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Scatterer Number Density Considerations in Reference Phantom Based Attenuation Estimation

Nicholas Rubert and Tomy Varghese

Department of Medical Physics, 1111 Highland Avenue, WIMR, University of Wisconsin – Madison, Madison, WI 53705

Abstract

Attenuation estimation and imaging has the potential to be a valuable tool for tissue characterization, particularly for indicating the extent of thermal ablation therapy in the liver. Often the performance of attenuation estimation algorithms is characterized with numerical simulations or tissue mimicking phantoms containing a high scatterer number density (SND). This ensures an ultrasound signal with a Rayleigh distributed envelope and an SNR approaching 1.91. However, biological tissue often fails to exhibit Rayleigh scattering statistics. For example, across 1,647 ROI's in 5 *ex vivo* bovine livers we find an envelope SNR of 1.10 ± 0.12 when imaged with the VFX 9L4 linear array transducer at a center frequency of 6.0 MHz on a Siemens S2000 scanner. In this article we examine attenuation estimation in numerical phantoms, TM phantoms with variable SND's, and *ex vivo* bovine liver prior to and following thermal coagulation. We find that reference phantom based attenuation estimation is robust to small deviations from Rayleigh statistics. However, in tissue with low SND, large deviations in envelope SNR from 1.91 lead to subsequently large increases in attenuation estimation variance. At the same time, low SND is not found to be a significant source of bias in the attenuation estimate. For example, we find the standard deviation of attenuation slope estimates increases from 0.07 dB/cm MHz to 0.25 dB/cm MHz as the envelope SNR decreases from 1.78 to 1.01 when estimating attenuation slope in TM phantoms with a large estimation kernel size (16 mm axially by 15 mm laterally). Meanwhile, the bias in the attenuation slope estimates is found to be negligible (< 0.01 dB/cm MHz). We also compare results obtained with reference phantom based attenuation estimates in *ex vivo* bovine liver and thermally coagulated bovine liver.

Keywords

Attenuation; Envelope SNR; Scatterer Number Density; Reference Phantom; Thermal Ablation; Liver; Multi-taper

Introduction

Ultrasound imaging is a low-cost, portable imaging modality, and attenuation coefficient estimation and subsequent imaging has the potential to be a valuable tool for several clinical applications, including distinguishing between different types of breast masses (Nam et al., 2013), characterizing bone density in order to assess the risk of osteoporosis and bone fracture (Sasso et al., 2008; Wear, 2003), diagnosing fatty infiltration in the liver (Narayana and Ophir, 1983), and indicating the extent of thermal ablation therapy in the liver and other abdominal organs (Bush et al., 1993; Gertner et al., 1997; Kemmerer and Oelze, 2012; Parmar and Kolios, 2006; Techavipoo et al., 2004). In this article we focus on attenuation estimation in the liver and its application toward assessing the extent of thermal coagulation following thermal ablation therapy. Attenuation in thermally coagulated regions has often been shown to approximately double relative to untreated normal liver tissue (Bush et al., 1993; Gertner et al., 1997; Kemmerer and Oelze, 2012; Parmar and Kolios, 2006; Techavipoo et al., 2004).

In particular, Parmar and Kolios (2006) found a relative increase in attenuation by a factor of 2.29 at a transmit center frequency of 5 MHz in *ex vivo* porcine liver following heating to 75° C for 60 minutes. Bush et al. (1993) estimated increases in attenuation coefficient in the frequency range of 3.0 to 8.5 MHz from 32% to 192% in high-intensity focused ultrasound (HIFU) lesions approximately 10 by 30 mm in cross-section in *ex vivo* pig livers. Gertner et al. (1997) found the attenuation coefficient estimated at 3.5 MHz increased by a factor of over 1.8 in eight store-bought bovine liver samples following 30 minutes of heating at 70° C in a saline bath. Techavipoo et al. (2004) examined the temperature dependence of sound speed and attenuation in canine liver tissue. It was found that the attenuation coefficient showed a slight decrease as tissue was heated up to 50° C and then an increase in attenuation as it was heated from 50° to 100° C. The relationship between temperature and attenuation was hypothesized to be due to tissue coagulation increasing tissue attenuation and temperature elevation decreasing tissue attenuation (Techavipoo et al. 2004). Kemmerer and Oelze (2012) found that the attenuation slope doubled from 0.2 dB/cm MHz to 0.4 dB/cm MHz over a frequency range of 9 to 25 MHz when rat liver tissue was exposed to a 70° C saline bath. In these experiments, gas bubbles produced due to heating were addressed very differently. In the saline bath experiments of Gertner et al. (1997), the authors stated that the tissue was degassed only prior to heating, while in the other saline bath heating experiments tissue degassing was not explicitly mentioned (Kemmerer and Oelze, 2012; Parmar and Kolios, 2006; Techavipoo et al., 2004). In the HIFU experiment described in Bush et al. (1993) tissue was degassed under vacuum both prior to and after HIFU exposure.

A variety of techniques have been applied to estimate attenuation within tissue. Several authors have proposed time domain algorithms (Ghoshal and Oelze, 2012; He and Greenleaf, 1986; Jang et al., 1988; Knipp et al., 1997). He and Greenleaf (1986) proposed the envelope peak method, where the ratio of the mean envelope peak to standard deviation of envelope peaks over depth was minimized by adjusting an attenuation-dependent gain function. Jang et al. (1988) also proposed applying an attenuation and depth dependent gain to the signal envelope in order to determine attenuation. In their method, the entropy difference between adjacent signal segments was minimized by adjusting the attenuation

estimate and an associated depth-dependent gain function. Knipp et al. (1997) proposed a method for estimating attenuation and backscatter in an unknown sample by comparing B-mode image values with values in a look-up table developed for individual transducers from phantoms with known attenuation and backscatter coefficients. This method had the disadvantage of requiring determination of an effective frequency to assign to the recorded B-mode image to account for frequency dependence of beamforming, backscatter, and attenuation. Ghoshal and Oelze (2012) recently proposed a time-domain algorithm where they explicitly modeled the diffraction pattern for a single-element circular transducer and iteratively solved for the attenuation coefficient in an unknown sample.

Frequency domain techniques are currently the more popular method for determining the attenuation coefficient in tissue. Frequency domain techniques are frequently described in analogy to two techniques explored by Kuc (1984): the spectral shift method and the spectral difference method. In Kuc's (1984) approach, the ultrasound signal prior to propagating through tissue is modeled by a pulse power spectrum, $P_i(f)$. The effect of traveling through a thickness of tissue, D , is to multiply the pulse power spectrum by an attenuation transfer function, $|H(f)|^2 = \exp(-4\pi\beta fD)$, where the attenuation coefficient is assumed to have a linear frequency dependence. After making a round trip through tissue, the pulse power spectrum is then given by $P_f(f) = P_i(f) \exp(-4\pi\beta fD)$.

In the spectral shift method, $P_i(f)$ is modeled by a Gaussian function, and the effect of attenuation is to shift the center frequency of the Gaussian spectrum by an amount proportional to β . The attenuation coefficient can then be obtained by tracking the center frequency of the ultrasound pulse with depth. In the spectral difference method, the logarithm of the ratio of the power spectral magnitude is examined. In order to estimate β , log spectral ratios are directly computed and no parametric model for the pulse spectrum is necessary. The spectral shift and spectral difference techniques as described by Kuc (1984) failed to account for the effect of transducer beam diffraction and focusing effects. Insana et al. (1983) developed correction factors to the spectral difference method to account for beam diffraction when making attenuation measurements in TM phantoms with known attenuation coefficients. Madsen et al. (1984) derived a frequency-domain model for the echo signal voltage for measurements of backscatter coefficients. This model was applied to estimation of backscatter using single element transducers, where beam diffraction was accounted for using the signal spectrum from reflections off a reference planar reflector. The signal model of Madsen et al. (1984) was later extended to backscatter coefficient and attenuation estimation with clinical array transducers by Yao et al. (1990). Yao et al. (1990) accounted for beam diffraction through division of tissue spectra by spectra acquired from a reference phantom with matched sound speed and the same ultrasound system settings.

The reference phantom method developed by Yao et al. (1990) may be considered a spectral difference method. In this method, amplitude changes in the frequency content of the ultrasound pulse are measured over depth and normalized by a reference spectrum from a well-characterized phantom with known attenuation and backscatter. Attenuation is then estimated from the logarithm of the resulting ratio (Yao et al., 1990). Kim and Varghese (2008) later implemented a hybrid method by incorporating the reference phantom method for normalizing spectra to reduce system dependent effects, and then applying a Gaussian

filter to the normalized spectra. The frequency shift of the power spectrum with depth was then detected using a cross-correlation algorithm (Kim and Varghese, 2008). This algorithm was shown to be less sensitive to artifacts than the reference phantom method when measuring attenuation in regions with inhomogeneous backscatter coefficient under diffuse scattering conditions (Kim and Varghese, 2008). Nam et al. (2011b) proposed a reference phantom method where the backscatter coefficient frequency dependence was modeled with a power law and attenuation was assumed to have a linear frequency dependence. In this algorithm, the backscatter and attenuation were estimated simultaneously by minimizing the squared error between the predicted spectral ratio and the measured spectral ratio.

The reference phantom method and its variations make several assumptions about the tissue being imaged. First, this method assumes that the tissue being imaged has a sound speed matched to the reference phantom and the assumed sound speed of the beamformer in the ultrasound system. Second, it assumes that scattering occurs from spatially randomly distributed scatterers and their number density is large enough that the envelope is Rayleigh distributed. The impact of sound speed variations on attenuation estimation has been evaluated previously. Omari et al. (2011) examined variations in sound speed from 1480 to 1600 m/s at center frequencies of 4 and 5 MHz, and they found that sound speed errors led to a bias in the attenuation estimate. Meanwhile, Nam et al. (2011a) found that bias in the attenuation estimate due to sound speed errors was worst when examining depths at and around the lateral focal depth.

However, little attention has been given to the impact of non-Rayleigh envelope statistics on the resulting attenuation estimate. In many of the simulation studies for attenuation estimation, a single scatterer size and an SND greater than 10 per mm is often used (Kim and Varghese, 2008, 2007; Kim et al., 2008; Kim, H. and Heo, S.W., 2012; Labyed and Bigelow, 2011; Nam et al., 2011a; Omari et al., 2011, 2013). This is done to yield Rayleigh scattering statistics. However, non-Rayleigh scattering statistics commonly occur in ultrasound imaging. For example, cysts and fibroadenoma in the breast have been shown to possess non-Rayleigh statistics using Nakagami imaging (Tsui et al., 2008). The Nakagami m-parameter has also been shown to range from 0.55 to 0.83 in rat liver as the Metavir score of that liver increased from 0 to 4, estimated from radiofrequency (RF) data recorded with a 6.5 MHz center frequency single-element transducer (Ho et al., 2012). The Metavir scoring system was developed in France for evaluating fibrosis and cirrhosis of the liver resulting from chronic viral hepatitis (Bedossa and Poynard, 1996). Under this scoring system, a pathologist evaluates the liver for damage and assigns a score of F0 through F4. A score of F0 indicates a healthy liver; F4 indicates cirrhosis; scores in-between F0 and F4, represent varying degrees of fibrosis. The Nakagami imaging study by Ho et al. (2012) indicated that healthy liver has a low SND, and increasingly severe liver fibrosis tends to increase the SND.

The main aim of this article was to investigate the effect of SND variations on the bias and variance of reference phantom attenuation estimates. To this end, SND variations and their impact on reference phantom attenuation estimates were examined with computer simulations and TM phantoms imaged with a clinical, linear array transducer. These results

were used to explain the result of reference phantom based attenuation estimates in normal *ex vivo* bovine liver and thermally coagulated *ex vivo* bovine liver.

Theory

Reference Phantom based Attenuation Estimation

In this article, we examine the reference phantom method of Yao et al. (1990). Yao et al. (1990) derived the following equation relating power spectra of sample and reference phantom to their attenuation and backscatter coefficients:

$$\frac{P_s(t_0, f)}{P_r(t_0, f)} = \frac{B_s(f)}{B_r(f)} \exp((\alpha_s(f) - \alpha_r(f))4z) \quad (1)$$

In equation (1), $P_s(t_0, f)$ denotes a time-varying spectrum of an RF signal from tissue and $P_r(t_0, f)$ is a time-varying spectrum of an RF signal from a reference phantom. By a time-varying spectrum, we mean that RF data may be regarded as approximately stationary over a small time window centered on t_0 . Then for fixed t_0 , $P_s(t_0, f)$ is the power spectrum estimated over frequency centered on the location t_0 . $B_s(f)$ is the backscatter coefficient of a collection of monopole scatterers in tissue, and $B_r(f)$ is the same quantity in the reference phantom. $\alpha_s(f)$ is the attenuation coefficient at frequency f of the unknown sample and $\alpha_r(f)$ is the attenuation coefficient at frequency f of the reference phantom. z is the imaging depth. Imaging depth and time are assumed to be related through a known and constant sound speed, c . In order to compute the attenuation coefficient of the unknown sample at a particular frequency, it's necessary to take the logarithm of equation (1). This yields:

$$\ln\left(\frac{P_s(t_0, f)}{P_r(t_0, f)}\right) = \ln\left(\frac{B_s(f)}{B_r(f)}\right) + (\alpha_s(f) - \alpha_r(f))4z \quad (2)$$

To compute the attenuation coefficient at a particular frequency, we computed a linear fit to (2) over depth. We then computed the attenuation coefficient at a single frequency, f , from the resulting slope and the known attenuation coefficient of the reference phantom. In this article, we have assumed a linear frequency dependence of attenuation with an intercept of 0. Therefore, attenuation in a sample is given by: $\alpha_s = \beta_s f$. To determine attenuation slope in the sample, we performed a second linear fit to the attenuation coefficient as a function of frequency, fixing the intercept at 0. The estimated attenuation slope, β_{est} , is then given by:

$$\beta_{est} = \frac{\sum_i \alpha_s(f_i) f_i}{\sum_i f_i^2} \quad (3)$$

Equation (3) is derived by minimizing the sum of square differences between $\alpha_s(f_i)$ and $\beta_{est}(f_i)$.

To compute a power spectrum, we used Thomson's multi-taper method (Thomson, 1982). In this method, an individual power spectrum is computed by averaging multiple Fourier spectra, each computed with a separate orthogonal window function. We denote the i^{th} A-

line in a 2-D ROI containing M A-lines by x_i . An estimate for the time-varying spectrum is then given by the following expression:

$$S_{k,i}(t_0, f) = \left| \sum_{t=0}^{N-1} h_k(t) x_i(t_0 + t) \exp(-i2\pi ft) \right|^2 P(t_0, f) = \frac{1}{M} \frac{1}{K} \sum_{i=0}^{M-1} \sum_{k=0}^{K-1} S_{k,i}(t_0, f) \quad (4)$$

In equation (4), h_k is the k^{th} Slepian sequence. The power spectrum at a particular time point t_0 is given by an average over K tapers and M A-lines for a 2-D ROI. Improvements to attenuation estimation with the use of the MT method when compared to other gating windows have been discussed by Rosado-Mendez et al. (2013). When computing a power spectrum with a Multi-taper estimator, improvement in estimation variance over just a single taper is expected when the spatial extent of the parameter estimation region is constrained (Rosado-Mendez et al., 2013). All array and FFT operations were carried out using the Python library Numpy (Oliphant, 2007).

Envelope SNR

If the ultrasound signal is a result of scattering from a large number of uniformly distributed microscopic inhomogeneities throughout a region of interest, then the ultrasound RF echo signal envelope is a random signal with values given by a Rayleigh probability density function (PDF) (Wagner et al., 1983). The Rayleigh PDF for the RF echo signal envelope is derived by modeling the ultrasound signal as an analytic signal whose real and imaginary components are Gaussian distributed. The Rayleigh distribution is characterized by the ratio of the mean to the standard deviation, i.e. the SNR. For a Rayleigh PDF the SNR is given by $(\pi/4 - \pi)^{1/2}$, which is approximately 1.91.

A Rayleigh PDF is only achieved as a limiting case as the SNR becomes large. As the number density of uniform scatterers drops below what is required for a Rayleigh distributed envelope, the envelope SNR of a random signal begins to drop. Throughout the article we characterize the signals we examine according to the envelope SNR parameter. We estimated envelope SNR based on the following equation:

$$SNR = \frac{\mu_E}{\sigma_E} \quad \mu_E = \frac{1}{M} \sum_{i=1}^M \frac{1}{T} \sum_{t=1}^T E_i(t) \quad \sigma_E^2 = \frac{1}{M} \sum_{i=1}^M \frac{1}{T-1} \sum_{t=1}^T E_i(t) - \mu_E \quad (5)$$

In equation (5), $E_i(t)$ is the envelope value at the i^{th} A-line in a two-dimensional (2-D) region of interest (ROI) containing M A-lines, at time point t of T discrete points in the axial direction.

Materials and Methods

Simulations

Frequency domain simulations developed at UW-Madison were employed in this study (Li and Zagzebski, 1999). Two linear array transducers with different center frequencies (3.5 and 5.5 MHz with 65% bandwidth each) were simulated. Each transducer had a maximum aperture that included 128 elements. Each element was 0.2 mm wide laterally and 10.0 mm

wide elevationally. The simulated transducer had a lateral and elevational focus occurring at a depth of 30.0 mm. Dynamic receive focusing was simulated with an F/# of 2. Two sets of simulated phantoms with a sound speed of 1600 m/s were created. At this sound speed, the wavelengths corresponding to the transducer center frequencies were 0.457 mm and 0.291 mm, respectively. The two sets of numerical phantoms differed in their linear attenuation coefficients. One set had a linear attenuation coefficient of 0.50 dB/cm MHz. The other set of numerical phantoms had a linear attenuation coefficient of 1.00 dB/cm MHz. Numerical phantoms having SND's of 0.2 to 1.0 scatterers/mm³ in increments of 0.1, 2 to 128 in dyadic steps, and also 12 and 96 were simulated for each linear attenuation coefficient value. The scattering amplitude for all scatterers was identical. Scattering from small particles was simulated so the frequency dependence was given by f to the power of 2. For each SND, 1,000 independent A-lines were generated. Each A-line was formed using a completely independent and random realization of the scattering distribution.

Tissue Mimicking Phantoms

Tissue mimicking (TM) phantoms with low, variable SND's were manufactured in our laboratory in order to compare attenuation estimates obtained using a clinical linear array transducer (VFX 9L4) and the S2000 ultrasound system (Siemens Medical Solutions, Mountain View, CA, USA) with attenuation estimates obtained using the numerically simulated data. Four TM phantoms were manufactured containing agar, glass beads, and graphite powder. Graphite powder was utilized to obtain the desired attenuation coefficient and diffuse scattering, while the glass beads provided scattering with a low SND. Two size distributions of glass beads were utilized: 75 to 90 microns and 125 to 150 microns. All beads were made of soda lime glass and had a mass density of 2.52 g/cm³. We refer to the phantoms as phantoms A through D in the order of the envelope SNR estimated from them. Phantom A contained 1.286 g/liter of 100 to 125 micron glass beads (Potters Industries, Inc., Valley Forge, Pennsylvania, USA). Phantom B contained 0.278 g/liter of 125 to 150 micron glass beads. Phantom C contained 4 g/liter of 125 to 150 micron glass beads (Potters Industries, Inc., Valley Forge, Pennsylvania, USA). Phantom D contained 4 g/liter of 75 to 90 micron glass beads (Potters Industries, Inc., Valley Forge, Pennsylvania, USA). The glass bead diameters, concentrations used in phantom construction, and the estimated number density of glass beads are summarized in Table 1. We estimated the number density of glass beads by assuming that all beads in the distribution had a size equal to the mean of the diameter distribution. Phantom C and D have been previously analyzed (Omari et al., 2013).

Two reference phantoms were utilized, one for attenuation estimation in TM phantoms A through D and a second reference phantom for experiments with *ex vivo* bovine liver. We refer to the reference phantom used in the TM phantom experiments as phantom E and that used in the *ex vivo* bovine liver experiments as phantom F. The reference phantoms also contained agar, graphite powder, and glass bead scatterers. The glass bead diameters within the reference phantoms were in the 4 to 50 microns range (Potters Industries, Inc., Valley Forge, Pennsylvania, USA). For all TM phantoms, sound speed and attenuation were determined using through transmission measurement using three pairs of unfocused hydrophones at nominal center frequencies of 2.5, 5.0, and 7.5 MHz. The transmitting transducers were Panametrics, models C3003, V309, and V320. The receiving transducers

were manufactured by Aerotech Delta, models PN2794-1, PN2794-3, and PN2794-4. A single sound speed and attenuation slope estimate was measured with each of the transducers at their nominal center frequencies, and a linear attenuation coefficient was calculated by dividing the attenuation measurement by the transmit frequency.

The mean estimated sound speed in phantoms A through D was 1539, 1540, 1533, 1533, and 1533 m/s respectively, and the sample standard deviation among the three measurements was less than 1 m/s. The mean estimated sound speed in phantom E was 1580 ± 5 m/s, where a single sample standard deviation was computed. The estimated attenuation slope value in phantoms A through F were 0.49 ± 0.02 dB/cm MHz, 0.49 ± 0.02 dB/cm MHz, 0.68 ± 0.10 dB/cm MHz, 0.61 ± 0.05 dB/cm MHz, 0.57 ± 0.03 dB/cm MHz, and 0.57 ± 0.02 dB/cm MHz respectively, where the mean measurement and a single standard deviation between the three transducers was computed.

RF data was acquired during imaging of each of the TM phantoms on the S2000 scanner with the VFX 9L4 transducer. B-mode images of the TM phantoms are shown in Figure 1. 100 independent frames of RF data were recorded on each of the four TM phantoms with the transducer being translated by at least 2 mm between each frame. 15 planes of reference data were acquired from the reference TM phantom.

Ex vivo bovine liver

Ten independent bovine livers excised from ten separate animals were utilized in this study for reference phantom attenuation estimation, and five of these livers were thermally coagulated. Another ten bovine livers were utilized for through transmission measurements of attenuation and sound speed, and five of these livers were thermally coagulated. Thermal coagulations were produced by heating large sections of liver in a saline bath maintained at a temperature of 80°C for 45 minutes. All bovine livers were acquired immediately following animal sacrifice at a commercial meat production facility. Within 45 minutes, all livers were refrigerated until they could be utilized for experiments. Subsequent through transmission and clinical array imaging experiments were performed within 10 hours of refrigerating tissue.

For bovine liver, all the through transmission measurements were performed with three pairs of unfocused, single-element transducers with nominal center frequencies of 2.25 MHz, 3.50 MHz, and 5.0 MHz. The transmitting transducers were manufactured by Panametrics, model No.'s V306, V382, and V309. The receiving transducers were manufactured by Aerotech Delta, model No.'s PN2794-1, PN2794-2, PN2794-3. Tissue was degassed in saline solution in a low pressure vacuum (25 mm Hg), and allowed to warm or cool to room temperature (22°C) prior to through transmission measurements. Each tissue sample was cut in such a way as to fill a cylindrically shaped container with a length of 2.50 cm and a diameter of 8.00 cm. The through transmission attenuation estimates were made over a frequency range of 1.7 to 6.3 MHz in both normal bovine liver and thermally coagulated liver. For a single attenuation slope estimate corresponding to a single bovine liver sample, the attenuation coefficient was estimated in 0.1 MHz intervals over frequency ranges of 1.7 MHz to 2.9 MHz, 2.8 MHz to 4.2 MHz, and 4.0 to 6.3 MHz for the three transducers. Overlapping measurements were averaged. Estimates of attenuation slope of *ex vivo* bovine liver across

the five samples were 0.62 ± 0.06 dB/cm MHz and for thermally coagulated liver were 1.25 ± 0.07 dB/cm MHz. The error terms in the attenuation slope estimates indicate a single sample standard deviation calculated using five attenuation slope estimates.

Sound speed measurements were recorded with the same three transducers used to make attenuation measurements, but only at each transducer's nominal center frequency. A sound speed of 1598 ± 7 m/s was found in untreated liver tissue and a sound speed of 1592 ± 6 m/s was found in thermally coagulated tissue. The error terms represent a single sample standard deviation for the 15 measurements for either unheated liver or coagulated tissue (3 frequencies by 5 samples). In reporting the measurements, we are assuming that dispersion within the samples is negligible.

All imaging was performed with the VFX 9L4 transducer on the Siemens S2000 for reference phantom attenuation estimation. Tissue was imaged in saline solution at room temperature following degassing in a low pressure vacuum (25 mm Hg) and with an offset between tissue and transducer. The offset consisted of a plastic bag containing a mixture of ethylene glycol and water which matched the tissue sound speed (1590 m/s for the thermally coagulated liver and 1600 m/s for the unheated liver). The focal depth was set between 3 and 5 cm depending on the amount of fluid offset between tissue and transducer. Prior to any imaging, tissue in saline solution was degassed in a vacuum chamber. RF data was recorded while imaging a large number of independent scanning planes in all samples. For the normal liver this was 391, 316, 311, 333 and 296 imaging planes (1,647 total). For the thermally coagulated liver this was 250, 286, 317, 330, and 315 imaging planes (1,498 total).

Reference phantom data was recorded for both thermally coagulated liver tissue and untreated normal liver tissue. We utilized a beamformer sound speed of 1590 m/s for scanning and acquiring RF data recorded within thermally coagulated tissue and a sound speed of 1600 m/s when scanning untreated normal tissue. We assumed a linear attenuation coefficient of 0.58 dB/cm MHz in the TM reference phantom for computing attenuation estimates in untreated normal liver tissue and thermally coagulated liver tissue.

When collecting reference data, we utilized the same beamformer sound speed as was assumed in the tissue samples. So, a sound speed of 1600 m/s was used when collecting reference RF data from the TM phantom for analyzing untreated liver tissue and a sound speed of 1590 m/s for analyzing thermally ablated bovine liver tissue. While this leads to sound speed errors of 10 or 20 m/s relative to the reference phantom sound speed of 1580 m/s, we note that larger sound speed errors of 40 m/s have been examined and found to lead to biases in attenuation estimation of only approximately 0.1 dB/cm MHz (Nam et al., 2011a; Omari et al., 2011). For each set of reference data, 15 images were collected from independent imaging planes (separated by 2 mm or more) and power spectra were computed by averaging over all A-lines within an image and all 15 imaging planes. Thomson's multi taper method with a W of 1.0 MHz and a short-time gate length of 4 mm was used for all power spectrum calculations.

Results

Envelope SNR in numerical phantoms as a function of SND

Figure 2 presents plots of the envelope SNR as a function of SND for the more highly attenuating simulated signals at both center frequencies. Envelope SNR was computed for a 4 mm and a 12 mm gate length. We note that the longer gate length gives lower overall values for the envelope SNR. In addition, the SNR parameter estimated with a longer gate length is less sensitive to variations in SND when the SND is between 2 and 10. This is because when using longer gate lengths, the signal decays due to attenuation, introducing an additional component to the signal variance and making envelope SNR less dependent on SND. So, a short gate length performs better when using envelope SNR to distinguish between tissue with different SND. We estimated envelope SNR for both 3.5 MHz center frequencies and 5.5 MHz center frequencies. We find that envelope SNR is lower when examining 5.5 MHz signals than 3.5 MHz simulated signals. We show signal envelopes for several different values of SND in highly attenuating phantoms in Figure 3. As shown in the plots of envelope SNR, the signals created with 12 scatterers per mm^3 look similar to the signals created with 32 scatterers per mm^3 . However, the envelope variance shows a clear increase as the number of scatterers drops to 2 scatterers per mm^3 . In figure 3 (C), we see that an SND this low leaves the envelope signal with dead spots where the envelope is near zero, over the 10 mm signal length examined.

From figure 2, we note that the envelope SNR depends on the simulated center frequency in addition to the SND. This is because the envelope SNR is a function of the number of scatterers per resolution cell, which is a property of both the object being imaged and the imaging device. For the higher center frequency the resolution cell occupies a smaller volume than for the lower transmit frequency, so the number of scatterers per resolution cell changes as we alter the center frequency. We estimated the volume of a resolution cell in our simulations. We assumed that our ultrasound beam could be approximated by a short ellipsoidal cylinder over a small volume. To estimate the axial width of a resolution cell, we computed the correlation coefficient between a 4 mm segment of RF data and adjacent data using low attenuation slope, high SND phantoms (0.5 dB/cm MHz, 128 scatterers/ mm^3). We estimated the width of a resolution cell in the axial direction by the axial shift of the 4 mm data segment which reduced the normalized correlation coefficient below 0.2 when averaging over 100 A-lines. We estimated the lateral resolution by creating 10 numerical phantoms, translating the scatterers in 0.1 mm increments, and measuring the resulting normalized correlation coefficient between 4 mm segments. We estimated the elevational resolution in a similar manner, only shifting the scatterers in the elevational direction. Over a depth of 25 to 35 mm we found average resolution cell volume of $1.03 \pm 0.25 \text{ mm}^3$ for the 3.5 MHz transducer and average resolution cell volume of $0.41 \pm 0.09 \text{ mm}^3$ for the 5.5 MHz transducer. It is this difference in resolution cell volume which is responsible for the differences in envelope SNR observed in figure 2.

Attenuation estimates in numerical phantoms

For all attenuation estimates presented on numerical data, we utilized all 1,000 independent A-lines computed for the 0.5 dB/cm MHz phantom containing 128 scatterers per mm^3 to

form the reference power spectrum for a given center frequency. We performed all attenuation estimates in these simulations starting at a depth of 25 mm and using a 4 mm short-time window and a smoothing bandwidth, W , equal to 1.0 MHz. We estimate attenuation slope over the frequency range of 3.5 to 7.0 MHz for the 5.5 MHz center frequency and over the frequency range of 2.0 to 5.0 MHz for the 3.5 MHz center frequency. We first fixed the total data length at 33 wavelengths, which was 15.1 mm at a center frequency of 3.5 MHz and 9.6 mm at a center frequency of 5.5 MHz. The number of independent A-lines in each sample power spectrum was also fixed to 10 for each attenuation estimate.

We found that our attenuation results did not depend strongly on the W parameter in the multi-taper method or on the length of the short-time gate used. To be more precise, we present results when estimating attenuation slope in numerical phantoms with a scatterer number density of 4 scatterers/mm³ and an attenuation slope of 1.0 dB/cm MHz at a center frequency of 5.5 MHz. In this situation, with W fixed at 1.0 MHz, the attenuation slope estimate was 0.99 ± 0.22 dB/cm MHz, 0.97 ± 0.22 dB/cm MHz, and 0.95 ± 0.20 dB/cm MHz as the short time gate was varied between 3, 4, and 5 mm with the total parameter estimation region size kept the same. Within the same numerical phantoms, with the short time gate fixed at 4 mm, we found attenuation slope estimates of 0.90 ± 0.20 dB/cm MHz, 0.97 ± 0.22 dB/cm MHz, and 0.91 ± 0.19 dB/cm MHz as W was varied between 0.5, 1.0, and 1.5 MHz ($NW = 2, 4$, and 7). With this in mind, we perform all further calculations with the short time gate length fixed at 4 mm and W fixed at 1.0 MHz.

We plot the result of estimating attenuation in Figures 4 and 5 as the SND and thereby the envelope statistics were varied. Figure 4 and 5 (a) plot the mean values of the attenuation slope estimates, while Figures 4 and 5 (b) plot the variance of the attenuation slope estimates as a function of SND. Figure 4 and 5 (c) shows the mean attenuation slope estimates for a narrower range of SND and figure 4 and 5 (d) shows the variance in the attenuation slope estimates for the same narrow range of SND. These figures show that at an axial kernel length corresponding to 33 wavelengths and a lateral kernel width corresponding to 10 independent A-lines the attenuation estimate varied by a small amount until the envelope SNR is significantly altered from 1.91. From the previous section, this begins to occur when the SND begins to drop below 10 scatterers per mm³.

At a center frequency of 3.5 MHz, between an SND of 10 and 128 scatterers per mm³, the mean attenuation slope estimates were found to vary only between 0.49 and 0.51 dB/cm MHz in the less attenuating numerical phantoms and between 0.96 and 0.97 dB/cm MHz in the highly attenuating phantoms. The corresponding standard deviations were found to vary between 0.08 and 0.11 dB/cm MHz in both sets of phantoms. At a center frequency of 5.5 MHz, between an SND of 10 and 128 scatterers per mm³, the mean attenuation slope estimates were found to vary only between 0.47 and 0.51 dB/cm MHz in the less attenuating numerical phantoms and between 0.91 and 0.98 dB/cm MHz in the highly attenuating phantoms. The corresponding standard deviations were found to vary between 0.10 and 0.19 dB/cm MHz in the less attenuating phantoms and between 0.10 and 0.15 in the more attenuating phantoms.

The effect that decreasing SND below 10 scatterers/mm³ had on the attenuation estimates is interesting. The bias of the attenuation estimates shows no obvious trend with decreasing SND. In the highly attenuating phantoms the mean attenuation slope estimate remained within 16% of the true value of attenuation. In the less attenuating phantoms, the mean attenuation estimate remained within 20% of the true value of the attenuation slope, except for one outlying point. The figures demonstrate that a decreasing SND leads to a small underestimation of the attenuation slope. A precise relationship between SND and estimator bias is difficult to determine from our simulations though, as the bias rapidly oscillates when the scatterer number density is small. There is also some uncertainty in the bias in the attenuation estimate due to only using a small number (N=100) of realizations of independent attenuation slope estimates, which we have not characterized.

As seen in figures 4 and 5, we observed only a slight increase in the standard deviation of the attenuation slope estimates between 2 to 10 scatterers per mm³. Specifically, at a 3.5 MHz center frequency, the standard deviation remained between 0.11 and 0.13 dB/cm MHz in the less attenuating phantoms and between 0.10 and 0.14 dB/cm MHz in the more attenuating phantoms. At a 5.5 MHz center frequency, the standard deviation in the attenuation slope estimates increased from 0.19 to 0.29 dB/cm MHz in the less attenuating phantoms and from 0.15 to 0.29 dB/cm MHz in the more attenuating phantoms. As the SND drops below 2 scatterers per mm³, the estimation variance shows a dramatic increase, regardless of whether the sample phantoms have an attenuation coefficient of 0.5 or 1.0 dB/cm MHz and whether the center frequency is 3.5 or 5.5 MHz. Specifically, at a 3.5 MHz center frequency, the standard deviation increased from 0.14 dB/cm MHz to 0.37 dB/cm MHz in the less attenuating phantoms and from 0.14 dB/cm MHz to 0.40 dB/cm MHz in the more attenuating phantoms. At a 5.5 MHz center frequency, the standard deviation in the attenuation slope estimates increased from 0.28 to 1.03 dB/cm MHz in the less attenuating phantoms and from 0.29 to 1.10 dB/cm MHz in the more attenuating phantoms.

Note that these results indicate that we do not need fully developed speckle in order to obtain a relatively small variance in the attenuation estimate. At an SND as low as 4 scatterers per mm³, corresponding to an envelope SNR of approximately 1.3 at a center frequency of 5.5 MHz (according to figure 1), the standard deviation in the attenuation estimates is still near 0.2 dB/cm MHz for the highly attenuating phantoms. Figures 4 and 5 don't show rapid increases in the variance until the SND drops below 2 scatterers per mm³, which corresponds to an envelope SNR of approximately 1.2 at a center frequency of 5.5 MHz.

From figures 4 (b) and 5(b), we also note that the variance in the attenuation estimates is roughly comparable when examining fully developed speckle and matching the kernel dimensions of the attenuation estimator in units of wavelengths. The performance of attenuation estimation at different center frequencies diverges as the SND begins to drop and the envelope SNR falls. At low SND, the lower center frequency shows a smaller estimation variance than the higher center frequency for kernels matched axially in wavelengths. This is because of the larger resolution cell exhibited by the lower center frequency simulation. The lower center frequency simulation contains more scatterers per resolution cell and has a higher envelope SNR for the same SND.

We note that the results in figures 4 and 5 are dependent on the spatial resolution of the attenuation estimate that we are attempting to achieve. We next examine two low SND cases and vary the axial kernel length used to obtain the estimate. Figures 6 and 7 (A) and (B) present the mean and variance in the attenuation estimates as a function of the axial kernel length when the SND is fixed at 0.6 and 0.3 scatterers per mm^3 . From this figure, we see that a low estimation variance may still be achieved even if the SND becomes extremely low as long as resolution is sacrificed. We note that the maximum variation in the mean attenuation estimates among both low and highly attenuating phantoms as the axial kernel length is doubled over both center frequencies is 0.09 dB/cm MHz. However, the variance in the estimate shows a large decrease, especially when the SND is 0.3 scatterers per mm^3 and the transducer center frequency is high. At the 5.5 MHz center frequency and an SND of 0.3 scatterers per mm^3 , the standard deviation in the attenuation estimates decreases from 0.72 to 0.27 dB/cm MHz in the highly attenuating numerical phantoms as the axial kernel length is doubled. From all these simulations, we conclude that the SND is an important parameter in attenuation estimation. When SND becomes extremely low either estimator variance will significantly increase or estimator resolution must significantly decrease to counter the increased variance.

Reference phantom attenuation estimates in TM phantoms

For the purposes of the analysis in this section we assume sound speeds of 1540 m/s in TM phantoms A through E. We utilize this sound speed simply because it is commonly assumed by most commercial ultrasound systems, and the discrepancy with the measured sound speed is quite small. We reiterate that a much larger sound speed of 40 m/s is expected to cause an attenuation estimation bias of only 0.1 dB/cm MHz (Nam et al., 2011a; Omari et al., 2011). Estimates of envelope SNR within each phantom are displayed in Table 2. Envelope SNR estimates were made with 4 mm kernels axially, and were averaged over a 16 mm by 15 mm ROI in the center of each imaging plane. We note that our own results show an increase in envelope SNR with increasing glass bead number density. However, the effective SND is not necessarily the same as the estimated number density of glass beads even if our beads have a symmetric Gaussian distribution. This is because scattering is frequency dependent and some sizes will contribute to the signal more than others for a transducer with any given center frequency and bandwidth (Chen et al., 1994).

We performed attenuation estimation as described in the previous section. A linear attenuation coefficient of 0.56 dB/cm MHz was measured in the reference phantom. Power spectrum estimates were obtained using Thomson's multi-taper estimator with a short-time gate length of 4 mm and a smoothing bandwidth, W , equal to 1.0 MHz. We show results with a fixed axial kernel length and lateral kernel length in table 2. As in the previous section, the bias in the attenuation estimate is relatively independent of the envelope SNR. So, a low SND does not result in biased attenuation estimates. A low SND, however, does result in an increase in the variance of the attenuation estimates. We also estimate attenuation with variable gate lengths and display the results in figure 8. As in the simulations, a low SND is not an impediment to achieving an unbiased attenuation estimate and either a small estimation variance or a high spatial resolution. However, a low SND is an impediment to achieving a high spatial resolution and a low estimation variance

simultaneously. This is demonstrated in figure 8 where the bias in the attenuation estimate changes relatively insignificantly as the axial kernel width is varied, but the variance in the attenuation estimate increases rapidly as the axial kernel width is decreased. This is particularly true of the TM phantom with the low SND denoted by TM A. We also note that the differences in the attenuation estimation variance between different SND phantoms become most pronounced as the axial kernel length is reduced down to only 6 mm.

Reference phantom attenuation estimates in ex vivo bovine liver

We estimated the envelope SNR in thermally coagulated liver and untreated normal *ex vivo* bovine liver. We found a mean envelope SNR of 1.28 in thermally coagulated tissue, and a lower mean envelope SNR of 1.10 in untreated normal *ex vivo* bovine liver. For the envelope SNR estimates, one 16 mm by 15 mm ROI from each image was subdivided into non-overlapping 4 mm axial sub-regions and envelope SNR within a single ROI was the average of envelope SNR over the sub-regions. The simulations in combination with Table 3 and 4 inform us that *ex vivo* liver tissue exhibits envelope statistics on the threshold of the low SND regime described in the simulated data. With this in mind, we first examine the estimation variance estimated over all liver samples as a function of gate length in both coagulated and untreated liver in figure 9. A single attenuation estimate was made in each imaging plane around the focus depth of the transducer. Each attenuation estimation region was laterally centered in the image.

As in the simulations and TM phantom results, the estimation variance is a strong function of the parameter estimation region size, and the estimation bias varied much less than the estimation variance. This can be seen by comparing between figures 9A and 9B. Over the range of parameter estimation region sizes examined we found that the mean attenuation estimate in the liver varied only between 0.43 and 0.51 dB/cm MHz while the standard deviation in the attenuation estimate decreased from 1.80 to 0.52 dB/cm MHz. Meanwhile, the mean attenuation estimate in the thermally coagulated liver varied only between 0.96 and 1.12 dB/cm MHz while the standard deviation in the attenuation estimate in the thermally coagulated liver decreased from 1.65 to 0.50 dB/cm MHz. The large decrease in estimation variance with increasing axial kernel length suggests that a very small variance in the attenuation estimate may be achievable in the liver if larger sections of tissue were imaged, degrading the spatial resolution.

We display results of attenuation estimation with a large, fixed kernel within each of the individual livers in figure 10 and tables 3 and 4. Even at a large axial kernel length of 16 mm, the standard deviation in the estimates for attenuation in thermally coagulated tissue was approximately 50% of the mean value. This value was even higher in the untreated normal tissue where the coefficient of variation was approximately 100% at a large gate length. The lower performance in untreated normal liver could be expected since the envelope SNR was lowest prior to thermal coagulation.

A natural question to ask is whether the variance is due to biological variability or estimator variance. To answer this question, we keep the spatial resolution of the attenuation estimator fixed at an axial kernel length of 16 mm and examine statistical differences between the attenuation estimates across each of the livers from distinct animals. To be more precise, we

make the assumption that the attenuation estimates are normally distributed random variables and determine whether each group of estimates is likely to have come from distributions with the same mean and variance. If there is biological variability among the samples, then we would expect the distributions of attenuation estimates within each liver to show statistically significant differences.

We first performed Bartlett's test to determine whether statistically significant differences exist between the variance of the attenuation estimates within each of the five heated or unheated livers (Snedecor and Cochran, 1989). Bartlett's test yielded p -values of 0.9113 and 0.8397 within the unheated and heated livers, respectively. These p -values are extremely high, indicating that the attenuation estimates within each unheated or heated liver likely come from distributions with the same variance. We employed one-way ANOVA to determine whether or not all attenuation slope estimates came from distributions with the same means (Snedecor and Cochran, 1989). Within the unheated liver, we obtained a p -value of 0.684. Within the heated liver, we obtained a p -value of 6.29×10^{-8} .

These statistics indicate that at a fixed spatial resolution, the attenuation estimator variance is the same from one animal to the next. The statistics also indicate that the average attenuation measurement is roughly the same from one animal to the next prior to heating. So, biological variability is likely a small source of the variance in the attenuation estimates in tissue compared to the variance within a single animal due to low SND. The reason for the statistically significant differences in means following tissue heating is likely due to inconsistencies in sample volumes. There was some variability in the sections of the liver that were heated at one time so smaller pieces would have received a larger thermal dose than larger sections over the same heating time.

We also determine whether statistically significant differences exist between the results of the through transmission measurements and the results of the reference phantom measurements. We compared all reference phantom attenuation estimates at a fixed axial kernel length of 16 mm with all five through transmission measurements for heated and unheated liver. Welch's two-sample t -test (Snedecor and Cochran, 1989) indicated statistically significant differences between through transmission measurements and reference phantom measurements with p -values of 0.0002 in the cases of both heated and unheated liver.

Based on the results presented in this section, we remark that the error bars are large enough so as to obtain negative values for the attenuation coefficient. While a negative attenuation value is not physically possible, there is no constraint in the reference phantom algorithm that precludes it from obtaining such a result. We show a histogram of all the attenuation estimates in figure 11. Observe that the estimates take on an approximately Gaussian, symmetric appearance. Negative attenuation values are then just a logical consequence of the estimation variance being extremely high.

Discussion

As reported by previous authors, we found that the potential attenuation contrast between treated (coagulated) liver tissue and untreated liver tissue is high (Bush et al., 1993; Damianou et al., 1997; Gertner et al., 1997; Kemmerer and Oelze, 2012; Parmar and Kolios, 2006; Techavipoo et al., 2004; Worthington and Sherar, 2001). Using a linear model for the attenuation coefficient, we found an average attenuation coefficient of approximately 0.5 dB/cm MHz in *ex vivo* bovine liver prior to thermal coagulation and 1.0 dB/cm MHz following thermal coagulation. These results are slightly lower than the attenuation coefficients we reported from through transmission measurements (0.62 and 1.25 dB/cm MHz). We believe the through transmission measurements are over-estimates. Over-estimation occurred because it was difficult to slice tissue samples to perfectly flat cylindrical shapes the same size as their container. This led to a small gap between the saran cover of the sample holder and the tissue sample, additional reflection losses, and overestimation of the attenuation coefficient.

In this article, we demonstrated the difficulty in obtaining attenuation images in the liver with low variance and high spatial resolutions. This explains the large number of articles in the literature addressing the attenuation contrast between thermally coagulated liver and untreated liver (Bush et al., 1993; Gertner et al., 1997; Kemmerer and Oelze, 2012; Parmar and Kolios, 2006; Techavipoo et al., 2004), the large number of articles showing reference phantom based attenuation imaging results (Kim and Varghese, 2008, 2007; Kim et al., 2008; Kim, H. and Heo, S.W., 2012; Labyed and Bigelow, 2011; Nam et al., 2011a; Omari et al., 2011, 2013), but the limited results demonstrating attenuation images of thermal coagulations.

Imaging in an *ex vivo* setting is one of the first steps in validating algorithms to be applied to clinical abdominal ultrasound setting. In an *ex vivo* setting the confounding effects on the imaging of fat and muscle layers intervening between the liver and the transducer may be avoided. However, we found that the scattering we observed in the liver deviates significantly from the scattering model that has been frequently assumed when developing reference phantom and other algorithms for attenuation estimation. We hypothesize that the sparse scattering responsible for the low envelope SNR arises from the portal triads while hepatocytes provide the underlying low level scattering. This sort of scattering model is frequently employed when analyzing periodic scattering and MSS in the liver (Fellingham and Sommer, 1984; Machado et al., 2006; Rubert and Varghese, 2013, In Press; Varghese and Donohue, 1993).

The estimation variance for attenuation slope estimation is highly dependent on the envelope SNR. While our own results showed a low envelope SNR in an *ex vivo* setting, we note that the effect of excising liver from an animal on the envelope statistics of a liver has not been investigated. We noted that small differences in envelope SNR had a large effect on the attenuation estimation variance once the SNR began to significantly deviate from 1.91. *Ex vivo* porcine (Bush et al., 1993; Zhou et al., 2013), bovine (Bevan and Sherar, 2001a, 2001b; Dewall et al., 2010; Gertner et al., 1997; Luchies et al., 2012, 2012; Parker, 1983; Pereira and Maciel, 2001), rat (Kemmerer and Oelze, 2012), and human (Machado et al., 2006) liver

have all been used for the development of ultrasonic tissue characterization algorithms based on features extracted from RF data. However, we are not aware of any studies comparing estimates of envelope statistics in excised liver with estimates of envelope statistics in an *in vivo* setting. When comparing *ex vivo* and *in vivo* measurements of envelope SNR there are two effects to consider: the changing size of a resolution cell dependent on the propagation path and the immediate drop in blood pressure and loss of perfusion that occurs when a liver is excised.

In order to match the resolution cell size, comparisons could be made between RF data recorded with an animal under open surgery conditions and an excised liver using the same transducer and imaging settings at the same depth. An open surgery model would avoid the broadening of a resolution cell that occurs from phase aberration within layers of skin, fat, and abdominal muscle that are encountered when imaging the liver *in vivo*. With identical resolution cell volumes, the envelope SNR would reflect only differences in the state of tissue. The effect of the pathological state of tissue and the species under consideration also are likely to have an effect on envelope statistics. Envelope SNR or some other metric of scatterer number density must be well characterized in tissue in order to develop bounds on reference phantom based attenuation estimation variance.

Conclusion

The results in this article show that for tissue exhibiting a Rayleigh distributed envelope distribution a low-variance, high resolution attenuation image may be generated using the reference phantom method developed by Yao et al. (1990) in conjunction with a Multi-taper spectral estimate. Attenuation imaging holds great promise for delineating the extent of thermally coagulated tissue during thermal therapy in the liver. Using both through transmission measurements and reference phantom attenuation estimates we found that the attenuation slope approximately doubles following delivery of a large thermal dose to liver tissue. However, across a large number of ROI's (N= 1,647) in 5 *ex vivo* bovine livers we found an envelope SNR of 1.1. We found that the large variance in the envelope magnitude due to scattering results in a large variance in reference phantom attenuation estimates and is a currently an obstacle to the use of reference phantom based attenuation imaging for delineating the extent of thermally coagulated regions.

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Figure 1.

B-mode images of TM phantom labeled (A), (B), (C), and (D). Images recorded with 9L4 transducer on Siemens S2000 with a focal depth of 3.0 cm. Image (A) exhibits the lowest envelope SNR, and Image (B) exhibits the highest envelope SNR.

Figure 2.

(A) Envelope SNR versus SND calculated with a 4 mm and 12 mm gate starting from a depth of 30 mm in highly attenuating phantoms imaged at 3.5 MHz and 5.5 MHz center frequency. (B) Envelope SNR versus SND over a narrower range of SND

Figure 3. Simulated signal envelope from 1.0 dB/cm MHz phantoms imaged at a center frequency of 5.5 MHz. Depth of 30 mm to 40 mm. Scatterer number density of (A) 32, (B) 12, (C) 2, and (D) 0.5 per mm³

Figure 4.

(A) Mean and (B) variance of attenuation estimate in 1.0 dB/cm MHz phantoms as the SND is varied. For all estimates an axial kernel size of 33 wavelengths and 10 independent A-lines were used. (C),(D) show the same estimates as in (A),(B) but over a narrower range of SND. 33 wavelengths corresponds to 9.6 mm at 5.5 MHz and 15.1 mm at 3.5 MHz.

Figure 5.

(A) Mean and (B) variance in attenuation estimate in 0.5 dB/cm MHz phantoms as the SND is varied. For all estimates an axial kernel length of 33 wavelengths and 10 independent A-lines were used. (C),(D) show the same estimates as in (A),(B) but over a narrower range of SND. 33 wavelengths corresponds to 9.6 mm at 5.5 MHz and 15.1 mm at 3.5 MHz.

Figure 6.

(A) Mean and (B) variance of attenuation estimates in 1.0 dB/cm MHz phantoms as the axial kernel length is varied and the number of A-lines in the estimate is fixed at 10 when the SND is 0.6 scatterers per mm^3 . (C) Mean and (D) variance of attenuation estimates in 1.0 dB/cm MHz phantoms as the axial kernel length is varied and the number of A-lines in the estimate is fixed at 10 when the SND is 0.1 scatterers per mm^3 .

Figure 7.

(A) Mean and (B) variance of attenuation estimates in 0.5 dB/cm MHz phantoms as the axial kernel length is varied and the number of A-lines in the estimate is fixed at 10 when the SND is 0.6 scatterers per mm^3 (C) Mean and (D) variance of attenuation estimates in 0.5 dB/cm MHz phantoms as the axial kernel length is varied and the number of A-lines in the estimate is fixed at 10 when the SND is 0.3 scatterers per mm^3 .

Figure 8.

■ TM A
 ■ TM B
 ■ TM C
 ■ TM D

Attenuation slope estimates as a function of axial kernel length with the lateral kernel width fixed at 15.0 mm. (A) Mean estimate vs. axial kernel length in TM phantoms. (B) Standard deviation of estimates as a function of axial kernel length. Images recorded with 9L4 transducer on Siemens S2000 with a focal depth of 3.0 cm.

Figure 9. (A) Mean and (B) standard deviation of attenuation slope estimates within all untreated normal liver and all thermally coagulated liver as a function of gate length with the lateral extent of the kernel fixed at 15 mm. In the legend, U.L. indicates unheated liver and T.C. indicates thermally coagulated liver.

Figure 10.

Bar plot showing mean and variance of attenuation estimates in individual (A) *ex vivo* untreated normal bovine livers (B) and in individual thermally coagulated livers for a large kernel size (16 mm axially by 15 mm laterally). Error bars correspond to one standard deviation.

Figure 11.

Histogram showing approximately symmetric distributions of attenuation estimates in all untreated normal livers and all thermally coagulated livers. The kernel size was also 16 mm axially by 15 mm laterally.

Table 1

TM Phantom scatterer distributions. The glass bead number density was estimated by assuming each bead in the distribution had a diameter equal to the median of the diameter distribution (137.5 microns, 82.5 microns, and 27.0 microns).

TM Phantom	Glass Bead Diameters (microns)	Glass Bead Concentration (g/liter)	Estimated number density of glass beads (beads/mm ³)
A	125 to 150	0.643	0.25
B	75 to 90	0.278	0.50
C	125 to 150	4	1.55
D	75 to 90	4	7.20
E (TM phantoms reference)	4 to 50	4	355
F (<i>Ex vivo</i> liver reference)	4 to 50	4	355

Table 2

TM Phantom envelope SNR and attenuation slope estimates with a fixed kernel size (16 mm axially by 15 mm laterally). The mean and a single sample standard deviation are given.

TM Phantom	Envelope SNR	Through transmission attenuation slope (dB/cm MHz)	Attenuation Slope RPM Estimate (dB/cm MHz)	Attenuation Slope Coefficient of Variation
A	1.01 ± 0.05	0.49 ± 0.02	0.50 ± 0.25	50%
B	1.23 ± 0.03	0.49 ± 0.02	0.50 ± 0.10	20%
C	1.56 ± 0.03	0.68 ± 0.10	0.68 ± 0.08	11.7%
D	1.78 ± 0.02	0.61 ± 0.05	0.60 ± 0.07	11.7%
E (TM phantoms reference)	1.87 ± 0.02	0.57 ± 0.03	-	-
F (<i>Ex vivo</i> liver reference)	1.79 ± 0.02	0.57 ± 0.02	-	-

Table 3

Envelope SNR and attenuation slope estimates in individual untreated normal bovine livers and all untreated normal bovine liver for a large kernel size (16 mm axially by 15 mm laterally). The mean and a single sample standard deviation are given.

Sample Number	Envelope SNR	Attenuation Slope Estimate (dB/cm MHz)	Attenuation Slope Coefficient of Variation
1	1.07 ± 0.12	0.47 ± 0.53	113.0%
2	1.12 ± 0.12	0.43 ± 0.51	118.8%
3	1.04 ± 0.09	0.53 ± 0.49	94.0%
4	1.06 ± 0.10	0.47 ± 0.55	116.3%
5	1.19 ± 0.10	0.63 ± 0.44	70.0%
All Samples	1.10 ± 0.12	0.50 ± 0.51	102.1%

Table 4

Envelope SNR and attenuation slope estimates in individual heated bovine livers and all heated bovine livers for a large kernel size (16 mm axially by 15 mm laterally). The mean and a single sample standard deviation are given.

Sample Number	Envelope SNR	Attenuation Slope Estimate (dB/cm MHz)	Attenuation Slope Coefficient of Variation
1	1.35 ± 0.11	0.76 ± 0.52	68.9%
2	1.36 ± 0.13	1.02 ± 0.55	54.4%
3	1.24 ± 0.09	1.20 ± 0.39	32.5%
4	1.21 ± 0.13	0.84 ± 0.44	52.7%
5	1.25 ± 0.10	1.00 ± 0.45	44.5%
All Samples	1.28 ± 0.13	0.97 ± 0.49	50.1%