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Mean Scatterer Spacing Estimation in Normal and Thermally Coagulated Ex Vivo Bovine Liver

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Abstract

The liver has been hypothesized to have a unique arrangement of microvasculature that presents as an arrangement of quasiperiodic scatterers to an interrogating ultrasound pulse. The mean scatterer spacing (MSS) of these quasiperiodic scatterers has been proposed as a useful quantitative ultrasound biomarker for characterizing liver tissue. Thermal ablation is an increasingly popular method for treating hepatic tumors, and ultrasonic imaging approaches for delineating the extent of thermal ablation are in high demand. In this work, we examine the distribution of estimated MSS in thermally coagulated bovine liver and normal untreated bovine liver ex vivo. We estimate MSS by detecting local maxima in the spectral coherence function of radio frequency echoes from a clinical transducer, the Siemens VFX 9L4 transducer operating on an S2000 scanner. We find that normal untreated bovine liver was characterized by an MSS of approximately 1.3 mm. We examined regions of interest 12 mm wide laterally, and ranging from 12 mm to 18 mm axially, in 2 mm increments. Over these parameters, the mode of the MSS estimates was between 1.25 and 1.37 mm. On the other hand, estimation of MSS in thermally coagulated liver tissue yields a distribution of MSS estimates whose mode varied between 0.45 and 1.0 mm when examining regions of interest over the same sizes. We demonstrate that the estimated MSS in thermally coagulated liver favors small spacings because the randomly positioned scatterers in this tissue are better modeled as aperiodic scatterers. The submillimeter spacings result from the fact that this was the most probable spacing to be estimated if the discretely sampled spectral coherence function was a uniformly random two-dimensional function.

Keywords

mean scatterer spacing (MSS); thermal ablation; liver; spectral coherence; multi-taper

Introduction

Hyperthermic thermal ablation therapies are a rapidly evolving group of cancer treatments that kill tumor cells by raising tissue temperature well over 60°C for several minutes.

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Multiple technologies have been developed, including microwave ablation,¹ radio frequency (RF) ablation,² high intensity focused ultrasound (HIFU),³ and laser interstitial thermal therapy.⁴ Ultrasound imaging is a low cost, portable imaging modality with the potential to monitor the progress of tumor treatment using ablation therapy. However, the use of ultrasound for monitoring thermal ablation therapy in a clinical setting has been hindered by poor contrast between treated and untreated tissue on ultrasound B-mode images.⁵

Therefore, a large body of work has been devoted to the application of quantitative ultrasound (QUS)-based methods to monitor thermal ablation treatment. Research on monitoring thermal ablation using ultrasound may be divided into two groups: temperature estimation during treatment^{6–8} and estimating the extent of tissue coagulation following treatment.^{9–12} A variety of techniques have been applied to temperature estimation during thermal ablation therapy, including estimation of thermal strain,^{8,13–16} estimation of temperature-induced changes to mean scatterer spacing (MSS),⁷ sound speed,^{17–19} backscatter,²⁰ and the estimation of the acoustic nonlinearity coefficient,⁶ B/A .

Delineating the extent of tissue coagulation following thermal ablation motivates this paper. Tissue coagulation has been shown to cause large changes in some acoustic properties of tissue while leaving others unchanged. Specifically, tissue coagulation has been shown to cause an increase in tissue stiffness^{9,10} and the ultrasound attenuation^{11,12} coefficient. However, it has been found that the sound speed and backscatter coefficient are relatively unchanged by tissue coagulation.^{21,22} For example, Kemmerer and Oelze examined ex vivo rat liver tissue heated from 45°C to 70°C using a water bath. They found that the backscatter coefficient of their samples was relatively unchanged over the frequency range of 8–15 MHz.²¹ Gertner et al. heated ex vivo bovine liver using water baths ranging from 50°C to 70°C and also examined changes in backscatter coefficient. They found the mean change in backscatter coefficient in eight samples, over the frequency range of 2.5 to 5.0 MHz, to be insignificant relative to the standard deviation of the measurement.²³

While backscatter coefficient measurements have shown little contrast between ablated and unablated tissue, these measurements fail to account for differences in the spatial distribution of scatterers. When estimating the backscatter coefficient, tissue has been modeled in two equivalent ways: as a continuously varying random medium with a stationary correlation function²⁴ and as a discrete collection of a large number of scatterers whose positions are uniformly and randomly distributed in space.²⁵ Whether continuous or discrete, we refer to these models as diffuse scattering models.²⁶

The RF echo signal from a diffuse scattering medium will exhibit uncorrelated phase between its different frequency components.²⁶ However, the RF echo signal from the liver has been shown to exhibit correlated phase between characteristic frequency components.²⁶ A quasiperiodic scattering model, characterized by an MSS, has been shown to be compatible with this observation.²⁷ In addition to temperature,⁷ MSS has been shown to be altered by cirrhosis and fibrosis of the liver.²⁷ Specifically, Fellingham and Sommer found an MSS of 1.07 ± 0.16 mm among 14 normal subjects versus an MSS of 1.48 ± 0.24 mm among 15 cirrhotic patients.²⁷

Many algorithms have been implemented for estimation of MSS, and our laboratory has recently discussed these in another paper.²⁸ Briefly, a wide range of algorithms to analyze the periodicity of the RF signal from the liver exists and have utilized power spectral autocorrelation,²⁷ the cepstrum,²⁹ spectral coherence,²⁶ discrete Fourier transform (DFT) of a quadratic transformation of the RF signal,³⁰ singular spectrum analysis,³¹ and wavelet analysis.³² In addition, a comparison of MSS obtained by several algorithms has been performed by Machado et al.³³ In the study, Machado found that measuring MSS using SAC, SSA, and a quadratic transformation performed comparably when examining liver samples from patients with varying degrees of cirrhosis ex vivo. This study performed MSS measurement using a single-transmit frequency of 20 MHz. Other studies have performed spectral coherence or MSS measurements in the liver using transducers with transmit center frequencies of 3.0 MHz,²⁷ 3.3 MHz,³¹ 5.0 MHz,²⁷ and 6.0 MHz,²⁸ respectively.

In the context of thermal ablation, a wavelet-based MSS detection algorithm similar to that of Abeyratne and Tang has recently been applied to the task of distinguishing between ablated and unablated tissue.³⁴ Zhou et al. estimated MSS in 20 liver tissue samples heated in a water bath and 18 cases of microwave ablation of fresh ex vivo porcine liver samples. They found an MSS of 1.15 ± 0.12 mm in normal porcine liver and an MSS of 0.93 ± 0.07 mm in coagulated porcine liver tissue.

The remainder of this paper is organized as follows: We first discuss the scattering model employed when estimating MSS and a simulation used to verify our ex vivo findings. Sound speed measurements in normal untreated liver and thermally coagulated bovine liver tissue are presented. Finally, MSS estimates in untreated ex vivo bovine liver and thermally coagulated ex vivo bovine liver are presented. We hypothesize that the scattering distribution in untreated liver can be modeled by a quasiperiodic arrangement in many regions, while a diffuse or sparse aperiodic model of scattering better agrees with the data measured in thermally coagulated liver.

Theory

Spectral Coherence and Scattering Model

We assume that the ultrasound RF echo signal is produced by a large number of monopole scatterers. This is equivalent to assuming that microscopic fluctuations in tissue density are significantly smaller than fluctuations in compressibility. Monopole scattering makes the scattering intensity independent of the scattering angle and agrees well with experimentally measured data in the liver.³⁵ Following the work of Donohue et al.,³⁶ we divide these monopole scatterers into three groups: diffuse, periodic, and sparse. Diffuse scatterers are a large number of spatially uniformly distributed microscopic fluctuations in compressibility. Periodic scatterers are scatterers arranged in a quasi-regular pattern with a characteristic spacing, d . While the three-dimensional structure of periodic scatterers in the liver is debatable, the position of periodic scatterers in one dimension may be modeled by generating the inter-scatterer spacing from a Gamma distribution.²⁶ Finally, sparse scatterers are a very small number of aperiodic dominant scatterers lying within the A-line or a specular surface running through the A-line. Our signal model can, therefore, be written as:

$$x(t) = \sum_{i=1}^{N_P} \int d_i(\lambda - \tau_i) h(t - \lambda; \tau_i) d\lambda + \sum_{j=1}^{N_P} \int p_j(\lambda - \tau_j) h(t - \lambda; \tau_j) d\lambda + \sum_{k=1}^{N_S} \int s_k(\lambda - \tau_k) h(t - \lambda; \tau_k) d\lambda. \quad (1)$$

In the above equation, h is a time-varying system response function that characterizes overlying tissue attenuation and beamforming; d_i is a scattering function for the i th diffuse scatterer; p_j is a scattering function for the j th periodic scatterer; and s_k is a scattering function for the k th sparse scatterer. The ultrasound RF signal $x(t)$ is a random signal and has two equivalent characterizations. These are the autocovariance function in the time domain and the Loève spectrum in the frequency domain. The Loève spectrum is related to the autocovariance function of the signal through a Fourier transform.³⁷

$$\Gamma_L(t_1, t_2) = \int \exp(i2\pi(f_1 t_1 - f_2 t_2)) \gamma_L(f_1, f_2) df_1 df_2 \quad (2)$$

In the above equation, Γ_L represents the time-domain autocovariance and γ_L is the Loève spectrum of the signal. The Loève spectrum depends on the overlying attenuation, ultrasound beam-forming, and scatterer distribution. This dependence results in a rapid decay of the Loève spectrum as one moves from the power spectrum, where the frequency difference, $f_1 - f_2 = 0$, to regions of larger frequency difference in the bi-frequency plane. To compensate for this, spectral coherence is often computed instead of the Loève spectrum. Spectral coherence is computed by dividing the Loève spectrum at each complex-valued point in the bi-frequency plane by its magnitude, or equivalently, by dividing it by the corresponding values along the main diagonal. Spectral coherence is computed using:

$$\gamma_c(f_1, f_2) = \gamma_L(f_1, f_2) / \sqrt{\gamma_L(f_1, f_1) \gamma_L(f_2, f_2)}. \quad (3)$$

Donohue et al. have shown that if the ultrasound RF signal can be modeled by a time-varying linear system as in Equation (1), then the spectrum of a gated RF data segment may be regarded as a product of a scattering function and a function encompassing diffraction and overlying attenuation effects.³⁶ Dividing the Loève spectrum by the magnitude of the diagonal components reduces the dependence of the Loève spectrum to only the phase relationships between the scatterers.³⁶ Spectral coherence is a more useful quantity for analyzing periodicity than the Loève spectrum, as this doesn't give additional weight to the larger spacings near the power spectrum. Once the spectral coherence function was estimated in an ROI, MSS was calculated by first finding the maximum amplitude location in the bi-frequency plane. We denote the coordinates of this location by $(f_{1,\max}, f_{2,\max})$. An estimated MSS, d , was then calculated according to:

$$d = \frac{c_s}{2(f_{1,\max} - f_{2,\max})}. \quad (4)$$

In the above equation, c_s represents the sound speed in the tissue. We briefly discuss sound speed measurements in the experimental methods section.

Multi-taper Spectral Coherence Estimation

In this paper, we estimate the Loève spectrum using Thomson's multi-taper algorithm.³⁸ This algorithm windows data using a set of orthogonal tapers and averages the spectral estimate over these orthogonal tapers. Using Thomson's multi-taper method, an estimate of the Loève spectrum in a region of interest (ROI) containing N_A A-lines is given by

$$\begin{aligned}\hat{y}_{i,k}(f) &= \sum_{t=0}^{N-1} x_i(t) v_k(t) \exp(-j2\pi f t) \\ \hat{\gamma}_L(f_1, f_2) &= \frac{1}{N_A K} \sum_{i=0}^{N_A-1} \sum_{k=0}^{K-1} \hat{y}_{i,k}(f_1) \hat{y}_{i,k}^*(f_2).\end{aligned}\quad (5)$$

In the above equation, v_k denotes the k th discrete prolate spheroidal sequence (DPSS). The DPSS are also referred to as Slepian sequences. These sequences have the property that their Fourier transforms are optimally concentrated within a user-defined bandwidth, $2W$. The variable $x_i(t)$ represents the t th radio frequency data sample in the i th A-line in an ROI. Relative to an ultrasound B-mode image, the lateral extent of an ROI dictates the number of A-lines N_A , and the axial extent of an ROI decides the number of time points, N , in each windowed A-line. The variable t is restricted to take on integer values if the sampling frequency is normalized to be equal to one. In an ROI, the first periodic scatterer in a windowed data segment may be offset by a variable time delay, τ_i , between different radio frequency A-lines. This introduces a phase offset into the spectral calculation. We correct for this phase offset by multiplying each windowed FFT, $y_{i,k}(f)$, by a phase factor,³⁹ $\exp(j2\pi f \tau_i)$, determined by the time delay of the maximum value of the signal envelope within a gated data segment:

$$\tau_i = \arg \max |x_i(t) + j\mathcal{H}(x_i(t))| \quad (6)$$

The operator $\mathcal{H}$ denotes the Hilbert transform and j the imaginary unit. The estimation procedure described by Equation (5) is an averaging process, where the averaging is performed over both K tapers and N_A A-lines. The number of tapers used in the averaging procedure, K , is proportional to the smoothing bandwidth, W . The number of discrete frequency points spanning the bandwidth, W , in a standard FFT is denoted by NW in implementations of the multi-taper algorithm. The number of tapers in the calculation and the parameter, NW , are related by

$$K = 2 * NW - 1. \quad (7)$$

In this analysis, we fix the smoothing bandwidth to be 1.0 MHz for all calculations. When selecting a bandwidth parameter, the signal spectrum is regarded as a constant or linear over a region of width $2W$. Selecting the W parameter is, therefore, a trade-off between curvature bias and variance reduction³⁸ when estimating a power spectrum. It has been shown that Thomson's multi-taper algorithm for estimating the Loève spectrum has the remarkable property that the smoothing that occurs is strictly along the diagonal lines of constant frequency difference. This point has been discussed by David Thomson in the context of

radar signal processing.⁴⁰ Physically, this corresponds to lines of constant scatterer spacing within the bi-frequency plane. It is this property that makes a multi-taper spectral estimator attractive in the field of QUS.

Experimental Setup

Ultrasound Simulation

We performed frequency domain simulations of periodic, diffuse, and sparse scattering in order to make comparisons with our results *ex vivo*. We hypothesized that thermally coagulated liver would be better modeled by a strictly diffuse collection of scatterers, while normal untreated liver could be modeled as quasiperiodic. For all simulations, we created a linear array transducer having an aperture of 128 elements, each one being 0.2 mm wide laterally and 10 mm wide elevationally. The simulated transducer had a lateral and elevational focus occurring at a depth of 30 mm. We simulated a transmit pulse centered at 5.0 MHz and having a -10 dB bandwidth of 2.0 MHz. For all scatterers, we utilized a frequency dependence of f^2 for the scattering amplitude (yielding a backscatter coefficient with a frequency dependence of f^4).

We simulated two kinds of ROIs to model the untreated liver: diffuse plus periodic scattering and diffuse plus sparse scattering. To achieve diffuse scattering, a numerical phantom contained 32 uniformly randomly distributed scatterers/mm³. To simulate periodic scatterers, we also included a column of Gamma distributed scatterers along the lateral and elevational center of the simulated beamline. These Gamma distributed scatterers had an MSS of 1.3 mm and a fractional standard deviation in the spacing of 12%. The scattering amplitude for any given periodic scatterer was multiplied by a random constant over all frequencies selected from a Gaussian distribution centered at 300 with a standard deviation of 50. To create sparse scattering, three scatterers were added to a phantom. One was added between a depth of 20 and 50 mm, another between a depth of 30 and 40 mm, and another between a depth of 30 and 50 mm. These scatterers were spatially uniformly distributed, and their scattering amplitude was multiplied by a constant value selected from a Gaussian distribution with a mean of 50 and a standard deviation of 10.

We also simulated two kinds of ROIs to model ablated tissue: diffuse only and diffuse plus sparse scattering ROIs. These ROIs were created the same way as in the simulated normal liver tissue, except the attenuation coefficient was doubled. For ROIs representing normal untreated liver, a linear attenuation coefficient with an attenuation slope of 0.5 dB/cm MHz was assigned to each phantom. For ROIs representing thermally coagulated liver, a linear attenuation coefficient of 1.0 dB/cm MHz was used. A large, linear attenuation coefficient is what has been experimentally determined by other authors to correspond to thermal coagulation of liver tissue.²³ For these simulations, we neglected the small sound speed difference between thermally coagulated liver and normal untreated liver. We assumed a sound speed of 1600 m/s throughout all simulations.

Sound Speed Measurements

Sound speed measurements were made using a through transmission, broadband substitution method using five independent samples of normal untreated liver and five independent

samples of thermally coagulated liver.⁴¹ The sound speed of a sample was estimated using the following equation:

$$c_s = \frac{c_w}{1 + \frac{c_w \Delta t}{d}} \quad (8)$$

The temperature-dependent sound speed of water, c_w , was given by the formula of Kaye and Laby.⁴²

$$c_w = 1402.9 + 4.835T - 0.047016T^2 + 0.00012725T^3 \text{ m/s.} \quad (9)$$

The water in the tank was maintained at room temperature, 22°C. All sample thicknesses, d , were sliced to be equal to 2.6 cm. Three sound speed measurements were made for each sample using three transducers operating at center frequencies of 2.2, 3.2, and 5.20 MHz, respectively. For each transducer, a five-cycle burst was created using a function generator. The shift in the location of the third peak in the burst was tracked following insertion of the sample between the transmitting and receiving transducers to compute t . Over the 15 measurements (three frequencies by five samples), the sound speed in ex vivo bovine liver was estimated to be 1599.9 ± 6.4 m/s, and the sound speed in thermally coagulated ex vivo bovine liver was estimated to be 1591.6 ± 5.5 m/s.

Radio Frequency Data Acquisition

We estimated spectral coherence from radio frequency data acquired using the VFX 9L4 transducer and the Siemens S2000 system (Siemens Medical Solutions, Mountain View, California) in five independent ex vivo bovine livers and another five independent thermally coagulated ex vivo bovine livers. For all ex vivo measurements, we placed the tissue sample in a saline bath maintained at room temperature, 22°C. The liver was obtained from a slaughterhouse, and all the measurements were made within 12 hours of animal sacrifice. Within 45 minutes of animal sacrifice, the liver arrived at the laboratory, and it was refrigerated until a portion of it was sectioned. The portion of liver was then placed in the saline bath where it was allowed to warm to room temperature. Prior to imaging, tissue was degassed by placing the tissue within the saline bath into a vacuum chamber for 15 minutes. In order to thermally ablate the bovine liver, tissue was placed in an additional saline bath maintained at 80°C for 45 minutes. The coagulated liver was returned to its original saline bath at room temperature and degassed prior to imaging.

For imaging untreated bovine liver and thermally coagulated liver, a plastic bag containing an ethylene glycol and water offset was placed between the transducer and the tissue. This was done to increase the imaging depth to the point where a second row of elements on the 9L4 transducer activated to assist with elevational focusing. The ethylene glycol concentration was adjusted, along with the beamformer sound speed, until the sound speed matched that of the imaged tissue. For the untreated liver, a volume fraction of 18.2% ethylene glycol was used to yield a sound speed of 1600 m/s, and for thermally coagulated liver, a volume fraction of 16.6% ethylene glycol yielded a sound speed of 1590 m/s. B-

mode images of untreated and uniformly thermally coagulated liver are shown in Figure 1. The dark region in the image corresponds to the fluid offset between the tissue and the transducer. In this paper, we fix the lateral extent of our ROI at 12 mm for all coherence calculations in tissue. We varied the axial extent of the ROI from 12 to 18 mm. We refer to the axial extent of the ROI as the gate length or window size. The maximum gate length selected was chosen such that a homogeneous region could consistently be imaged. We computed all discrete Fourier transforms using the chirp-Z algorithm.⁴³ The number of discrete frequency points computed over a frequency range of 2.8–7.0 MHz was selected to be equal to the number of time points within a signal processing window. Any additional array operations, such as the outer product involved in calculating equation (5), were computed using Numpy.⁴⁴

Results

Measurements from Simulated Ultrasound RF Data

We simulated 12,000 independent signals from 12,000 numerical untreated liver phantoms as described above. In all, 10,000 of these phantoms contained periodic and diffuse scatterers, while 2,000 phantoms contained sparse and diffuse scatterers. We also simulated 12,000 independent signals from 12,000 numerical thermally coagulated phantoms. A total of 10,000 of these phantoms contained diffuse scatterers, while 2,000 contained sparse and diffuse scatterers. All the analyzed signal segments were gated centered around a depth of 40 mm. Correlations between adjacent A-lines were not considered in these simulations. MSS estimates were made by gating 12 or 16 mm of the randomly generated signals, computing spectral coherence, and detecting local maxima between a frequency range of $f_1 = 2.8$ to 7.0 MHz. Ten independent A-lines were used in each of the spectral coherence calculations to yield a convergent spectral coherence calculation. This yielded 1200 MSS estimates in simulated untreated liver tissue and 1200 MSS estimates in simulated thermally coagulated liver. Several examples of randomly generated signals are shown in Figure 2.

We first show histograms of MSS estimates in sparse ROIs in simulated untreated liver in Figure 3. We note that the MSS estimates in sparse scattering ROIs show a significant probability mass across all possible MSS estimates for a given gate length. We display the results of the MSS estimates in all simulated untreated liver ROIs and all simulated coagulated ROIs as histograms in Figure 4. We compute the mean, mode, and standard deviation of these measurements and display the results in Table 1. The central MSS of the histogram bin with the highest number of counts was the initial estimate for the mode of the distribution. A parabolic fit was performed around the maximum peak, and a subsample estimation was performed to refine the initial estimate of the distribution mode. This procedure is often used in estimating the cross-correlation peak with subsample precision in elastography,⁴⁵ and we employed it with all estimates of histogram modes in this paper. The histogram approximates a continuous probability density function, and numerically integrating the density assigned to each bin over all the bins in Figure 4 yields a total probability mass of 1.0.

Several results stand out in Figure 4. First, the mode of the MSS estimates corresponds to the MSS of the periodic scatterers. Second, the mean of the measured MSS increases with

increasing gate length. Finally, the mode of the estimated MSS in the simulated coagulated liver was less than 1 mm. The second result was due to the inclusion of the sparse scatterers, and the reason for the third result will be discussed in a later section.

Note from Figure 3 that the sparse scattering ROIs produce many of the spectral peaks corresponding to spacings greater than 1.3 mm, making up the long tail of the estimated MSS distribution in both simulated liver and simulated thermal coagulation. If the scattering in an ROI is sparse, a spectral peak will often occur near the power spectrum, where the frequency difference between the f_1 and f_2 coordinates is almost zero. The MSS associated with these spurious peaks tends to be large, approaching half the gate length. The longer the gate length, the more severe is the effect of this error on shifting the mean of the estimated MSS distribution. This is why the mean MSS measurement is further from the true MSS at a longer gate length than a shorter gate length.

Measurements in Ex Vivo Bovine Liver

For the analysis, we estimated the MSS in normal untreated ex vivo bovine liver across 1647 ROIs (391, 316, 311, 333, and 296 ROIs in five samples). We estimated MSS across 1498 ROIs in thermally coagulated ex vivo bovine liver (250, 286, 317, 330, and 315 ROIs in five samples). Each ROI was selected from a separate imaging plane and was separated from adjacent imaging planes by an elevational width of at least 1 mm. We varied the axial extent of an ROI from 12 mm up to 18 mm, in 2 mm increments. Local maxima were detected between a frequency range of $f_1 = 2.8$ to 7.0 MHz. In the analysis, we excluded regions containing large vessels with diameters greater than 1.5 mm. This was done to exclude some specular scattering from the results. However, intermediate size vessels less than this diameter were allowed in the analysis. An example image containing a vessel traversing the image laterally and having a 1 mm diameter is shown in Figure 5(A). Note that this results in specular scattering. We could have attempted to remove all the specular scattering from the analysis, but it is not possible to do this just from examining the B-mode image. We demonstrate another ROI in Figure 5(C) that results in a coherence peak near the power spectrum. This ROI is an example of sparse scattering. It contains a small number of aperiodic scatterers dominating the signal, and the MSS estimated from this ROI was 6.34 mm. However, it would be extremely difficult to ascertain from the B-mode image whether this ROI should be labeled as sparse, periodic, or diffuse.

In Figure 6(A), we show an example of an ROI in untreated liver we may confidently consider periodic. Figure 6(A) shows the B-mode image of untreated bovine liver, starting from a depth of 5 cm, and an 18 mm by 12 mm ROI. Figure 6(B) shows the corresponding spectral coherence function. This ROI may be considered periodic because it exhibits a spectral peak corresponding to a scatterer spacing of 1.57 mm, which allows for a large number of scatterer pairs to be contained in the ROI. We contrast this with the sparse and specular cases, where the estimated MSS approaches half the gate length and allows only two scatterer pairs to be contained within the gated A-line segment. In addition to considering an upper limit on what should be considered a true periodicity, the results in thermally coagulated liver also inform us that a lower limit on periodicity should also be considered. In Figure 6(D), we show a spectral coherence peak in thermally coagulated liver

occurring away from the power spectrum, corresponding to an estimated MSS of approximately 0.5 mm.

The effect of the multi-taper estimation procedure is visible in Figures 6(B) and 6(D). The multi-taper algorithm smoothes over a bandwidth equal to twice the smoothing parameter, W , only along diagonal lines of constant frequency difference. This gives the spectral coherence estimate a highly smoothed appearance along one direction and a rapidly fluctuating appearance in the perpendicular direction.

Figure 7 shows histograms of the resulting MSS estimates in the normal untreated bovine liver and the histograms of MSS estimates in the thermally coagulated liver. We present these results in order to show that the estimator for MSS is biased and that the bias depends on the gate length. We also display the estimated mean, standard deviation, and mode of the estimated MSS over multiple gate lengths in Table 2. Table 2 demonstrates the gate length dependence of the mean and standard deviation of the MSS estimates in both untreated liver and thermally coagulated liver. At a gate length of 12 mm, the mean of the MSS estimates in the untreated liver was 2.45 mm, while at a gate length of 18 mm, the mean of the MSS estimates was 3.06 mm. In the thermally coagulated liver tissue, the mean of the MSS estimates was 2.31 mm at a gate length of 12 mm and 3.01 mm at a gate length of 18 mm. In addition, the mode of the MSS estimates in the thermally coagulated liver showed large fluctuations between 0.45 mm and 1.0 mm as the gate length was varied. In the case of the normal untreated liver, the estimated MSS showed a consistent mode varying only between 1.25 mm and 1.37 mm. The results presented in Table 1 have previously demonstrated in our numerical phantoms that the mode corresponded to the true MSS of the periodic scatterers.

MSS Estimation and Sampling Effects

This section illustrates why diffuse scattering tends to result in spectral coherence peaks that correspond to submillimeter spacings. When computing the spectral coherence function, we discretely sample this function over an evenly spaced grid with respect to frequency. Thus, larger spacings are coarsely sampled, while small spacings are finely sampled. A plot of MSS bin width versus estimated MSS is shown in Figure 8. This figure plots the MSS bin width corresponding to spacings derived from the frequency differences present in the discretely sampled bi-frequency plane over the square region from (2.8 MHz, 2.8 MHz) up to (7.0 MHz, 7.0 MHz). We also assume a gate length of 12 mm or 18 mm, a sampling frequency of 40 MHz, and a sound speed of 1590 m/s. These parameters are identical to the thermally coagulated liver case. With these settings, the smallest possible MSS that could result from a spectral peak in this region of the bi-frequency plane corresponds to an MSS of 0.19 mm. The plots demonstrate that a much smaller spacing difference between diagonally adjacent peaks corresponding to an MSS under 1 mm exists than between adjacent spectral peaks corresponding to an MSS over 1 mm. If a spectral coherence function was a randomly uniformly distributed two-dimensional function, this sampling effect would favor the smallest spacings, tending toward 0.19 mm.

In addition, the frequency axes are rotated 45 degrees with respect to lines of constant frequency difference and constant scatterer spacing. This means that spectral coherence is

calculated over shorter lines of constant scatterer spacing or constant frequency difference as the scatterer spacing gets smaller. If spectral coherence was a uniformly distributed random two-dimensional function, the effect of the estimation region shape would be to favor the largest scatterer spacings.

Now let us consider the expected result if spectral coherence were a uniformly distributed, randomly generated function. In Figure 9, we show the resulting MSS distribution if the spectral coherence function was a randomly generated function whose pixels are uniformly distributed. This shows that if a spectral peak was equally likely anywhere in the bi-frequency plane, with the constraint that no peak may occur too close to the power spectrum, the two competing sampling effects favor a small spacing between 0.5 mm and 1.0 mm.

The actual estimated MSS distribution shown in Figure 6 demonstrates a heavier tail favoring larger spacings than what is expected from the considerations in this section. This is because, for diffuse scattering, the spectral coherence function tends to decay as one moves away from the power spectral region of zero frequency difference.

Discussion

In both ex vivo bovine liver and thermally coagulated liver, we found that the distribution of estimated MSS had a mean that steadily increased with the axial extent of our ROI. However, in normal untreated ex vivo bovine liver, the mode of the distribution of MSS estimates remained between 1.25 and 1.37 mm. We hypothesize that ex vivo bovine liver exhibits a true periodicity at an MSS of approximately 1.3 mm and that some ROIs in normal untreated liver will fail to be periodic. Based on the simulations of periodic and sparse scattering, we assert that the mode of the estimated MSS distribution should be regarded as the true MSS. The tail of the MSS distribution may be attributed to falsely calculating MSS in regions where no periodicity exists. In the future, our lab will explore parametric images of MSS estimates for delineating thermal ablations. To account for sparse scattering noise, prior information about the expected MSS in untreated liver may need to be used to decide on a cutoff spacing above which MSS estimates will be dismissed as sparse scattering noise. This cutoff spacing would be chosen based on prior information about tissue, namely, the true MSS in tissue estimated from the mode of an estimated MSS distribution and not based on the spatial resolution of the MSS estimator. Further studies will be needed to determine the minimum spatial resolution that can be achieved for an MSS estimator while balancing a cutoff spacing chosen independently of the gate length.

There were some small fluctuations in the mode in our MSS estimates in untreated ex vivo liver. These fluctuations may be due to the variance inherent in estimating the mode of an unknown probability distribution with only a small number of samples. We also note that there is a discrepancy between our simulations and the data measured ex vivo. The peak of the MSS distribution in the simulations is narrower than what was observed ex vivo. There may be several reasons for this discrepancy: first, there may be some variance between different animals or between different anatomical sections of the liver within a single animal. Unfortunately, verifying this would require examining a very large number of animals. Second, our periodic scattering model is only a very crude approximation to the

actual three-dimensional scatterer arrangement within the liver. In the future, experiments with large numbers of animals and comparisons with data from micro-CT⁴⁶ may shed light on this problem. Finally, no pathological analysis was performed on the livers used in this study. All the animals were raised to maturity under the same conditions prior to sacrifice for commercial meat production. Therefore, no pathology was expected in any animal, but this is still a source of experimental uncertainty.

Meanwhile, the mode of the MSS estimates in coagulated liver ranged between 0.5 and 1.0 mm, with significant probability density below 1.0 mm. We hypothesize that this is because thermally coagulated liver is better modeled by a diffuse and sparse aperiodic scattering model. While a large difference exists between the MSS estimated in normal untreated liver and MSS estimated in thermally coagulated liver, it must be stressed that an MSS of 0.5 mm in thermally coagulated liver corresponds to an algorithmic artifact rather than anything physical within the tissue. If thermally coagulated liver is better modeled as aperiodic, then any value for estimated MSS within this tissue must be regarded as an artifact, and one should avoid estimating MSS within the ROI altogether.

To our knowledge, the only other reported measurements of MSS in *ex vivo* bovine liver are in a paper by Pereira et al.³¹ In this paper, spacings of 3.1 to 4.8 mm were found in MSS measurements using SSA in *ex vivo* bovine liver and a transducer with a transmit center frequency of 3.3 MHz. In human liver, measurements of MSS have yielded values of 1 ± 0.16 mm, but this depends on the pathological state of the tissue.²⁷ We find that the mode of our MSS estimates show better agreement with previously reported MSS values in human liver than the values reported by Pereira et al.³¹ Concerning thermal ablation, a study has recently been published that contends that thermal ablation resulted in only a small decrease in MSS from 1.15 to 0.93 mm in *ex vivo* pig liver.³⁴ We hypothesize that this result is likely dependent on signal processing parameters and may not hold if imaging parameters are changed. For example, the authors did not demonstrate that they measure the same MSS if the gate length was changed or the type of wavelet used was altered. All the results presented in this study used an analysis window length fixed at 7 mm³⁴ and a fifth order bi-orthogonal spline wavelet. It is difficult to say whether algorithmic changes would result in coagulated liver exhibiting a smaller or larger MSS than normal untreated liver tissue when using a wavelet algorithm, since the tissue may not have a true MSS associated with it to estimate.

The issue of what features of the liver microanatomy contribute to the scattering has been an open question in ultrasound for some time. Following thermal ablation with an RF applicator, it has been found that the liver cells and connective tissue retain their morphology in regions close to the RF applicator where extremely large thermal doses are delivered.⁴⁷ Meanwhile, RF ablation has been found to result in complete destruction of microvasculature.⁴⁷ We hypothesize that the changes to the scattering model observed in this paper are due to destruction of small blood vessels and bile ducts.

Conclusion

Quantitative ultrasound is a potentially powerful tool for diagnosis of disease in a clinical setting. MSS has been investigated as a possible parameter for tissue characterization for over two decades. This work examined MSS estimation in untreated ex vivo bovine liver and MSS estimation in thermally coagulated bovine liver. We found that bovine liver can be modeled by a quasiperiodic distribution of scatterers with an approximate MSS of 1.3 mm. Meanwhile, the estimates of MSS in thermally coagulated liver tended toward submillimeter values. MSS may serve as a useful parameter for producing parametric images of treated versus untreated liver in the future, with the understanding that the ultrasound echo signal in thermally coagulated liver corresponds primarily to scattering from diffuse scatterers exhibiting a Rayleigh distributed signal envelope.

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Figure 1. B-mode images of (A) ex vivo liver and (B) uniformly thermally coagulated ex vivo liver. The dark region corresponds to the water/ethylene glycol offset.

Figure 2.

(A) A simulated radio frequency signal from periodic scatterers in the liver. (B) A simulated signal from sparse, resolvable scatterers in the liver. (C) A simulated diffuse scattering signal from a thermally ablated region.

Figure 3. Histograms of MSS estimates for sparse scattering ROIs in simulated liver for (A) 12 mm and (B) 16 mm gate lengths.
Note. ROI = region of interest.

Figure 4.

Histograms of MSS estimates for all the ROIs in simulated untreated liver (U.L. in caption) and simulated thermally coagulated regions (T.C. in caption). The blue histogram bars represent simulated liver, while the red histogram bars represent estimates from thermally coagulated regions for (A) 12 mm and (B) 16 mm ROIs.

Note. ROI = region of interest.

Figure 5.

(A) B-mode image illustrating liver tissue exhibiting specular scattering. The green arrow indicates approximate depth of specular scattering surface. The red box indicates the analysis ROI. (B) The corresponding spectral coherence function, with local maximum near power spectrum, is denoted by black arrow. (C) B-mode image demonstrating sparse scattering. Note the isolated bright points within the ROI. (D) Corresponding spectral coherence function with local maximum near the power spectrum, corresponding to an MSS of 6.34 mm, denoted by the black arrow.

Note. ROI = region of interest; MSS = mean scatterer spacing.

Figure 6.

(A) 18 mm by 12 mm ROI in ex vivo bovine liver. (B) Corresponding spectral coherence function. The arrow identifies a spectral peak corresponding to an MSS of 1.29 mm. (C) 18 mm by 12 mm ROI in thermally coagulated ex vivo bovine liver. (D) Corresponding spectral coherence function. The arrow identifies a spectral peak corresponding to an MSS of 0.5 mm.

Note. ROI = region of interest; MSS = mean scatterer spacing.

Figure 7.

Histogram of MSS estimates in untreated liver (U.L. in caption) and thermally coagulated liver (T.C. in caption) as the gate length is varied over (A) 12 mm, (B) 14 mm, (C) 16 mm, (D) 18 mm.

Note. MSS = mean scatterer spacing.

Figure 8.

Estimated MSS resulting from frequency differences present in the discretely sampled bi-frequency plane, assuming a sound speed of 1590 m/s, sampling frequency of 40 MHz, and square ROI with dimensions 2.8 MHz to 7.0 MHz. (A) 12 mm and (B) 18 mm gated signals. *Note.* MSS = mean scatterer spacing; ROI = region of interest.

Figure 9.

Estimated MSS distribution, with each pixel value in the bi-frequency plane given by a uniformly distributed random variable. Signal processing parameters were identical to those used when analyzing thermally coagulated liver. (A) 12-mm gate length. (B) 18-mm gate length.

Note. MSS = mean scatterer spacing.

Table 1

Parameters of the Estimated MSS Distributions in Simulated Tissue.

Gate Length (mm)	$M \pm SD$ in Simulated Untreated Liver (mm)	$M \pm SD$ in Simulated Heated Tissue (mm)	Mode in Simulated Untreated Liver (mm)	Mode in Simulated Heated Tissue (mm)
12	1.49 ± 1.19	1.25 ± 1.12	1.27	0.45
16	1.62 ± 1.51	1.47 ± 1.55	1.30	0.43

Note. MSS = mean scatterer spacing.

Table 2

Parameters of the Estimated MSS Distributions in Ex Vivo Liver and Thermally Coagulated Liver.

ROI dimension (mm)	<i>M</i> ± <i>SD</i> of MSS Estimates in Normal Untreated Liver (mm)	<i>M</i> ± <i>SD</i> of MSS Estimates in Coagulated Liver (mm)	Mode of MSS Estimates in Normal Untreated Liver (mm)	Mode of MSS Estimates in Coagulated Liver (mm)
12	2.45 ± 1.51	2.31 ± 1.63	1.25	0.84
14	2.78 ± 1.75	2.48 ± 1.86	1.35	0.45
16	3.00 ± 2.01	2.74 ± 2.11	1.37	1.00
18	3.06 ± 2.15	3.01 ± 2.50	1.27	0.53

Note. MSS = mean scatterer spacing; ROI = region of interest.