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Quantifying Backscatter Anisotropy Using the Reference Phantom Method

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Abstract

Acoustic properties can be exploited to infer and evaluate tissue microstructure. However, common assumptions are that the medium of interest is homogeneous and isotropic, and that its underlying physical properties cause diffuse scattering. In this paper, we describe how we developed and tested novel parameters designed to address isotropy/anisotropy in backscattered echo signal power in complex biological tissues. Specifically, we explored isotropy/anisotropy in backscattered power in isotropic phantoms (spherical glass beads), an anisotropic phantom (dialysis phantom with rod-like fibers), and an *in vivo* human tissue with well-described anisotropy (bicep muscle). Our approach uses the Reference Phantom Method to compensate for system-transfer and diffraction losses when electronically beamsteering a linear array transducer. We define three parameters to quantify the presence and orientation of anisotropic scatterers, as well as address magnitude of anisotropy. We found that these parameters can detect and sense the degree of anisotropy in backscatter in both phantoms and bicep muscle. Bias of the summary anisotropy parameters, induced through a speed of sound mismatch of sample media and reference phantom, was less than 0.2 dB if the speed of sound was within ± 20 m/s of the sample media. In summary, these new parameters may be useful for testing the assumption of isotropy as well as providing more detailed information about the underlying microstructural sources of backscatter in complex biological tissues.

I. Introduction

Common assumptions in quantitative ultrasound (QUS) approaches for describing tissue microstructure are that the medium of interest is homogeneous and isotropic, and that its underlying physical properties cause diffuse scattering.^{1;2} However, some biological tissues clearly show anisotropy in acoustic properties (backscatter and attenuation coefficients).^{3–9} We previously reported a simple metric of backscattered echo signal power anisotropy, the *excess backscattered power loss* (eBSPL).¹⁰ While this method was sensitive to tissue anisotropy in general, summarizing both backscatter and attenuation coefficient anisotropy, it did not attempt to quantify orientation or magnitude of structural alignment. We sought to refine our previous approach by developing novel summary parameters that illustrate the relative contributions of the backscatter and attenuation coefficients to final anisotropy-related metrics.

Most previous research of backscatter coefficient and attenuation anisotropy relied upon the use of single element transducers that interrogated tissue while either the tissue or transducer were independently rotated.^{3–9} The use of single element transducers allows for accurate measurements of attenuation, ultrasonic velocity, and the backscatter coefficient. Independent rotation of the tissue or transducer allows for accurate measurement of angle-dependent QUS behavior. Single element transducers are, these days, mostly relegated to the laboratory as the majority of clinical scanners use multi-element array transducers. The ability to detect and quantify anisotropy using clinical array transducers is therefore desirable. It is currently possible to directly measure the anisotropy of the backscatter coefficient and attenuation using the reference phantom method assuming 1) that the ultrasound's propagation path to the region of interest in the sample media is sufficiently homogeneous and 2) the scattering arising from the tissue is incoherent.¹¹ Inhomogeneous media creates a bias in both attenuation and the backscatter coefficient¹² and is routinely encountered in practice. Coherent scattering, which is a possibility if there is spatial periodicity in the locations of scattering sources, will confound the interpretation of the backscatter coefficient.^{13–15} We aimed to create a simple measure of scattering anisotropy that remained robust in instances where the media may be inhomogeneous or coherently scattering.

Angle dependence of the backscatter coefficient and attenuation is reported in skeletal^{3,4} and cardiac muscle,^{5–8} as well as the renal cortex.⁹ Regardless of the parameter used to describe it, backscatter in soft tissues tends to be highest when the acoustic beam is perpendicular (normal) to the aligned structure and lowest when the acoustic beam is parallel to the aligned structure. For instance, Mottley and Miller reported an integrated backscatter that was $4\times$ (6 dB) higher for parallel compared to normal incidence in fresh canine myocardium,⁶ and Topp and O'Brien report a backscatter coefficient of nearly $10\times$ (10 dB) greater for normal compared to 45° incidence in rat skeletal muscle.⁴ Similarly, comparing normal to parallel incidence in fixed human myocardium, Wickline *et al.* found integrated backscatter to be $28\times$ (14.5 dB) higher,⁸ and Hall *et al.* found the power-law frequency dependence of the backscatter coefficient to be $3\times$ higher (1.8 vs 0.4).⁷ Consistent with the above observations, acoustic attenuation in fresh muscle is highest when the acoustic beam is parallel to the aligned structure and lowest when the acoustic beam is perpendicular (normal) to the aligned structure. For example, attenuation was nearly $3\times$ higher in bovine skeletal muscle,³ and $2.2\times$ higher in canine myocardium,⁵ at parallel compared to normal incidence, as well as $2\times$ higher at 45° compared to normal incidence in rat skeletal muscle.⁴ These trends in backscatter and attenuation angle dependence persist in other anisotropic tissues, such as tendon,¹⁶ trabecular bone¹⁷ and renal cortex.⁹

Based on the Reference Phantom Method,¹¹ an approach that accounts for system effects to provide system-independent estimates of backscatter and attenuation coefficients,^{18–20} we developed a simple normalization method that allows measurement of the angle of normal incidence on an aligned structure via linear array beam steering. We derived the asymmetric backscattered power difference (*aBSPD*) parameter to assess for potential underlying aligned scattering structures (not parallel to the transducer face) in the medium of interest through asymmetry in power loss from negative to positive steering angles, and demonstrated that this parameter differentiates anisotropic from isotropic scattering media.

We next defined the average power loss caused by anisotropy in the scattering medium (mean backscattered power difference, *mBSPD*) and demonstrated that it becomes higher as the scattering medium exhibits greater acoustic anisotropy. After confirming measurement reliability in phantoms, including measurement of the bias induced by through speed of sound mismatch, we tested the parameters *in vivo* in a well-described human tissue (bicep muscle).

In summary, we found that our novel parameters identify anisotropy in backscatter, characterize the angle dependence of backscatter, and address the magnitude of anisotropy. They may thus be useful to quantify the presence, orientation, and magnitude of scattering anisotropy in biological tissues with complex microstructure.

II. Methods

The following sections present derivations of parameters that quantify the orientation and magnitude of the changes in backscatter echo signal power with the angle of interrogation. (Table I summarizes terms.)

A. Parameter Derivation

Isotropy (and anisotropy) in backscattered echo signal power can be ascertained by electronically steering the acoustic beam away from the normal angle (w.r.t. the transducer face). By definition, isotropic materials should have equivalent backscattered echo signal power regardless of beam interrogation angle. However, under conditions of diffuse scattering, beam steering with array transducers induces a loss in backscattered echo signal power because of two system-dependent and angle-dependent mechanisms. The first loss is due to the decreasing effective aperture with the cosine of the beam steering angle. The second loss is due to a decreasing normal component of force exerted on the transducer face as the beam steering angle is increased. Both of these are symmetric about normal incidence (assuming a flat linear array transducer, which allows all beams to be equivalent and steered at the same angle). The Reference Phantom Method¹¹ can be used to account for these system-dependent losses and ensure that the (normalized) backscattered echo signal power is equivalent among all angles in isotropically scattering media.

1) Backscattered Power Difference (BSPD)—This derivation is based on the Reference Phantom Method for attenuation and backscatter coefficient estimation.¹¹ The method assumes the same speed of sound in the test medium (sample) and reference phantom, no multiple scattering, (locally) homogeneous attenuation, and a depth, z , into the test medium of at least one aperture distance from the transducer. With these assumptions,

$$\text{PSD}(f, z, \theta) = G(f, z, \theta) D(f, z, \theta) \text{BSC}(f, z, \theta) \times \exp\{-4\alpha(f, \theta)z\} \quad (1)$$

where $\text{PSD}(f, z, \theta)$ is the power spectral density at frequency f from a power spectral estimation region (PSER, see Fig. 1) at axial depth z and beam steering angle θ . G represents the signal transduction and processing in the system. D represents the diffraction pattern of the acoustic beam. The value of the Reference Phantom Method is that, under the

assumed conditions, G and D are (individually) equivalent in the reference phantom and the test medium (and thus cancel if the ratio of power spectral densities is analyzed). BSC is the backscatter coefficient and α is the attenuation coefficient in the medium.

The BSPD, defined in Eqn. 2, describes the difference between the integrated echo signal power spectra of a test medium (sample; subscript s) and that of the reference phantom (subscript r) (see Fig. 2). Power spectral densities of both sample and reference phantom are computed in a PSER at equivalent depth z and at steering angle θ , and integrated over a bandwidth (BW) well above the spectral noise floor. To simplify the following equations, we drop the z -dependence from the PSD and BSPD variables.

$$\text{BSPD}(\theta)[dB] = \frac{1}{\text{BW}} \int_{\text{BW}} 10 \log_{10} \left(\frac{\text{PSD}_s(f, \theta)}{\text{PSD}_r(f, \theta)} \right) df \quad (2)$$

We relate changes in BSPD to changes in BSC and attenuation with steering angle by substituting Eqn. 1 into Eqn. 2,

$$\text{BSPD}(\theta) = \frac{1}{\text{BW}} \int_{\text{BW}} 10 \log_{10} \left(\frac{\text{BSC}_s(f, \theta)}{\text{BSC}_r(f, \theta)} e^{-4z(\alpha_s(f, \theta) - \alpha_r(f, \theta))} \right) df \quad (3)$$

$$= \frac{1}{\text{BW}} \int_{\text{BW}} 10 \log_{10} \left(\frac{\text{BSC}_s(f, \theta)}{\text{BSC}_r(f, \theta)} \right) df - 4C z(\theta) \left(\int_{\text{BW}} \alpha_s(f, \theta) df - \int_{\text{BW}} \alpha_r(f, \theta) df \right) \quad (4)$$

where $C = 0.2303$ converts from 10 base-10 to natural logarithm.

The method requires a reference medium with homogeneous, independently measured acoustic properties (sound speed, backscatter and attenuation coefficients).²¹ (Note that if the path between the transducer and a parameter estimation region (PER, see Fig. 1) includes heterogeneous media, α_s is the effective attenuation to the PER.¹⁸)

BSPD can be positive or negative, describing either a higher or lower $\text{PSD}(f, z, \theta)$ from the test medium compared to the reference phantom. BSPD magnitude is proportional to the backscatter coefficient and attenuation of the test medium and reference phantom. If the assumptions of the Reference Phantom Method are not violated, a positive BSPD indicates that the BSC of the sample is higher and/or its attenuation lower than that of the reference phantom. On the other hand, a negative BSPD value corresponds to a lower BSC and/or higher attenuation in the test medium relative to the reference phantom. Of note, this approach does not estimate the specific contributions of BSC and/or α to the change in BSPD.

2) Normalized BSPD (nBSPD)—Our primary goal was to develop parameters to detect the presence of anisotropic scattering sources; primarily scattering arising from aligned rod-like scattering structures. Empirical evidence of the angle dependence of backscatter and

attenuation in tissues composed of aligned structures,^{3-7;16;17} suggests that the BSPD will be highest at normal, and lowest at parallel, incidence with respect to the aligned structure. Thus, it should be possible to measure the angle of normal incidence with an aligned structure by identifying the angle of highest BSPD in the beam steering range.

Our other goal was to measure the magnitude of echo signal power anisotropy caused by an anisotropic rod-like scattering structure. We begin by assuming that, if there is an aligned structure, the magnitude of BSPD with beam steering angle will symmetrically decrease about the angle of normal incidence, as is known to occur with rod-like scattering structures.^{22;23} Because the magnitude of BSPD at the angle of normal incidence depends on the attenuation and backscatter coefficient of the sample, to quantify anisotropy, we remove the overall magnitude information of the BSPD through normalization. Specifically, we characterize echo signal anisotropy as the loss of BSPD relative to the highest BSPD in the beam steering range. We quantify the presence and magnitude of anisotropy by normalizing all the BSPD(θ) estimates by the maximum BSPD value over all steering angles (see Fig. 3). The normalization angle (θ_{norm}) and normalized BSPD ($n\text{BSPD}$) are defined:

$$\theta_{\text{norm}} = \arg \max_{\theta \in \text{Beam Steering Range}} (\text{BSPD}(\theta)) \quad (5)$$

$$n\text{BSPD}(\theta) = \text{BSPD}(\theta_{\text{norm}}) - \text{BSPD}(\theta) \quad (6)$$

where θ_{norm} is the angle at which the greatest BSPD occurs within the beam steering range. If there is an aligned structure, θ_{norm} will be the angle of normal incidence (or the angle closest to it). If not, the θ_{norm} will be about the same among all steering angles. The $n\text{BSPD}$ does not depend on the overall magnitude of backscatter and therefore facilitates evaluation of anisotropy in the sample because one can be confident that power losses are due to anisotropic scatterers instead of other factors. Further, the $n\text{BSPD}$ will always be a positive value because it is a measure of the difference between the maximum BSPD and all lesser values of the BSPD at other steering angles. A caution, however, is that this approach requires the sound speed in the reference phantom to be closely matched to that of the test medium.²⁴

3) Asymmetric BSPD (aBSPD)—As stated above, isotropic scattering should result in equivalent normalized backscattered echo power among all beam steering angles. Thus, an easy test to determine the presence of an anisotropic rod-like scattering structure is to measure the symmetry of BSPD about zero degree (normal) incidence with respect to the (flat linear) transducer face because anisotropic rod-like scattering structures oriented at any angle other than parallel to the transducer will result in BSPD asymmetry. To obtain a summary measure of BSPD symmetry (about $\theta = 0^\circ$), we integrated $n\text{BSPD}$ among positive beam steering angles and similarly among negative beam steering angles, then computed the difference between the integrals (where θ_{Range} refers to the largest angle in our beam steering range).

$$aBSPD = \frac{1}{2(\theta_{\text{Range}})} \left(\int_{\theta=0^\circ}^{\theta_{\text{Range}}} nBSPD(\theta) d\theta - \int_{-\theta_{\text{Range}}}^{\theta=0^\circ} nBSPD(\theta) d\theta \right) \quad (7)$$

(Note, due to the $\alpha(\theta)$ term in Eq. 3, we are restricted to analyzing only beam steering ranges that are symmetric about 0° .)

In Fig. 4(a) the backscatter coefficient and attenuation are symmetric with respect to 0° and $aBSPD = 0$. A zero magnitude $aBSPD$ indicates symmetric backscattered power with respect to the 0° angle (suggesting either isotropic sources of backscatter, or that the dominant rod-like scattering sources are aligned parallel to the transducer face). In Fig. 4(b) an aligned structure is oriented at 10° w.r.t. the transducer and thus an asymmetry of the backscatter coefficient and attenuation results in backscattered power being lost predominantly on the negative steering angles and $aBSPD < 0$. A non-zero value of $aBSPD$ suggests the presence of an aligned structure while the sign of $aBSPD$ indicates the general orientation of scattering sources (with respect to the transducer face), as discussed below.

4) Mean BSPD (mBSPD)—We expect large changes in the BSC and attenuation of a sample when there is an aligned structure and the angle of interrogation is varied. In contrast, we expect little to no change in BSC and attenuation in the case of spherical scattering. Thus, the average $nBSPD$ can also be used to differentiate between isotropic and anisotropic rod-like scatterers.

To obtain the mean $nBSPD$ ($mBSPD$; Eqn. 8), we integrate the $nBSPD$ over all beam steering angles and divide by twice the angular range of integration. The magnitude of this parameter indicates the average normalized backscattered power difference among all beam steering angles.

$$mBSPD = \frac{1}{2(\theta_{\text{Range}})} \left(\int_{\theta=0^\circ}^{\theta_{\text{Range}}} nBSPD(\theta) d\theta + \int_{-\theta_{\text{Range}}}^{\theta=0^\circ} nBSPD(\theta) d\theta \right) \quad (8)$$

$mBSPD$ will always be positive and non-zero because it integrates $nBSPD$ (which is always positive as described above). Figure 4 shows two examples of angle-dependent backscattering. If there is little or no loss of power (beyond the system-dependent losses) when acoustic beams are steered away from the angle with highest BSPD, then small values of $mBSPD$ would be expected, as shown in Fig. 4(a). Accordingly, small values of $mBSPD$ are anticipated in the case of spherical scattering structures. On the other hand, large $mBSPD$ values would be expected of anisotropic scattering structures because of the large loss of power when acoustic beams are steered away from the angle with highest BSPD, as shown in Fig. 4(b).

B. Tests of Anisotropy Parameters

1) Isotropic Phantom—To test our parameters, we first applied them to an isotropic phantom. This experiment consisted of collecting a large number of independent frames of

radiofrequency echo signals (RF frames) from one reference phantom, and partitioning them into ‘sample media’ and ‘reference phantom’ samples. We expected that the Reference Phantom Method would compensate for the geometric system-dependent losses with beam steering angle, the BSPD parameter would be equivalent among all angles, the a BSPD and the m BSPD would be approximately zero (anisotropy is not present). For this test, we used a homogeneous phantom composed of a water-based gel containing graphite powder (51 g/L; 9039 Superior Graphite, Chicago, IL) and glass beads (6 g/L, 3000E beads, ~5–20 μ m; Potter’s Industries, Malvern, PA) with a speed of sound of 1540 m/s and a linear attenuation slope of $0.67 \text{ dB}\cdot\text{cm}^{-1}\text{MHz}^{-1}$ in the 2.5–10 MHz frequency range. The phantom was housed in an acrylic block that had a 25 μ m thick polyvinylidene chloride (Dow Chemical, Midland, MI) scanning window.

A Siemens Acuson S2000 ultrasound system (Siemens Healthcare, Ultrasound Business Unit, Mountain View, CA, USA) equipped with an 18L6 linear array transducer operating at a nominal frequency of 10 MHz was used to acquire radiofrequency (RF) echo signal data from the phantoms. We used the Axius Direct Ultrasound Research Interface²⁵ for beam steering and collection of raw RF echo signals which were sampled at 40 MHz. All beamforming on the system assumed a speed of sound of 1540 m/s. For each scanning configuration, RF echo data were collected with acoustic beams steered from -28° to $+28^\circ$ in steps of 4° . Negative beam steering angles refer to beams transmitted towards the left-hand side of the B-mode image while positive beam steering angles refer to beams transmitted towards the right-hand side following the convention used by the Axius Direct Ultrasound Research Interface. The data were downloaded and analyzed off-line using MATLAB (Mathworks, Natick, MA).

The transducer was placed in contact with the phantom and coupled with standard transducer coupling gel. One hundred sets of beam-steered RF echo signal data (456 lines (5.8 cm) and 2076 axial time samples (4 cm) per frame) were acquired, each after translating the transducer $\approx 300 \mu$ m in the elevation direction. The ROI for each set of beam-steered data was a 2 cm axial and 2 cm lateral square located at the center of the RF frame acquired at zero degree beam steering. Each ROI began at 1 cm axial depth to ensure that we were at least one (elevational) aperture away from the transducer face.

2) Isotropic Phantoms with Speed of Sound Mismatch—We expect that a speed of sound mismatch will produce a bias in BSPD estimates when using the RPM²⁴ so we explored the bias magnitude using three isotropic phantoms with different sound speeds.

For this test, we used three homogeneous phantoms composed of equal parts of water-based gel containing graphite powder (51 g/L; 9039 Superior Graphite, Chicago, IL) and glass beads (6 g/L, 3000E beads, ~5–20 μ m; Potter’s Industries, Malvern, PA). The three phantoms had sound speeds of 1500, 1540, and 1560 m/s respectively. The SOS was controlled by varying the ratio of n-propanol to water when creating the gel; higher concentrations of n-propanol increases the speed of sound.²⁶ The linear attenuation slope between the three phantoms were within 5% of each other being, on average, $0.67 \text{ dB}\cdot\text{cm}^{-1}\text{MHz}^{-1}$ in the 2.5–10 MHz frequency range. All the phantoms were housed in an

acrylic block that had a 25 μm thick polyvinylidene chloride (Dow Chemical, Midland, MI) scanning window.

The data acquisition system for these three phantoms was the same as that in the isotropic phantom experiment described above.

With three different speeds of sound (1500 m/s, 1540 m/s, and 1560 m/s), we generated nine different SOS mismatch combinations. To ease the representation of results, we define the SOS as the speed of sound of the sample media minus the speed of sound of the reference phantom. In equation form:

$$\Delta\text{SOS} = c_{\text{Sample Media}} - c_{\text{Reference Phantom}} \quad (9)$$

A SOS = +20 m/s, in this study, indicates that the sample media's speed of sound is 1560 m/s and the reference phantom's speed of sound is 1540 m/s. Each SOS phantom had 20 individual RF frames used as the 'sample' data and the other 80 RF frames used as the 'reference phantom' data. For example, the SOS = +20 m/s used the 20 individual RF frames of the 1560 m/s phantom as the 'sample' and 80 RF frames of the 1540 m/s phantom as the 'reference phantom.' SOS ranged from -60 m/s to +60 m/s in steps of 20 m/s with three estimates of 0 m/s.

3) Anisotropic (Dialysis) Phantom—We next tested the parameters in an anisotropic phantom; a phantom meant to simulate the scattering arising from rod-like scattering sources. In the anisotropic phantom we expected that the normalization angle would be equal to the angle of normal incidence with the underlying rod-like scatterers, the $a\text{BSPD}$ would be non-zero (anisotropic scattering is present) when the phantom is tilted away from parallel to the transducer face, and the $m\text{BSPD}$ would be large (significant backscatter anisotropy) and remain roughly constant regardless of tilt angle. This phantom was composed, in part, of a dialysis filter (Hemoflow F8 Polysulfone; Fresenius Medical Care, Waltham, MA) containing pseudo-aligned pseudo-straight Polysulfone rods (280 μm diameter see Fig. 5). The filter was filled with an animal hide gelatin mixture, removed from its mold, and then suspended in mineral oil.

The data acquisition system was the same as described above. The anisotropic phantom was mounted to a rotary stage capable of tilt in 1° increments (relative to the transducer face). The transducer was mounted to a holder that secured the transducer orientation relative to the fiber alignment. Both beam steering angle and angle of phantom orientation were relative to the transducer face. Five sets of RF echo frames (456 A-lines (5.8 cm width) and 3116 axial time samples (6 cm depth) per frame) were acquired at 21 (tilt) orientations from -20° to 20° in steps of 2° . Each frame of RF echo data was acquired after translating the transducer $\approx 300 \mu\text{m}$ in the elevation direction for each tilt angle. Each imaging plane ROI was a $1 \text{ cm} \times 1 \text{ cm}$ square region centered in the middle of the anisotropic phantom (the center of the ROI was 3 cm deep and 3 cm from the lateral edge of the data field).

4) In vivo Tissue (Bicep Muscle)—Finally, we tested the parameters on *in vivo* data acquired from different imaging planes of a known anisotropic tissue (bicep muscle)^{3;4} to explore whether we could detect anisotropy (with *a*BSPD), orientation (angle of normal incidence), and magnitude of anisotropy (with *m*BSPD).

Using an 18L6 linear array transducer we acquired 10 independent sets of beam-steered RF echo frames (456 lines (5.8 cm width) and 2076 axial time samples (4 cm depth) per frame) at three different positions on a human bicep. The three imaging locations included muscle fibers that were tilted with respect to the transducer face, muscle fibers that were parallel to the transducer face, and muscle fibers that were perpendicular to the imaging plane. A sketch of the locations of the three bicep imaging planes is shown in Fig. 6. The ROI for each imaging plane was approximately 1.5 cm axially by 3 cm laterally. The number of independent BSPD PERs was on average 3 axial by 7 lateral. Representative B-mode images with ROIs are shown in Fig 13. Analysis of the angled muscle fiber imaging planes was restricted to only angled muscle fibers below the hyperechoic fascia shown in Fig. 13(a). Analysis of the parallel and transverse muscle fibers was restricted to homogeneous regions in the first 1.5 cm below the skin. For each muscle fiber orientation, we combined backscatter anisotropy estimates from the 10 independent frames of RF echo data for statistical analysis. We additionally acquired 20 sets of beam-steered RF frames from a 1560 m/s reference phantom to compute backscatter anisotropy parameters (the speed of sound in skeletal muscle⁴ is approximately 1580 m/s). The 1560 m/s reference phantom construction was the same phantom as used in the Section II.B.2. Data acquisition for the 1560 m/s reference phantom was the same as for the isotropic phantoms as described above.

5) Power Spectral Density Estimation—A multitaper method described by Thomson²⁷ was used to estimate the power spectrum for all phantom and tissue echo signal data analysis. Previous work investigating acoustic attenuation demonstrated the benefits of the multitaper PSD estimator when limiting the size of the power spectral estimation regions (PSER) and parameter estimation regions (PER, illustrated in Fig. 1) for improved spatial resolution.²⁸ Power spectrum estimates are highly dependent on the amount of uncorrelated data included in the estimates (the axial window size and number of uncorrelated lateral and elevational estimates averaged, in the case of ultrasound echo data) which introduce a tradeoff in bias and variance. The PSER and PER are chosen to optimize the tradeoff between bias and variance by knowing the acoustic pulse length and lateral correlation length.²⁸

The bandwidth selected for data analysis was the range that had power at least 10 dB above the spectral noise floor. The noise floor was estimated by averaging the echo signal power between 13–20 MHz, a range far outside the observed echo signal bandwidth. Based on these criteria, the 4–9 MHz frequency range was chosen for all data analysis.

Following the methods described by Thijssen,²⁹ the axial and lateral RF echo signal correlation lengths were estimated to be 205 and 287 μm , respectively, in the reference phantom. For these correlation cell sizes, previous work²⁸ suggests using 4 mm $\times$ 4 mm PSERs and a 4 mm $\times$ 4 mm BSPD PER (since only a single PSER is needed for BSPD

estimation) to provide relatively low bias and variance parameter estimates when using the multitaper method (4 tapers with $N=209$ RF samples for $5.2 \mu\text{s}$ signal segments²⁷).

In all cases, all PERs were defined at least one aperture away from the lateral edges of the RF data frame in order to avoid incomplete aperture artifacts that occur at the edge of transducers.

6) Angular Range Selection—It is possible to beam steer linear array beams through any arbitrary angular range with (nearly) arbitrary angular step sizes. In a linear array acquisition a larger angular beam steering range will reduce the lateral and axial area shared by all steered acoustic beams. This geometric relationship is illustrated (using the 18L6 transducer geometry) in Fig. 7. As the angular beam steering range is increased, the shared area between all beam-steered acoustic beams decreases. This geometric phenomenon creates a trade-off between the axial depth and lateral extent where we can measure our QUS parameters for the maximum beam steering angle selected. Ideally, we would like to include as large a beam steering angle range as possible to have greater sensitivity to aligned structures. Realistically, our transducer's lateral extent and the chosen axial depth of interest will restrict the range of angles we may include. In this study, we used $\pm 28^\circ$ as our angular range, allowing the 18L6 transducer (which has a 5.7 cm lateral extent) to interrogate up to 5 cm into our phantoms and tissues.

7) Statistical Analysis of Parameter Estimates—A one-sample Student's t-test was used to test for significant deviations from zero for the BSPD, normalization angle, and *a*BSPD estimates from the isotropic phantom, anisotropic phantom, and *in vivo* tissue. A one-sample Kolmogorov-Smirnov test³⁰ on *a*BSPD and *m*BSPD values was used to test the hypothesis of normally distributed data at the 5% confidence level. Use of the Kolmogorov-Smirnov test allowed us to determine whether the *a*BSPD and *m*BSPD were approximately normally distributed. A one-way ANOVA test for normally distributed data was used to determine statistical significance of normalization angle, *a*BSPD, and *m*BSPD estimates between the different muscle fiber orientations in the bicep muscle experiment. The ANOVA tests were used to test whether we could statistically differentiate between the three muscle fiber orientations using the normalization angle, *a*BSPD, or *m*BSPD.

III. Results

A. Tests of BSPD Parameters

1) Isotropic Phantom—Figure 8 shows the mean and standard deviation of the BSPD versus beamsteering angle using 20 uncorrelated frames of RF echo signals from the 1540 m/s phantom as 'sample' data and 80 uncorrelated frames of RF echo signals from the same phantom as the 'reference phantom' data. BSPD values were averaged laterally and axially for each RF frame. The average BSPD (over all angles) was -0.22 ± 0.31 dB. This small negative bias is discussed in the Appendix.

The average normalization angle for the isotropic phantom was $-3.25 \pm 9.81^\circ$ and was not significantly different from zero ($p > 0.05$). The *m*BSPD values measured in the isotropic

phantom were 0.41 ± 0.04 dB while the $aBSPD$ values were -0.03 ± 0.08 dB and were not significantly different from zero ($p > 0.05$).

2) Isotropic Phantoms with Speed of Sound Mismatch—Figure 9 shows the $BSPD$ versus beamsteering angle for $SOS = \pm 20$ m/s. We see that the $BSPD$ is negative for sample media that has a faster speed of sound and positive for media with a slower speed of sound (compared to the reference phantom). The magnitude of the $BSPD$ becomes larger at larger beamsteering angles.

Figure 10 shows $mBSPD$ versus SOS . The $mBSPD$ increases for both positive and negative speed of sound mismatches. Notably, having a slower speed of sound in the sample media, compared to the reference phantom, causes larger changes in the $mBSPD$ than having a faster speed of sound. The $aBSPD$ and normalization angle were not significantly different from zero for all SOS ($p > 0.05$ for $aBSPD$ and normalization angle for all SOS).

3) Anisotropic (Dialysis) Phantom—Figure 11 shows B-mode images of the anisotropic (dialysis) phantom (shown in Fig. 5) in three orientations. The $nBSPD$, $mBSPD$, and $aBSPD$ were estimated from five RF frames, using each anisotropic phantom RF frame as the “sample” frame and 20 RF frames from the 1540 m/s phantom as the reference phantom.

Figure 12(a) shows the median and inner-quartile range (IQR) of the normalization angle of $nBSPD$ versus phantom tilt angle. The median normalization angle was not significantly different from the dialysis tilt angle for any of the 21 angles measured ($p > 0.05$ for all angles), indicating that the highest $BSPD$ is at the angle of normal incidence with the anisotropic phantom rods. Figure 12(b) shows the median and IQR of $mBSPD$ versus anisotropic phantom tilt angle, averaged both axially and laterally. $mBSPD$ increased nearly symmetrically with increased tilt of the dialysis phantom. Figure 12(c) shows the median and IQR of $aBSPD$ versus anisotropic phantom tilt angle. The $aBSPD$ is nearly zero at, and antisymmetric about, the 0° phantom tilt angle. Large positive $aBSPD$ values are associated with fibers tilted in the negative direction, and large negative $aBSPD$ values are associated with fibers tilted in the positive direction.

4) In vivo Tissue (Bicep)—Figure 13 shows representative B-mode images of the three bicep muscle fiber orientations measured. Values for the average normalization angle, $mBSPD$, and $aBSPD$ for the angled, parallel, and transverse muscle fibers are plotted in Fig. 14. The angled muscle fiber’s normalization angle ($-13.8 \pm 1.3^\circ$) appears consistent with the muscle fiber orientation seen in Fig. 13(a), and the parallel muscle fiber’s normalization angle ($-1.5 \pm 0.7^\circ$) appears consistent with the fiber orientation seen in Fig 13b. Both parallel and angled muscle fiber normalization angle values were significantly different than zero ($p < 0.01$) and suggests a rod-like structure in the image plane. The transverse muscle fiber’s normalization angle ($-0.8 \pm 1.8^\circ$) was not significantly different than zero ($p > 0.05$), as expected for scatterers with circular cross section in the image (beam steering) plane. The $mBSPD$ values for muscle fibers oriented at about -15° in the image plane (9.06 ± 0.56 dB) and for those nearly parallel to the transducer face (8.20 ± 0.40 dB) also strongly suggest the presence of anisotropic scattering structures. The $mBSPD$ values among the parallel and

angled muscle fibers were significantly different ($p < 0.01$). The $mBSPD$ values for muscle fibers oriented transverse to the imaging plane (3.12 ± 0.42 dB) were significantly lower than those for the parallel and angled muscle fibers in the imaging plane ($p < 0.01$ for both comparisons). Average $aBSPD$ for the angled, parallel, and transverse muscle fibers were 5.28 ± 0.48 dB, 1.02 ± 0.32 dB, and 0.10 ± 0.25 dB respectively. We found that $aBSPD$ values were positive, significantly different from zero ($p < 0.01$ for both angled and parallel fibers), and consistent with the respective normalization angles for both the angled and parallel muscle fiber imaging planes, but the $aBSPD$ values for the transverse muscle fiber orientation were not significantly different from zero ($p > 0.05$).

IV. Discussion

A. Quantifying Backscattered Power Difference

When the sample media and reference phantom were the same isotropic (spherically scattering) phantom, the BSPD was a small negative value for all beam steering angles. The log of the average power spectra of N independent A-lines is a biased estimator whose bias depends solely on N .³¹ We derive the bias and standard deviation of the BSPD in Appendix A. Having $N=14$ independent A-lines in our PSER, the estimated bias of the BSPD is -0.16 dB. Our estimate of -0.22 ± 0.31 dB for the bias in BSPD estimates is consistent with theory.

Eq. 1–3 shows that a BSPD of zero implies that the sample media and reference phantom have equivalent diffraction, system transfer, attenuation, and backscatter coefficient. If we remove the bias discussed above, the average BSPD is not significantly different from zero for all angles ($p > 0.05$). A BSPD of zero for all angles suggests that the Reference Phantom Method correctly compensated for the system-dependent geometric losses induced by beam steering in the isotropic phantom (Fig. 8). The remaining fluctuations in BSPD versus angle are caused by variance in power spectral estimates which is composed of both fluctuations in speckle brightness³² and statistics of the power spectral estimation method.³³ (How this affects estimation of the magnitude of anisotropy is discussed in Section D below.)

B. Quantifying the Presence of Anisotropy

The isotropic phantom results (Fig. 8) illustrates the lower bound on BSPD estimates and parameters derived from them. The largest of the BSPD values (that are all essentially zero) is selected as the normalization value. The other values are (slightly) higher than that, after normalization, and the average among those values is reported as the $mBSPD$. Summing the normalized $mBSPD$ values separately for the positive and negative beam steering angles provides an $aBSPD$ estimate that is also essentially zero. Although there is an error in diffraction correction in the Reference Phantom Method when the sound speed in the reference phantoms doesn't match that of the sample, even a 60 m/s difference in sound speed results in an $mBSPD$ of 1 dB, and the BSPD values are symmetric about zero degrees.

Conversely, the anisotropic (dialysis) phantom experiment demonstrated that, for any non-zero dialysis phantom tilt angle, the $aBSPD$ is non-zero as shown in Fig. 12(c). In other words, for any tilt angle, measurable asymmetry in BSPD among positive and negative beam steering angles suggests anisotropic scattering. Further, the positive or negative value of the

α BSPD indicates general direction of the fibers, with ‘negatively’ oriented fibers producing positive α BSPD and vice versa. However, the zero degree dialysis phantom tilt angle, as shown in Fig. 12(c), demonstrates a limitation of relying on BSPD symmetry to detect anisotropy. Specifically, rods parallel to the transducer face will result in symmetric losses about zero degree incidence and an α BSPD equal to zero. (Strategies to deal with this confounding behavior are discussed below in Subsection IV-F.)

In the *in vivo* bicep muscle experiment, we found that angled muscle fibers generated non-zero α BSPD values with a sign consistent with the ‘negative’ angular orientation of the fibers as seen on the B-mode. The parallel muscle fibers had small, but significantly non-zero negative α BSPD values. Under experimental control we could generate symmetric BSPD from anisotropic media, but under *in vivo* conditions muscle fibers that appear parallel in B-mode were not perfectly parallel to the transducer face and generated non-zero α BSPD. For transverse fibers we found α BSPD values that were not significantly different from zero, indicating isotropic scattering. Our results are consistent with the idea that muscle fibers are transversely isotropic. When imaged with 2D beam steering, a transverse plane of fibers should more closely resemble isotropic (spherical) scatterers than anisotropic (rod-like) scatterers. This suggests that 3D beam steering would properly characterize transverse isotropy.

C. Quantifying the Orientation of Anisotropy

We suggested above that fluctuations in BSPD versus angle in the spherically scattering phantom are primarily from speckle fluctuations and power spectral estimation variance. If all angles have the same speckle fluctuations and power spectral estimation noise, the angle of highest BSPD should be random and uniformly distributed among all angles in the beam steering range. Using a beam steering range of $\pm 28^\circ$, a uniform distribution has a mean of 0° and standard deviation of 16.17° . The estimated mean and standard deviation of the normalization angle in a BSPD PER for the isotropic phantom was -0.32° and 18.08° respectively. This agrees well with theory.

The anisotropic phantom experiment suggests that the orientation of anisotropic scatterers could be determined through the normalization angle. Specifically, we found that the measured normalization angle was statistically equivalent to the anisotropic phantom tilt angle for all tilt angles (Fig. 12(a)).

In bicep muscle, we found that the normalization angle matched the dominant orientation of the muscle fibers visually assessed in the B-mode images (Fig. 13). The normalization angle of parallel muscle fibers was non-zero, indicating nonparallel alignment to the transducer face, and consistent with detection of anisotropy via the α BSPD. Further, the mean normalization angle of transverse muscle fibers was near zero, as expected from isotropic scattering.

D. Quantifying the Magnitude of Anisotropy

In the isotropic phantom, we found a small non-zero magnitude of m BSPD. Because fluctuations in the BSPD will occur at all beam steering angles, there will (nearly) always be a non-zero m BSPD. This finding indicates that in any measure of the m BSPD there will be

a component due to PSD estimate variance in addition to a component due to anisotropic scattering structures.

Results from the dialysis phantom demonstrated $mBSPD$ was symmetric about the 0° phantom tilt angle, remained consistent in the $\pm 10^\circ$ tilt angle range, and increased beyond that. We noted nearly complete loss of signal at beam steering angles greater than 45° incidence with the phantom. As tilt angle increased beyond 10° , there were more beam steering angles that had little to no backscattered power than beam steering angles that reflected power from the dialysis filter. Thus, $mBSPD$ increased as beam steering angles with small angles of incidence with the phantom were replaced with beam steering angles with large angles of incidence with the phantom. For truly system and user independent parameters, the $mBSPD$ needs to be independent on the angle of the underlying aligned structure; the dialysis phantom experiment suggests that interpretation of the $mBSPD$ is confounded when there is a complete loss of power on one beamsteering angle. It is important to note, although the $mBSPD$ was not the same for all tilt angles, the $mBSPD$ indicated a deviation from isotropic scattering conditions ($mBSPD \ll 0.41$ dB) for all dialysis phantom tilt angles.

$mBSPD$ was significantly lower in the transverse muscle fiber imaging planes compared to the other muscle fiber orientations, further evidence that the $mBSPD$ differentiates between rod-like and circular-cross section scattering sources. We observed significantly different (≈ 1 dB difference) $mBSPD$ values between angled and parallel muscle fibers, suggesting that the two muscle groups have different angle-dependent losses of power. This could mean that the angled and parallel muscle groups have slightly different composition.

Care must be taken when interpreting $mBSPD$ findings, because anything that causes a change in speckle fluctuations (either in sample media or reference phantom) or power spectral estimation noise, will change (increase or decrease) the $mBSPD$. Possible sources of bias and variance include speed of sound mismatch between sample media and reference phantom (discussed in Subsection IV-E below) and power spectral estimation parameters (PSER size and windowing method). Any choice of power spectral estimation parameters that decrease power spectral variance (such as increasing PSER size) will decrease variance of the $mBSPD$. Quantifying the effect of power spectral estimation method on BSPD variance (and therefore $mBSPD$ variance) is discussed in Appendix A. That said, our estimate of $mBSPD$ noise in the isotropic phantom (≈ 0.4 dB) is considerably lower than $mBSPD$ measurements in *in vivo* isotropic media (transverse muscle fiber ≈ 3 dB), *in vivo* anisotropic media (parallel and angled muscle fibers ≈ 8 – 9 dB), or anisotropic phantom media (≈ 20 dB). This observation suggests that power spectral noise contributes much less to bias than the modest violations of isotropy encountered through *in vivo* data acquisition.

E. Bias of Anisotropy Due to Speed of Sound Mismatch

We found that modest differences in speed of sound between the sample media and reference phantom caused bias in the BSPD and in the $mBSPD$. Figure 9 shows that a negative SOS (slower speed of sound in the sample) causes a positive BSPD bias while a positive SOS (faster SOS in the sample) causes a negative BSPD bias.

A uniform speed of sound mismatch did not cause significant bias in the $aBSPD$ or normalization angle. Figure 9 shows that a uniform SOS mismatch causes symmetric behavior of the BSPD about the zero degree beamsteering angle. It is consistent that measures of BSPD symmetry ($aBSPD$ and normalization angle) are unbiased by a uniform SOS mismatch. As long as the reference phantom's speed of sound is within ± 20 m/s of the sample media the increase in the $mBSPD$ is < 0.2 dB and there is no appreciable change in $aBSPD$ or normalization angle.

The speed of sound is known to be anisotropic in some tissues composed of aligned structures (myocardium³⁴, skeletal muscle⁴). Speed of sound anisotropy is modest in most tissues; the kidney reported no significantly different change from perpendicular to parallel incidence,⁹ the fresh bovine myocardium has a < 9 m/s ($< 0.5\%$) variation between perpendicular and parallel incidence,³⁴ and fresh rat skeletal muscle has a < 8 m/s ($< 0.5\%$) variation between perpendicular and 45° incidence.⁴ The results in Figure 9 demonstrate that the bias in BSPD estimates, introduced with a uniform ± 20 m/s SOS mismatch, is minimal compared to the change in BSPD observed in the bicep muscles (> 8 dB from -28° to $+28^\circ$ for parallel and angled muscle fibers).

F. Strategies and Limitations Detecting the Presence, Orientation, and Magnitude of Anisotropy

The purpose of this paper was to develop and test an objective approach to evaluating anisotropy in backscatter assessed with beam steering from a linear array. We found that it is possible to determine the presence of anisotropy in an unknown media via the $aBSPD$, because non-zero $aBSPD$ values indicate asymmetric power loss (i.e. anisotropic backscattered echo signal power). An $aBSPD = 0$, however, does not directly indicate isotropic scattering, because both spherical scatterers and rods oriented parallel to the transducer face will cause symmetric BSPD. Differentiating between rod-like scatterers oriented parallel to the transducer face and spherical scatterers requires additional assessment; for instance, the media may be measured in a transverse plane to compare the $mBSPD$ values (as we demonstrated in bicep muscle), or it may be tilted to evaluate whether the $aBSPD$ becomes non-zero (as we demonstrated in the anisotropic phantom).

A general limitation of this approach is that, in order to compare media or tissue, care must be taken to ensure that analysis is restricted to a specific beam steering angle range because the angular range used will affect the magnitude of both $aBSPD$ and $mBSPD$. This requires reporting the angular ranges used when measuring these anisotropy parameters. Another general limitation is the assumption that the experimentally measured trends of anisotropy in the BSC and attenuation in skeletal muscle, myocardium, and kidney are consistent in all media composed of aligned structures. A violation of this assumption would change the behavior of the BSPD versus beamsteering angle and interpretation of the BSPD parameters. The dialysis phantom experiment (Fig. 12) highlighted the need to ensure that there is enough SNR in the analysis bandwidth to have a meaningful interpretation of the BSPD parameters. In addition we are exploring the potential bias in BSPD parameters caused by large aligned media angles.

The intent of the methods reported here is to provide an objective measure, with few assumptions, to demonstrate (an)isotropy in backscattered power to inform careful experimental methods and data interpretation if the material under investigation demonstrates anisotropy. We are investigating whether we can determine if the backscatter or attenuation coefficient dominates the BSPD changes with beam steering angle due to an aligned scattering structure. If either coefficient underwent much larger changes then we could simplify our derivation and our anisotropy parameters would mainly reflect changes in the dominant coefficient. We hope to understand whether the *m*BSPD provides information about organization of the scattering structures.

V. Conclusions

Our novel backscatter anisotropy parameters, *a*BSPD and *m*BSPD, detected the presence, orientation, and the magnitude of anisotropy in phantoms and a simple human tissue. These parameters may allow objective quantification of anisotropy in complex biological tissues.

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Appendix A: Bias of the Log averaged Ratio of Power Spectra

Lizzi *et al.*,³¹ presented a derivation of the probability density function (PDF) for the power spectral density (PSD, the power spectral value at a specific frequency channel) for echo signals resulting from diffuse scattering. Further, they presented the PDF for the log of the average PSD for *N* uncorrelated lines of echo data.

$$\text{PSD}_{\text{av}}(f_m) = \frac{1}{N} \sum_{n=1}^N P_n(f_m) \quad (10)$$

where PSD_{av} is the average power spectrum, P_n is the power spectrum for the single n^{th} A-line, and f_m is the frequency at channel m . The mean and standard deviation of $\text{PSD}_{\text{av}}(f_m)$ are μ_m and $\frac{\sigma_m}{\sqrt{N}}$ respectively.

The mean, standard deviation, and approximate values (assuming large *N*) of the log of the *normalized* $\text{PSD}_{\text{av}}(f_m)$ are:

$$\mu_{dBav}(N) = 10 \log_{10}(\mu_m) + 4.34 [\Psi(N) - \ln(N)] \xrightarrow{N \gg 1} 10 \log_{10}(\mu_m) - \frac{4.34}{2N} \quad (11)$$

$$\sigma_{dBav}(N) = 4.34 \sqrt{\zeta(2, N)} \xrightarrow{N \gg 1} \frac{4.34}{\sqrt{N}} \quad (12)$$

where μ_{dBav} is the estimated mean and σ_{dBav} is the standard deviation of the normalized PSD at frequency f_m . μ_m is the expected mean power of the sample media at frequency channel m (the value approached as N approaches infinity). $\Psi(N)$ denotes the Psi (digamma) function, and $\zeta(2, N)$ is the generalized Riemann Zeta function. The second term on the far right in Eqn. 11 is the estimation bias of the mean PSD from using a limited sample size.

They then used those derivations to demonstrate the bias and variance in estimates of spectral parameters (specifically the spectral slope and intercept) for a linear fit of the normalized power spectrum. The spectral normalization in their case used the PSD of the echo signal from a planar interface as is commonly done when data acquisitions employ a single-element transducer. Employing the PDF for the PSD from the random echo signal in the description of the statistics of spectral parameter estimates appropriately treated the echo signal from a planar interface as a deterministic signal.

Alternatively, the spectral parameters reported here are obtained using the Reference Phantom Method (see Eqn. 1) which is commonly done when data acquisitions employ a clinical imaging system and an array transducer. As such, spectral normalization is performed based on a the *average* PSD of $L \gg 1$ uncorrelated lines of echo data from an independently-calibrated “reference phantom” that creates diffuse scattering.

$$\text{PSD}_r(f_m) = \frac{1}{L} \sum_{l=1}^L P_l(f_m) \quad (13)$$

Therefore, the moments of the PDF for the ratio of two random variables need to be computed. Since power spectral values are always positive, a simple approach for computing the mean and variance of the ratio of two power spectra is to use a bivariate Taylor series expansion about the expected values for each PSD.³⁵ The results show that, to a second-order approximation (assuming that the echo signals from the sample media and reference phantom are independent of each other),

$$E \left\{ 10 \log_{10} \left(\frac{\text{PSD}_s(f_m)}{\text{PSD}_r(f_m)} \right) \right\} \approx 10 \log_{10}(\mu_m^s) - \frac{\sigma_m^{s2}}{2\mu_m^{s2}} - 10 \log_{10}(\mu_m^r) + \frac{\sigma_m^{r2}}{2\mu_m^{r2}} = \mu_{dBav}(N) - \mu_{dBav}(L) \quad (14)$$

$$\text{Var} \left\{ 10 \log_{10} \left(\frac{\text{PSD}_s(f_m)}{\text{PSD}_r(f_m)} \right) \right\} \approx \frac{\sigma_m^2}{\mu_m^2} + \frac{\sigma_m^2}{\mu_m^2} = \sigma_{dBav}^2(N) + \sigma_{dBav}^2(L) \quad (15)$$

Substituting Eqn. 11 into Eqn. 14 obtains

$$\text{E} \left\{ 10 \log_{10} \left(\frac{\text{PSD}_s(f_m)}{\text{PSD}_r(f_m)} \right) \right\} \approx 10 \log_{10}(\mu_m^s) - \frac{4.34}{2N} - 10 \log_{10}(\mu_m^r) + \frac{4.34}{2L} \quad (16)$$

where μ_m^s and μ_m^r are the expected mean echo signal power at frequency channel m in the sample and reference media, respectively.

Substituting Eqn. 12 into Eqn. 15 obtains

$$\text{Var} \left\{ 10 \log_{10} \left(\frac{\text{PSD}_s(f_m)}{\text{PSD}_r(f_m)} \right) \right\} \approx \frac{4.34^2}{N} + \frac{4.34^2}{L} \quad (17)$$

If the echo signals representing sample media and reference phantom are independent samples acquired from the same phantom (see Sec. III-A1 and Fig. 8) then the expected mean PSD of the sample media is equal to the expected mean PSD of the reference phantom ($\mu_m^s = \mu_m^r$). With that, and recognizing that $L \gg N$ (L is generally 20–100× N), we may simplify Eqn. 16:

$$\text{E} \left\{ 10 \log_{10} \left(\frac{\text{PSD}_s(f_m)}{\text{PSD}_r(f_m)} \right) \right\} = 10 \log_{10}(\mu_m^s) - 10 \log_{10}(\mu_m^r) - \frac{4.34}{2N} + \frac{4.34}{2L} = -\frac{4.34}{2N} + \frac{4.34}{2L} \xrightarrow{L \gg N} -\frac{4.34}{2N} \quad (18)$$

Substituting the expected value of the log ratio into the equation for the BSPD (Eq. 2), provides an estimate of the bias when the sample media and reference phantom are equivalent:

$$\begin{aligned} \text{E} \{ \text{BSPD}(\theta) \} &= \frac{1}{\text{BW}} \int_{\text{BW}} \text{E} \left\{ 10 \log_{10} \left(\frac{\text{PSD}_s(f, \theta)}{\text{PSD}_r(f, \theta)} \right) \right\} df \\ \text{E} \{ \text{BSPD} \} &= \frac{1}{\text{BW}} \int_{\text{BW}} -\frac{4.34}{2N} df = -\frac{4.34}{2N} \quad (19) \end{aligned}$$

This demonstrates that the BSPD estimates will have a slight negative bias that is angle-independent, but does depend on the number of uncorrelated echo signals, N , used to estimate the sample media power spectra.

The variance of BSPD estimates is independent of frequency and will be reduced through frequency averaging in BSPD estimation. The total variance will be the variance of the log ratio of power spectra divided by the number of independent frequency channels in the chosen bandwidth. We may write the variance of the BSPD as:

$$\text{Var}\{\text{BSPD}(\theta)\} = \frac{1}{M} \text{Var} \left\{ 10 \log_{10} \left(\frac{\text{PSD}_s(f, \theta)}{\text{PSD}_r(f, \theta)} \right) \right\}$$

$$\text{Var}\{\text{BSPD}\} = \frac{1}{M} \left(\frac{4.34^2}{N} + \frac{4.34^2}{L} \right) \xrightarrow{L \gg N} \frac{1}{M} \frac{4.34^2}{N} \quad (20)$$

where M is the number of independent frequency channels in the signal bandwidth:

$$M = \frac{\text{BW}}{\Delta f} \quad (21)$$

where BW is the bandwidth in MHz, f is separation between independent frequency channels. f may be computed for power spectra estimated using the rectangle window following Appendix B of Lizzi *et al.* 2006. Defining decorrelation as occurring at 20% correlation, assuming a speed of sound of 1540 m/s, a 4 mm PSER axial length, and a 5 MHz bandwidth, $f = 0.18$ MHz and $M = 26$. Calculating M for spectral windows other than the rectangle window requires estimation of the reduction of spectral variance at each frequency bin and reduction of the number of independent frequency bins caused by the window.³³

Figure 15 shows plots of the theoretical bias and standard deviation of the BSPD versus number of independent A-lines, N for power spectra estimated using a rectangle window and experimentally measured values of bias and variance using data from the isotropic phantom experiment (Section II-B1). The number of independent A-lines in the isotropic phantom experiment was $N = 14$; determined by the length of the lateral power spectral estimation region size (4 mm) divided by the experimentally measured lateral pulse echo correlation length (287 μm) (See Section II-B5).

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Fig. 1.

An illustration of the different “windows” used in data selection and analysis. The box with solid lines defines the region of interest (ROI) within which parameters are estimated in a data field. Within the ROI are power spectral estimation regions (PSER) represented in (a) by (white) dashed, dotted, and dash-dot lines which define the axial range and lateral width where power spectra are estimated A-line by A-line and averaged laterally. The white solid circles indicate the center of the PSER which is the assigned location of the power spectral estimate. The amount of PSER overlap defines the density of dots. Also within the ROI are, in (b), parameter estimation regions (black dashed and dotted boxes) which similarly define the axial range of (potentially overlapping) PSERs (white solid circles) which are used for estimating the acoustic parameter of interest (such as attenuation). The black dots indicate the center of the PER which is the assigned location of the parameter estimate.

Fig. 2.

Diagram of BSPD estimation for one angle and depth. The “sample” PSD is shown by the solid curve. The “reference” phantom PSD is shown by the dashed curve. The bandwidth of integration is demarcated by the vertical solid lines. The BSPD is the shaded area between the two curves.

Fig. 3.

Hypothetical plots from one PER of (a) BSPD and (b) n BSPD versus beam steering angle. Each point on the BSPD and n BSPD curves represents the frequency-integrated backscattered echo signal power difference (relative to isotropic scatterers) at one beam steering angle (the black shaded area in Fig. 2). The normalization angle (θ_{norm}) for the n BSPD is 0° for anisotropic scattering and arbitrary for isotropic scattering. The solid line represents a scenario in which the test medium has isotropic spherical scattering sources but lower attenuation than the reference phantom. The dashed line represents a scenario in which there is an anisotropic scattering source which causes a loss of power as the beam is steered away from normal incidence.

(a) Scattering structures aligned parallel to the transducer face

(b) Scattering structures tilted 10° relative to the transducer face

Fig. 4.

Hypothetical plots of (a) symmetric $nBSPD$ and (b) asymmetric $nBSPD$ versus steering angle. Each point represents the $nBSPD$ at one beam steering angle (the black shaded area in Fig. 2). The shaded area in this figure represents negative beam steering angle $nBSPD$ values. Non-shaded areas represent positive beam steering angle $nBSPD$ values. In (a) $\theta_{\text{norm}} = 0^\circ$, $mBSPD \approx 1\text{--}2$ dB, and $aBSPD \approx 0$ dB. In (b) $\theta_{\text{norm}} = +10^\circ$, $mBSPD \gg 1\text{--}2$ dB, and $aBSPD \ll 0$ dB.

Fig. 5.
A photograph of the Polysulfone fibers that provide anisotropic, pseudo-aligned backscattering sources in the dialysis phantom.

Fig. 6.

A sketch of the imaging planes for the *in vivo* bicep muscle experiment. On the left is an anterior view of the bicep, while the right is a coronal view. On the anterior view, the three imaging planes are represented by the solid, dashed, and dash-dot lines. On the coronal view the solid line and dashed line represent the approximate imaging region B-mode images, the dash-dot line represents the imaging plane of the transverse view. The solid line represents the imaging plane and ROI with angled muscle fibers, the dashed line represents the imaging plane and ROI with parallel muscle fibers, and the dash-dot line represents the transverse plane with muscle fibers aligned perpendicular to the angled and parallel imaging planes.

Fig. 7.

A plot demonstrating the decrease in overlapping acoustic beams with increasing beam steering angles for the 18L6 transducer. The colorbar represents the maximum angle used in the angular beam steering range. Lighter colors represent area interrogated by the widest range of beam steering angles, while darker colors represent more narrow beam steering angle range. We see that a more narrow beam steering angle range provides deeper penetration into the sample. There is a clear trade-off in terms of beam steering angles used and axial and lateral extent of the backscatter anisotropy parameter estimation region.

Fig. 8.

A plot of BSPD versus beam steering angle for the isotropic phantom. Each point represents the average BSPD from 20 frames of RF data. Error bars represent the standard deviation of the BSPD among 20 RF echo signal frames.

Fig. 9.

A plot of the BSPD versus steering angle for $\text{SOS} = \pm 20$ m/s. Each point represents the average BSPD of the 20 independent RF frames. The errorbars represent the standard deviation among the 20 independent RF frames. The dots represent $\text{SOS} = -20$ m/s while the x's represent $\text{SOS} = +20$ m/s.

Fig. 10.

A plot of $m\text{BSPD}$ versus ΔSOS . Each point represents the average $m\text{BSPD}$ of the 20 independent RF frames. The errorbars represent the standard deviation among the 20 independent RF frames. The dot, x, and asterisk represent $m\text{BSPD}$ calculated using the sample media being RF frames from the 1500 m/s phantom, 1540 m/s phantom, and 1560 m/s phantom respectively.

Fig. 11.

B-mode images from three anisotropic (dialysis) phantom orientations. Solid boxes represent the ROIs for the dialysis phantom tilt angles.

Fig. 12.

(a) Plot of the normalization angle of the m BSPD versus anisotropic (dialysis) phantom orientation. (b) Plot of m BSPD versus anisotropic (dialysis) phantom orientation. (c) Plot of a BSPD versus anisotropic (dialysis) phantom orientation. Each data point represents the median normalization angle, m BSPD, or a BSPD from one RF frame of the anisotropic phantom. The solid black line in (a) represents equal values of anisotropic phantom tilt angle and normalization angle. For each box plot, the interior line is the median value of the estimated parameter, the notches represent the 95% confidence interval for the median value, the ends of the boxes represent 25 to 75% of the range of values (the interquartile range; IQR). The bars (“whiskers”) represent extreme values not calculated as statistical outliers ($1.5 \times \text{IQR}$), while the crosses are statistical outliers ($>1.5 \times \text{IQR}$).

Fig. 13.

B-mode images for the three bicep muscle fiber orientations. Solid boxes represent the ROIs for the three muscle fiber orientations.

Fig. 14.

Box plots of (a) normalization angle (b) *mBSPD* and (c) *aBSPD* for each muscle fiber orientation group. Each box represents the median and IQR of the normalization angle, *mBSPD* or *aBSPD* of laterally and axially averaged estimates from one RF frame of the bicep muscle.

Fig. 15.

Plots of (a) bias (b) standard deviation of BSPD estimates vs the number of independent A-lines. The solid lines represents the predictions given in Eq. 19 and 20, respectively. The cross and error bars represents the average value of the BSPD from the experiment described in Section II-B1.

TABLE I

Summary table of the acronyms defined or mentioned in this paper.

Parameter Name	Acronym	Equation #	Brief Description
Power Spectral Estimation Region	PSER	N/A	The PSER defines an axial range and lateral width where power spectra are estimated A-line by A-line and averaged laterally. The power spectrum estimated from a PSER is spatially located at the center of the PSER.
Parameter Estimation Region	PER	N/A	The PER defines an axial range of power spectral estimation regions (that can be overlapping and can include only a single PSER or many PSERs) which are used for estimating an acoustic parameter of interest. In this paper there is only a BSPD PER, which happens to be a single PSER.
Backscattered Power Difference	BSPD	2	Log of sample power spectrum divided by reference power spectrum integrated over the bandwidth significantly contributing to the backscattered echo signal.
Normalization Angle	θ_{norm}	5	Beam steering angle which maximizes the value of the BSPD in the selected beam steering range.
Normalized Backscatter Power Difference	$n\text{BSPD}$	6	Difference between the maximum BSPD in the beam steering range and the BSPD at each angle.
Asymmetric Backscatter Power Difference	$a\text{BSPD}$	7	The average of the difference between the $n\text{BSPD}$ integrated over positive beam steering angles and the $n\text{BSPD}$ integrated over negative beam steering angles.
Mean Backscatter Power Difference	$m\text{BSPD}$	8	Average $n\text{BSPD}$ over all beam steering angles.