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Experimental Validation of Longitudinal Speed of Sound Estimates in the Diagnosis of Hepatic Steatosis (Part II)

Rebecca E. Zubajlo¹, Alex Benjamin¹, Joseph R. Grajo³, Kanakaraju Kaliannan⁴, Jing X. Kang⁴, Atul K. Bhan⁵, Kai E. Thomenius¹, Brian W. Anthony^{1,*}, Manish Dhyani^{2,6}, Anthony E. Samir²

¹Device Realization and Computational Instrumentation Laboratory, Department of Mechanical Engineering, Massachusetts Institute of Technology, Cambridge, MA 02139, USA

²Center for Ultrasound Research and Translation, Department of Radiology, Massachusetts General Hospital, Boston, MA 02139, USA

³Department of Radiology, Division of Abdominal Imaging, University of Florida College of Medicine, Gainesville, FL 32610, USA

⁴Laboratory for Lipid Medicine and Technology, Massachusetts General Hospital, Harvard Medical School, Charlestown, MA 02129, USA

⁵Department of Pathology, Massachusetts General Hospital, Boston, MA 02139, USA

⁶Department of Internal Medicine, Steward Carney Hospital, Dorchester, MA 02124, USA

Abstract

This study validates a non-invasive, quantitative technique to diagnose steatosis within tissue. The proposed method is based on two fundamental concepts: 1) the speed of sound (SOS) in a fatty liver is lower than that in a healthy liver and 2) the quality of an ultrasound image is maximized when the beamformer's speed of sound matches the speed in the medium under examination. The method uses image brightness and sharpness as quantitative image-quality metrics to predict the true sound speed and capture the effects of fat infiltration, while accounting for the transmission through subcutaneous fat. *Ex vivo* testing on sheep liver, mice livers, and tissue-mimicking phantoms indicated the technique's ability to predict the true speed of sound with errors less than 0.5% and to quantify the inverse correlation between fat content and SOS.

Keywords

quantitative; ultrasound; NAFLD; beam-formation; sharpness; brightness; FFT; PCA; subcutaneous; fat; sound speed corrections

*Corresponding Author: Manish Dhyani: Tel: +1 617 852 8909, Dhyani.manish@mgh.harvard.edu.

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Introduction

Overview of Non-Alcoholic Fatty Liver Disease (NAFLD)

Non-alcoholic fatty liver disease (NAFLD) is a chronic disease defined as the presence of hepatic steatosis (> 5%) in the absence of any known cause and minimal alcohol use (Williams et al.). NAFLD is the most common liver disease with an estimated one billion patients globally (Loomba and Sanyal 2013) with an estimated prevalence of 17-51% in the general population with even higher rates in western countries (Bellentani et al. 2010; Chalasani et al. 2012). More specifically, in the United States NAFLD afflicts roughly 45% of the population, and by 2020, NAFLD is projected to be the leading indicator and cause of liver transplantations in the world (Idowu et al. 2015; Rinella 2015).

Moreover, NAFLD has been recognized as an independent cardiovascular disease risk factor with a 70% overall mortality increase, due to a roughly 300% increase in cardiovascular disease mortality (Athysos et al. 2015); it has also been associated with severe metabolic impairments such as insulin resistance, hypertension, obesity, and Type 2 diabetes mellitus (Kelishadi et al. 2009; Schwimmer et al. 2008). The prevalence of NAFLD can be as high as 90% in patients with diabetes or obesity as co-morbidities (Bellentani et al. 2010; Chalasani et al. 2012).

The spectrum of NAFLD is divided into two categories; (1) simple steatosis (NAFL), which makes up 70-75% of cases, is defined by excess liver fat without inflammation and (2) steatohepatitis (NASH), which makes up 25-30% of cases, is defined by excess liver fat with inflammation and/or fibrosis (Chalasani et al. 2012; McPherson et al. 2015). Historically, it was believed that NAFL is non-progressive, and does not result in liver fibrosis, whereas NASH results in chronic liver injury and progressive fibrosis (Chalasani et al. 2012). However, recent longitudinal paired biopsy studies have shown that even patients with NAFL can progress (McPherson et al. 2015). In light of this data, the early diagnosis of NAFL is highly relevant for effective prognosis and patient management.

A liver biopsy is the accepted reference standard for the diagnosis of NAFLD and distinction of NAFL and NASH. However, biopsies are costly, invasive, associated with morbidity and uncommon mortality, and are subject to significant variability and sampling error. In a disease that affects roughly 30% of the population, it is impractical for diagnosis and risk stratification. As a result, clinicians prefer to use alternative tests such as serum biomarkers (e.g. liver enzymes), and radiological imaging tests (McPherson et al. 2015; Vajro et al. 2012). These techniques have low sensitivity and specificity, which is further exacerbated by the fact that as many as 50% of patients with NAFLD have normal liver chemistries (Yan et al. 2007). Several blood-based combination diagnostic tests have been introduced in recent years such as the FibroTest, SteatoTest, and NashTest (Lassailly et al. 2011; Poynard et al. 2005). Both the FibroTest and the NashTest have been used for fibrosis and necrosis rather than diagnosing steatosis (Poynard et al. 2005). The SteatoTest shows promise for diagnosing steatosis less invasively than a biopsy but has a negative predictive value of 0.86 (Poynard et al. 2005). The lower negative predictive value is not optimal for a progressive disease when the disease is treated in the early stages with exercise and diet changes.

There is a need for a methodology that allows for the quick and reliable quantification of liver fat at the point of care. This paper proposes and evaluates several methods for the estimation of liver fat by the use of the longitudinal speed of sound in liver tissue. This is distinct from the frequent use of shear wave speed estimation for liver fibrosis, a much more advanced stage of NAFLD.

Background and Motivation for using Speed of Sound to Quantify Steatosis

Fat has a relatively lower longitudinal speed of sound than healthy liver tissue. Consequently, the expected speed of sound in a fatty liver will be lower than that in a healthy liver (Ghoshal et al. 2012; Kumagai et al. 2014). Initial *ex vivo* results have demonstrated this expected speed reduction in human liver samples; however, the relative contribution of the amount of water and fat in the tissue were confounding factors in these studies. O'Brien et al. showed a speed of sound reduction of about 50 m/s in rats with liver lipid content ranging from a normal of 2-5% to slightly more than 20% (Ghoshal et al. 2012).

Estimating the speed of sound *in vivo* is a non-trivial task; most commercial ultrasound machines use an assumed, fixed speed of sound (1540 m/s) in clinical settings. This assumption is limiting, because the speed of sound in human tissues is known to vary over a range from less than 1450 to over 1600 m/s (Anderson et al. 2000; Chalasani et al. 2012). Various techniques have been developed in the last decade to estimate the true speed of sound; some of the commonly used parameters include backscatter coefficients, speckle size, echo amplitude, and attenuation coefficients (Anderson et al. 2000; Chalasani et al. 2012; Ghoshal et al. 2012; Weijers et al. 2016). All of these methods exploit the fact that when the assumed speed of sound in the beamformer matches that in the tissue being examined, a resulting measure of the quality of the B-mode image is maximized. More narrowly, in speckle-dominated B-mode images (e.g. those of the liver parenchyma), speckle brightness is maximized and speckle size is minimized when the appropriate speed of sound is used by the beamformer during image formation.

The use of speckle to assess quality of beamformation arises from the work of Smith and Wagner (Wagner et al. 1983). They demonstrated, by the use of speckle statistics, that speckle size is dependent on the use of an accurate speed of sound during beamformation and also that the smallest speckle dimensions occur near the focus. This concept was extended by Nock and Trahey who optimized speckle characteristics by adjusting beamformation delays until the brightness was the greatest at the desired focus (Nock et al. 1989). In our work, we apply a universal speed of sound correction as opposed to the individual channel-by-channel correction of Nock and Trahey.

In this study, we quantify the speed of sound by analyzing two different B-mode image metrics: the sharpness of a region of interest (ROI) around the expected focal zone and the brightness of the image around the expected focal zone. The sharpness of the B-mode image is quantified using two different methods: i) the proportion of Fast-Fourier Transform (FFT) coefficients above a defined threshold of the DC component of the image spectrum (De and Masilamani 2013) and ii) the singular values of spatial, intensity gradient of the image (Zhu and Milanfar 2009). The brightness of the image is obtained using a spatial average of the envelope of the corresponding IQ data for a fixed band around the intended focal zone.

The above metrics were chosen for their ease of implementation in the real-time processing of ultrasound data, thereby offering a technique that can be performed on a conventional scanner at the point of care. These metrics can be readily extracted from B-mode images and radiofrequency (RF) data with minimal computational cost and no calibration, thereby making them compatible with existing commercial ultrasound machines (Chalasani et al. 2012). The overarching goal is to utilize this ability to estimate the true speed of sound in livers to obtain a quantitative measure of the corresponding fat content. It is worth noting that when using the aforementioned metrics, the presence of subcutaneous fat skews the prediction. While there are existing methods that are able to account for the presence of subcutaneous fat, we present a simple and accurate technique to account for the transmission through a layer of subcutaneous fat (Chalasani et al. 2012; Weijers et al. 2016) and hope to offer a comparison between the techniques as part of our future work.

Materials and Methods

Explanation of Image Metrics and Accounting for Subcutaneous Fat

The following image-quality measures were used to estimate the speed of sound: focal zone Image Brightness (brightness), Image Sharpness: FFT Coefficients (FFT), and Image Sharpness: Principal Component Analysis (PCA). A detailed explanation on the metrics and how each of them are calculated can be found in our submission titled “A Novel Approach to the Quantification of the Longitudinal Speed of Sound and its Potential for Tissue Characterization (Part - 1)” as part of this two-part submission. Using these metrics, absorption and scattering will change due to intervening layers (fat, skin, muscle, etc.). On each transmit for an assumed speed of sound, the sound wave is passing through the same tissue so these confounding effects will be present in all data sets that are compared to obtain the true speed of sound in the tissue. The only factor changing in these data sets is the assumed speed of sound on transmit and receive.

A common problem that arises during the estimation of the longitudinal speed of sound *in vivo* is the inhomogeneity of the underlying tissue. Ultrasound typically propagates through a layer of subcutaneous fat before entering the organ of interest. This propagation can skew the speed of sound prediction within the organ of interest (Weijers et al. 2010; Weijers et al. 2012). A correction metric, similar to the one presented by Weijers et al. (2012), shows that the image can be segmented into the two layers (organ layer and skin/subcutaneous fat layer) and then linearly proportioned to account for the transmission through the occluding layer of subcutaneous fat. Weijers et al. assumes that even though the speed of sound within the organ of interest is unknown, the speed through the occluding layer (subcutaneous fat) is well-known (2012). We propose a method that is similar to the one proposed by Weijers et al. (2012) but extracts the speed of sound in the successive layers by shifting the intended focal zones accordingly. The correction presented here uses a similar method of using the envelope statistics to manually segment the layers, and linearly proportion the speed in each layer to account for the transmission through the occluding layer. For example, if the configuration is a bilayer setup (Layer 1 over Layer 2), the proposed algorithm is first used to extract the speed of sound in Layer 1 (occluding layer) and then in Layer 2 (layer of

interest); the manual image segmentation method described above is then used to correct for the transmission through Layer 1 (linear proportioning). This is captured by equation [1].

$$\text{SoS}_{\text{total}} = (\text{Layer 1 Percentage})(\text{SoS}_{\text{layer1}}) + (\text{Layer 2 Percentage})(\text{SoS}_{\text{layer2}}) \quad (1)$$

Experiments

The proposed method was tested in the following experimental configurations: i) sheep liver (N=1) ii) sheep liver immersed in olive oil (to simulate transmission through subcutaneous fat) iii) bilayer phantom (tissue mimicking phantoms of different speeds of sound in a stacked configuration) iv) fatty and control mice liver (N = 2) v) mice livers with varying grades of fat infiltration (N = 14).

Both layers in the bilayer phantom were calibrated phantoms manufactured by the Computerized Imaging Reference Systems Inc. (CIRS) (CIRS, Norfolk, Virginia, USA). The sheep liver was obtained from a local butcher shop in Cambridge, Massachusetts under Massachusetts Institute of Technology Committee on Animal Care approval (E17-07-0320), and the olive oil was obtained at a local retail store (Savenor's Market; Cambridge, MA, USA). The mouse liver samples were obtained from the Laboratory for Lipid Medicine & Technology at Massachusetts General Hospital Charlestown, MA, USA under Massachusetts General Hospital or Harvard Committee of Animal Care approval number 2010N000038 (Massachusetts General Hospital Charlestown, MA, USA).

The fatty mice were FAT-2 transgenic mice, which ranged in levels of fat infiltration into their liver based on a specialized high fat diet (Pai et al. 2014). They had significantly higher total fat and triglyceride (TG) fraction than the healthy control wild-type mice. The healthy mice were fed a regular diet and had low omega-6 essential fatty acids. The FAT-2 mice were able to create omega-6 polyunsaturated fatty acids (PUFA) endogenously, and therefore had high omega-6 PUFA. The high tissue omega-6 PUFA is a major contributor to fatty liver (Kaliannan et al. 2016).

The imaging of all specimens, except the 14 mouse livers, was performed on a General Electric (GE) Logiq E9 System with a 9L probe (GE, Boston, MA, USA). RF and B-mode images were collected from the machine with differing assumed speeds of sound in the beamforming process ranging from 1400 to 1620 m/s (1400, 1420, 1480, 1500, 1540, 1580, 1620 m/s). The center frequency was held constant at 9 MHz with a single focal zone within the depth of the tissue under examination.

Ground truth speed of sound measurements were obtained using the experimental setup in Figure 1. A mechanical fixture firmly held the ultrasound probe at a known distance from a surface. The specimen was placed between a fixed surface and the ultrasound probe. The distance measurement was obtained using a linear magnetic encoder accurate to 0.001 inches and a lead screw accurate to 0.050 inches. The travel time was calculated from the envelop of the reflected signal from the interface between the tissue and an air gap between the sample and the acrylic plate. The true speed of sound was then calculated using equation [2], taking into account the round-trip travel distance.

$$\text{Speed of Sound} = \frac{\text{Total Travel Distance}}{\text{Travel Time}} \quad (2)$$

If the error propagation is assumed to be correlated (additive) with a unit time of 1 second, the total error in the ground truth would be plus or minus 0.051 m/s, which is negligible on this time scale.

A similar setup was utilized for the 14 mouse livers as seen in Figure 2; the key difference being a VisualSonics Vevo 2100 system (Toronto, Ontario, Canada). A 40 MHz probe provided by VisualSonics Vevo (256 elements) was utilized (40 MHz on transmit and 32 MHz on receive) (Toronto, Ontario, Canada). The probe was in direct contact with the excised livers and held by a XY stage allowing hands-free imaging of the sample. The data was collected with standard settings for that system. RF and B-mode data was collected from the machine with varying speeds of sound ranging from 1400 to 1600 m/s in increments of 10 m/s. The livers were approximately 4 mm in depth.

Post data acquisition, the samples were frozen in dry ice and transported to the Pathology Department at the Massachusetts General Hospital for fat quantification. A small sample of the liver was extracted and the fat was quantified with a histology method similar to human biopsy sample fat quantification. The histology grading was performed with a standard Oil Red O stain and H&E stain. This protocol was used for the sheep liver samples and the mice liver samples. The rest of each sample was then frozen and transported to MGH Charlestown Campus for biochemical analysis.

A biochemical analysis was performed on the livers as described by Kaliannan et al (2013). This method quantifies the total hepatic fat and triglyceride (TG) quantification purposes rather than just a small section like the histology protocol. The tissue was mixed with a chloroform:methanol solution (2:1 volume mixture). The sample was then vortexed and incubated for 5 minutes. Additional chloroform:methanol solution was then added and vigorously homogenized for 3 minutes. The sample was centrifuged at $2,000 \times g$ for 10 minutes and the supernatant layer containing lipids was collected. The additional chloroform was allowed to evaporate and mass of the lipids was recorded. For TG quantification, lipids were mixed with methanol for resuspension. An aliquot was taken and vortexed with 1 M magnesium chloride and then centrifuged for 5 minutes. A colorimetric, guinoneimine dye assay was then performed to quantify the TG based upon lipoprotein lipase-mediated release of glycerol (Sigma: Serum Triglyceride Determination Kit) (Sigma-Aldrich, Natick, MA, USA) (Kaliannan et al. 2013).

Results

Sheep Liver

The true speed of sound in the sheep liver was 1540 m/s (± 0.051 m/s) as obtained by the experimental setup in Figure 1. Experiments performed on the sheep liver estimated the speed of sound to be: (1) 1580 m/s using brightness (2) 1540 m/s using the FFT metric, and (3) 1540 m/s using the PCA metric as seen in Figure 3c; the predicted values correspond to a

normalized metric of 1.0. When compared, the predictions of two of the three metrics were found to match the ground truth measurement with the caveat that the ground truth measurement had an associated uncertainty of 0.051 m/s. The sheep liver histology grading was < 5% fat as seen in the Oil Red O stain (Figure 3a) and H&E stain (Figure 3b).

Sheep Liver with a Simulated Fat Layer

The true speed of sound in the simulated fat layer (olive oil) was 1400 m/s ($\pm 0.5\%$) as measured using the setup described in detail above (Figure 1), and the true speed of sound in the sheep liver was measured to be 1540 m/s ($\pm 0.5\%$). The B-mode image of the experimental setup (Figure 4) shows the “fat” layer on top, and the sheep liver submerged in oil. The speed of sound in the sheep liver, as predicted by the brightness, FFT and PCA metrics independently, was found to be 1500 m/s (2.59% error) as seen in Figure 4. The skewed prediction was largely due to the transmission through a layer of olive oil with a relatively lower speed of sound. Upon accounting for this transmission, the corrected sound speed was found to be 1548 m/s (0.52% error), thereby indicating the robustness and accuracy of the proposed algorithm.

Bilayer Phantom

The ability of the proposed method to determine the speed of sound in sequential layers of tissue was tested using two phantoms with different sound speeds in stacked configuration. Phantom A (top layer) and Phantom B (bottom layer) were both certified by CIRS to have true speeds of 1440 m/s and 1540 m/s, respectively. The predicted speed of sound in Phantom B using the FFT metric was 1500 m/s and the PCA metric was 1500 m/s. Unfortunately, the brightness metric did not yield results consistent with the FFT and PCA metrics. A mean of both metrics resulted in a predicted speed of 1500 m/s. When compared to the ground truth value provided by CIRS, the prediction yielded a relative error of 2.59% as seen in Figure 5. The skewed prediction was largely due to the transmission through Phantom A with a relatively lower speed of sound. Upon accounting for this transmission, the corrected sound speed was found to be 1551 m/s (0.71% error), thereby indicating the robustness and accuracy of the proposed algorithm.

Fatty and Control Mice Liver

Having tested the efficacy of the algorithm in predicting the true speed of sound in tissue despite the presence of subcutaneous fat, the next step involved demonstrating the effect of fat infiltration on the predicted speed of sound in liver. To that end, the metrics were utilized to predict the speed of sound in a healthy mouse liver (*ex vivo*) and the liver of a mouse that was subjected to a high-fat diet for a prolonged duration of time as described in the methods.

Figure 6 shows the predicted speed of sound in the fatty and normal livers along with pathological proof, which validates the extent of fat infiltration in each of the samples. As is evident in the figure, the control sample was devoid of large fat vacuoles while the fatty sample demonstrated a larger concentration of these vacuoles. The proposed method readily captured this fact with all three metrics independently predicting a speed of 1500 m/s for the control specimen and 1460 m/s for the fatty specimen. The absolute fat content in the livers were found to be ~5% and ~15% for the control and fatty specimens, respectively.

Batch Study of Mice Liver

To further test the validity and applicability of the algorithm, a set of 14 mice livers was gathered; the fat content of each of the livers was determined by the Massachusetts General Hospital Pathology Laboratory, thereby providing a ground truth for the fat content within the livers. All three metrics were utilized to predict the speed of sound in each of the *ex vivo* livers. Figure 7 demonstrates the Pearson's correlation between the absolute fat content in the livers and the predicted speed of sound using the PCA ($R^2 = 0.75$) and FFT metrics ($R^2 = 0.75$). As can be seen in the figure, the speed of sound is inversely correlated with the corresponding fat content; the batches are defined as follows: Batch 1 (< 5% Fat), Batch 2 (5 – 10 % Fat), Batch 3 (10 – 15 % Fat), Batch 4 (15 – 20 % Fat) and Batch 5 (> 20% Fat).

Tables 1 and 2 summarize the predicted speeds of sound obtained using the FFT and PCA metrics. While the standard deviations for the predicted speeds of sound may seem large (max 2.8%), the observation can be explained as follows: firstly, the sample sizes were relatively small; secondly, within the individual batches, the amount of fat in each of the associated livers varied, which in turn, caused variations in the predicted speed of sound. Unfortunately, the brightness metric did not yield results consistent with the FFT and PCA metrics. This can largely be attributed to the fact that the mice livers were compressed (albeit by undocumented amounts) during the testing process and compression has been known to artificially boost the echogenicity of scatterers and interfaces within tissue. This provides a stronger case for the use of image sharpness metrics over brightness metrics to predict the speed of sound *in vivo*.

Conclusion/Discussion

In the context of NAFLD, which has an estimated prevalence of 30% in the US population, ultrasound imaging remains the likely choice for an imaging biomarker. Despite this fact, the use of conventional B-mode ultrasound imaging is plagued by several shortcomings. Conventional ultrasound imaging is only accurate when steatosis is moderate-to-severe (i.e., defined as histologic degree ≥ 33%) (Lee et al. 2010; van Werven et al. 2010) and is insensitive to lesser grades of hepatic steatosis (de Moura Almeida et al. 2008; Kinner et al. 2016; Lee et al. 2010; Palmentieri et al. 2006; van Werven et al. 2010). In addition, ultrasound evaluation is subjective, with significant inter-observer variability, rendering it ineffective for the evaluation of early therapeutic responses in patients undergoing NAFLD treatment. In light of this fact, the development and validation of a robust and accurate ultrasound method to accurately quantify steatosis at its earliest stages would be of great clinical importance. In this study, we have aimed to use several markers embedded in conventional ultrasound images to predict the true speed of sound within the liver parenchyma and use this ability to quantify the degree of fatty infiltration.

The proposed method involved the iterative estimation of the true speed of sound in the liver parenchyma using two image quality metrics: image brightness around the expected focal zone and image sharpness around the intended focal zone (FFT and PCA metrics as explained above). The algorithm's capability to extract the true speed of sound was tested in tissue-mimicking phantoms, *ex vivo* sheep and mice livers; comparison to ground truth measurements indicated that the proposed method was able to predict the speed of sound to

within 0.5% error. The algorithm's potential to non-invasively and quantitatively diagnose NAFLD was tested by using it to predict the speed of sound in the livers of mice of two kinds: 1) healthy and 2) those subjected to a high fat diet for a prolonged duration of time. Not only was the algorithm able to accurately capture the true speeds of sound in the livers (less than 0.5% error), but it was also able to capture the downshift in the speed of sound of the liver parenchyma due to fatty infiltration; lastly, when tested on mice livers with fat percentages ranging from 3% to 25%, the algorithm was able to capture the proposed inverse correlation between fat content and the estimated speed of sound.

Another strength of the proposed method lies in the fact that it can account for the transmission of ultrasound waves through layers of subcutaneous fat; this is a widespread limitation in estimating the bulk speed of sound *in vivo*. The proposed method utilizes a straightforward approach that segments the underlying RF data to predict the speed of sound in successively deeper layers of tissue. The validity and robustness of this approach was tested by predicting the speed of sound in healthy sheep liver submerged in homogenous oil (to simulate a layer of fat). The speeds of sound in both layers were accurately predicted to within 1%, thereby lending credibility to the proposed technique's potential to non-invasively and accurately diagnose NAFLD at point of care. So far, the proposed method is capable of predicting the bulk speed of sound in *ex vivo* liver tissue with/without the presence of occluding layers and capturing the relationship between the predicted speed of sound and the underlying fat content. However, the method does not have the requisite spatial resolution to generate longitudinal speed of sound maps. The generation of longitudinal speed of sound maps would account for tissue heterogeneity as well as local fibrosis as would be seen in the case of NASH. The work going forward would involve extending the proposed method to produce spatial maps that are similar to SWE techniques that would allow for a far more robust and accurate diagnosis of the full spectrum of NAFLD.

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Figure 1:

(a) Diagrammatic representation of the pulse-echo setup for determination of *ex vivo* ground truth speed of sound values. (b) Image of the experiment being performed to determine ground truth speed of sound in an *ex vivo* sheep liver using the General Electric (GE) Logiq E9 (9 MHz probe).

Figure 2: Measurement setup on the Visualsonics Vevo 2100 ultrasound system (40 MHz probe); the probe was in direct contact with the *ex vivo* mice livers and held by an imaging station that allows for the hands-free imaging of the sample.

Figure 3:

Pathology results of the sheep liver show a lack of large fat vacuoles within the parenchyma as seen with Oil Red O (a) and H & E stain (b). The ground truth measurements indicated that the speed of sound within the liver was 1540 m/s ($\pm 0.5\%$), and estimations using the brightness metric, fast Fourier transform (FFT) metric and principal component analysis (PCA) metric were found to be 1540 m/s each (c).

Figure 4:

Speed of sound prediction in a lean sheep liver (1500 m/s); the b-mode image in the lower left corner depicts the olive oil layer (true speed of sound = 1400 m/s ($\pm 0.5\%$)) sitting atop the liver parenchyma (true speed of sound = 1540 m/s ($\pm 0.5\%$)). As seen in the graph, the predicted speed of sound value was 1500 m/s, skewed by transmission through the layer of oil.

Figure 5:

Predicted speed of sound in the lowest layer i.e. phantom B (1500 m/s), skewed by the transmission through phantom A (true speed = 1440 m/s). The roughly 2.59% error was corrected by accounting for this transmission and resulted in a renewed estimate of 1551 m/s (0.71% error) for phantom B.

Figure 6: Predicted speed of sound in the control specimen (1500 m/s) along with pathology results that demonstrate a lack of fat vacuoles (top); predicted speed of sound in the fatty specimen (1460 m/s) consistent with the presence of large fat vacuoles in the liver parenchyma (bottom).

Figure 7:

Correlation ($R^2 = 0.75$) between predicted speed of sound using sharpness metrics and absolute fat content. Batch numbers are as follows: Batch 1 (< 5% Fat), Batch 2 (5 – 10 % Fat), Batch 3 (10–15% Fat), Batch 4 (15 – 20 % Fat) and Batch 5 (> 20% Fat).

Table 1:

Predicted speed of sound in mice livers using FFT metric

Batch #	1	2	3	4	5	Metric
Mean	1487	1483	1490	1465	1445	FFT
Std. Dev	1.9%	1.4%	2.8%	1.4%	0.49%	FFT
Sample Size	4	4	2	2	2	FFT

Table 2:

Predicted speed of sound in mice livers using PCA metric

Batch #	1	2	3	4	5	Metric
Mean	1490	1480	1490	1465	1445	PCA
Std. Dev	2.3%	1.2%	2.8%	1.4%	0.49%	PCA
Sample Size	4	4	2	2	2	PCA