולוגיות, שבה נעשה שימוש בקור עז כדי להקפיא ולהשמיד רקמת גידול או רקמות אחרות בצורה מדויקת. ניטור מדויק של הטמפרטורה הוא חיוני כדי להבטיח הרס מוחלט של הרקמה החולה תוך שמירה על רקמות בריאות מסביב. עם זאת, שיטות קיימות הינן מוגבלות על-ידי פולשניות, רזולוציה מרחבית מוגבלת, או עלות גבוהה. בעבודה זו פיתחנו שיטת אולטרסאונד לניטור מרחבי וזמני של הטמפרטורה במהלך הקפאה טיפולית, המבוססת על חישוב השינויים המקומיים במהירות הקול הנגרמים במהלך הטיפול.

האלגוריתם משערך שינויים מקומיים במהירות הקול מתוך אות אולטרסוני על-ידי מעקב אחר תזוזות פיקסלים בתמונת האולטרסאונד, הנגרמים על ידי שינוי בטמפרטורה. זאת על-ידי שילוב זרימה אופטית עבור חישוב תזוזת הפיקסלים בתמונה, מידול מתמטי של הקשר בין תזוזת הפיקסל לשינוי בטמפרטורה, ולבסוף פתרון מתמטי של בעיית רגולריזציה הפוכה למציאת השינוי במהירות הקול כתוצאה מהקירור וייצוג חזותי של התוצאה. ניסויי הקפאה טיפולית בוצעו בפנטומים מדמי רקמה העשויים מאגר וברקמת חזה הודו באמצעות מערכת הקפאה ייעודית המבוססת על חנקן נוזלי, אשר פותחה לצורך מחקר זה. במקביל, בוצעו מדידות טמפרטורה פולשניות באמצעות מדי טמפרטורה ששימשו כמדד ייחוס.

התוצאות מראות כי קיים קשר מובהק בין שינוי בטמפרטורה (ΔT) בזמן קירור לבין שינוי במהירות קול. באגר, הקשר היה לינארי עם מקדם של $-20.70 \text{ ns} \cdot \text{m}^{-1} \cdot ^\circ\text{C}^{-1}$ ($R^2=0.988$), בעוד שבחזה הודו הקשר היה אקספוננציאלי: $34.04 \times \exp(0.075(-\Delta T)-1) \text{ ns} \cdot \text{m}^{-1} \cdot ^\circ\text{C}^{-1}$ ($R^2=0.950$). באמצעות השיטה, ניתן לעקוב אחר שינויי טמפרטורה בטווח רחב, כאשר הטמפרטורה המינימלית שנוטרה הייתה $39.4 \pm 5.6^\circ\text{C}$ בחזה הודו. ניתוח כמותי של התוצאות הראה שורש שגיאה ריבועית ממוצעת (rmse) של 1.7°C באגר ו- 4.2°C בחזה הודו. ניתוח בלנד-אלטמן הראה התאמה של השיטה עם מדידות מד-החום, כאשר ההטייה הממוצעת הייתה 1.48°C באגר ו- 2.91°C בחזה הודו, וגבולות ההסכמה היו בין -3.37°C לבין $+6.34^\circ\text{C}$ באגר, ובין -8.11°C לבין $+2.28^\circ\text{C}$ בחזה הודו, עם 95% מההפרשים שנמצאו בטווח זה.

עבודה זו מציגה גישה חדשה לניטור מרחבי ולא פולשני של טמפרטורה במהלך הקפאה טיפולית באמצעות אולטרסאונד, ומרחיבה את יכולות האולטרסאונד הכמותי לניטור תהליכים תרמיים בזמן אמת.

אוניברסיטת תל אביב

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חיבור זה הוגש כעבודת גמר לקראת התואר "מוסמך אוניברסיטה" בהנדסה ביו רפואית

על-ידי

גאיה לם

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אב תשפ"ו

אוניברסיטת תל אביב

הפקולטה להנדסה ע"ש איבי ואלדר פליישמן

בית הספר לתארים מתקדמים ע"ש זנדמן - סליינר

פיתוח אלגוריתם לניטור טמפרטורה במהלך הקפאה טיפולית

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