1pBAb12. A two-component speckle model for detection of microbubble signals in linear contrast-enhanced ultrasonography

Matthew R. Lowerison*

*Corresponding author’s address: Robarts Research Institute, London, N6A 5K8, Ontario, Canada, mloweri@robarts.ca

Contrast-enhanced ultrasound (CEUS) serves oncology by imaging tumor blood supply to enable quantification of longitudinal vascular changes and monitoring of treatment responses. Unfortunately, the linear subtraction methods commonly used for preclinical imaging are susceptible to registration errors and motion artifacts that lead to reduced contrast-to-tissue ratios. In this presentation, an alternative approach is proposed to improve discrimination between the contrast and tissue signals by comparing the first-order speckle statistics of images acquired before and after injection of microbubbles. The microbubble signal component is modeled as a temporally varying random process superimposed on a Rayleigh-distributed speckle signal representing backscatter from tissue. Images were acquired at 18 MHz from a murine orthotopic (mammary fat pad) xenograft breast cancer model following a bolus injection of microbubbles. Images were processed using gold-standard pulse inversion (nonlinear CEUS), conventional linear subtraction, and the proposed statistical method. In comparison to conventional linear CEUS, the statistical method produced a wash-in curve that showed closer agreement to the gold-standard nonlinear CEUS data. The statistical method eliminates the subtraction of a baseline image from linear CEUS image processing, which should streamline the imaging workflow, improve the robustness of image quantification, and enable real-time perfusion imaging with linear CEUS.

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INTRODUCTION

Contrast-enhanced ultrasound (CEUS) is a perfusion imaging method used in clinical oncology throughout Europe and Asia to monitor and quantify tumor vasculature [1]. It is an attractive modality for longitudinal treatment monitoring due to its high accessibility, noninvasiveness, and lack of ionizing radiation [2]. The intravenous contrast agent, a saline suspension of shell-stabilized gas-filled microbubbles, is non-toxic and restricted to the intravascular space, further limiting patient risk [3].

In a preclinical setting, the advantage of CEUS lies in its low relative cost, high spatial and temporal resolution, and versatile surface-ligand based tissue targeting. Unfortunately, the conventional baseline subtraction methods used for small-animal imaging are susceptible to registration errors and motion artifacts, which lead to reduced contrast-to-tissue ratios. These problems were addressed in clinical CEUS by the introduction of nonlinear contrast imaging methods, but the large established base [4] of preclinical scanners with linear-mode only transducers indicates that many users of preclinical CEUS would still benefit from improvements to linear CEUS processing. This paper proposes an alternative approach, called the *k*-method, which quantifies the change in image speckle statistics due to the incursion of microbubbles as a means of circumventing the artifacts associated with conventional linear CEUS processing methods.

In the proposed method, the echo signal from circulating microbubbles is modeled as a temporally varying random process that is superimposed onto a Rayleigh distributed signal from tissue backscatter. The microbubble signal component can be viewed as a second population of diffuse scatterers in the active vasculature within the tissue volume. Addition of the microbubble signal component is equivalent to adding additional random-walk steps to the speckle signal from a subset of the image data corresponding to perfused pixels. The relative contribution of the microbubble-enhanced subpopulation to the final image speckle depends on the contrast signal and therefore can act as a surrogate marker for the total vascular volume fraction of the imaged tumor.

MATERIALS AND METHODS

Experimental Image Acquisition

Nonlinear CEUS images were acquired using a Vevo 2100 high-frequency imaging system (VisualSonics Inc., Toronto, Canada) following a bolus (50 μL) tail vein injection of MicroMarker (VisualSonics Inc., Toronto, Canada) microbubble solution (2 x 10⁹ microbubbles/mL). The probe used was a MS-250 linear array transducer, with a center frequency of 20 MHz, that is capable of pulse-inversion sub-harmonic imaging. Two-dimensional “wash-in” cine loops were acquired from a translational breast cancer model in nude mice: a murine orthotopic xenograft of a human breast cancer cell line (MDA-MB-231LN) into the right inguinal mammary fat pad. The images analyzed in this experiment were taken 35 days post tumor inoculation.

Mathematical Model

The model represents the distribution of speckle due to tissue backscatter as a random-walk on the complex plane, yielding a Rayleigh probability density function for the envelope detected signal. The incursion of microbubbles into the imaging plane is assumed to be equivalent to adding additional random-walk steps to an active vasculature sub-population of tissue (Fig. 1). Superimposing this approximately Gaussian secondary component onto the tissue echoes has the effect of increasing the variance of the signal magnitude from a contrast-enhanced sub-volume relative to the unenhanced volumes.
Each step of the random walk is assumed to be independent. Thus, the relationship between the received complex echo signals from a contrast-enhanced sub-volume relative to a tissue sub-volume can be expressed as the summation

$$N_{\text{Enhanced}}(0, \sigma_E^2) = N_{\text{Tissue}}(0, \sigma_T^2) + N_{\text{Contrast}}(0, \sigma_C^2)$$  \hspace{1cm} (1)

where each $N(0, \sigma^2)$ distribution is a 2-D Gaussian on the complex plane centered at the origin and $\sigma_E^2 = \sigma_T^2 + \sigma_C^2$. The increase in variance due to the contrast-enhancement depends on the step size and number of steps in the contrast random walk. The step size and number represent the relative echo strength and concentration of microbubbles, respectively.

Envelope detection generates a Rayleigh probability density function for the signal magnitude from both the unenhanced and contrast-enhanced sub-volumes within the tumor. The statistical character of the entire enhanced tumor is expressed as a $k$-degree mixture distribution of Rayleigh PDFs:

$$PDF_{\text{Tumor}} = \sum_{i=1}^{k} W_i(t) \ast \text{Rayleigh}(\sigma_i),$$  \hspace{1cm} (2)

where the time-dependent variable $W_i(t)$ denotes the relative weighting of each Rayleigh component. Prior to contrast enhancement of the image, $W_i(t < \text{time of injection})$ only has non-zero values for the tissue-specific Rayleigh components and thus the tumor PDF is equal to the baseline tissue backscatter.

The random square norm (intensity) of a circular symmetric complex normal variable (the RF echo signal) has a generalized $\chi^2$ (Chi-squared) distribution. The signal magnitude therefore follows a $\chi$ (Chi) probability density function. It has been shown [5] that a $k$-component zero-mean Gaussian mixture distribution, where each mixture component differs with respect to variance, can also be described using a $\chi^2$ probability density function with a correction for reduced degrees of freedom. Therefore, the envelope-detected signal in the tumor model follows a $\chi$ probability density function with reduced degrees of freedom. The use of this model for linear CEUS is henceforth named the $k$-method. It is worth noting that the lower limit of this model, a single-component 2-D Gaussian, has two degrees of freedom yielding an exponential function for signal intensity ($\chi^2 \sim \text{Exp}(\frac{1}{2})$) and a Rayleigh distribution for signal magnitude ($\chi_2(x) \sim \text{Rayleigh}(1)$).
Data analysis

Contrast wash-in cine loops were exported as log-compressed, magnitude signal digital data files. The nonlinear contrast signal and corresponding linear B-mode images were analyzed independently of one another. Manually segmented regions of interest surrounding the tumor were rigidly registered between the linear and nonlinear dataset via translation and scaling. All analysis was performed using MATLAB (version R2008a, The MathWorks Inc., Natick, MA).

RESULTS

Appropriateness of Statistical Model Fit

Two representative frames from a contrast wash-in cine loop, one pre-injection and one post-injection (near peak), are shown in Fig. 2. The B-mode images and corresponding nonlinear CEUS images were taken from a transverse plane at approximately the center of the tumor mass. The manually segmented region of interest is shown as a red outline. This region was used to calculate the nonlinear and linear subtraction contrast signals, as well as the image histograms (Fig. 3).

The raw image histograms for the sample frames are shown in Fig. 3. A fitted scaled Chi distribution for this data set is overlain as a red curve. Chi distribution parameters (degrees of freedom, $k$, and scale, $s$) were estimated using the coefficient of variation of the sample data. The fitted Chi probability density function shows qualitative agreement to the raw histogram.

![FIGURE 2. Sample nonlinear CEUS images of a tumor (day 35), taken a) before injection of contrast agent and b) near peak enhancement post injection. Gray-scale B-mode images are on the left of each image, showing the manually segmented region of interest in red. Sepia tone images (right side of each panel) display nonlinear contrast signal power.](image)

![FIGURE 3. Histogram data of manually segmented regions of interest for the above sample images. Chi-distribution fit is displayed in red and shows qualitative agreement to the experimental data.](image)
Contrast Image Processing Comparison

For the purposes of comparison, a monoexponential curve was fit to the nonlinear CEUS, conventional linear subtraction CEUS, and the proposed statistical-model-based linear CEUS wash-in data taken from three mice. Due to the change in unit scale, the relevant index of comparison between these methods is the wash-in rate, or exponential growth rate.

The results obtained from processing a cine loop acquired during a contrast bolus for each mouse is shown in Fig. 4. The baseline for image subtraction for conventional linear CEUS processing was 100 frames in the same plane taken immediately prior to injection. A monoexponential fit was determined using a least squares nonlinear regression. The statistical k-method demonstrates more robustness than linear subtraction, as evidenced by the closer fit and reduced signal variation. It is worth noting that the wash-in rate of the k-method is consistently slower than the nonlinear contrast data.

**FIGURE 4.** A comparison of CEUS methods for three mice. Effective degrees of freedom shows less variability than linear subtraction, but a slower wash-in rate than the gold-standard nonlinear contrast ultrasound.
DISCUSSION

In this study, we propose an alternative approach of linear CEUS processing that relies on the statistical character of ultrasound speckle by modeling tissue and contrast agent as a variable random walk on the complex plane. We hypothesize that the reflected echo magnitude can be described using a Chi distribution with reduced degrees of freedom. The statistical distribution fit was found to be qualitatively accurate. The k-method, a measure of effective degrees of freedom, was found to be a more consistent index for the wash-in curve than the conventional linear subtraction method, when compared to nonlinear CEUS as a gold standard.

It was found that, generally, the statistical method of linear contrast analysis would underestimate the wash-in rate of the tumor as compared to nonlinear CEUS. The k-method measures effective degrees of freedom, an index of the complexity of the mixture weighting function, as opposed to indicator mass as in nonlinear methods. As such, it would indicate the concentration heterogeneity of the microbubble population.

The time of highest contrast enhancement does not necessarily coincide with the peak concentration heterogeneity. A physical interpretation of the wash-in delay for bolus imaging is that the contrast signal peak is relatively homogenous: the speckle contribution from microbubbles is mostly due to the initial injection concentration in fast flowing vasculature. Following the bolus, when total indicator mass begins to drop, the mixture weighting function complexity continues to increase as the slow flow vascular components perfuse with contrast agent. An alternative method of contrast imaging, destruction-reperfusion, may simplify the relationship of concentration complexity to indicator mass. Destruction-reperfusion employs continuous contrast infusion to fully perfuse the vascular bed, along with high-energy ultrasound pulses to generate a “negative bolus” by bursting microbubbles. Therefore, the point of maximum enhancement, when the tumor is completely perfused, should also coincide with a leveling off in the increase of model complexity over baseline.

The statistical k-method of indicator analysis shows promise in improving the robustness of linear CEUS, with the important caveat that curve interpretation differs from conventional indicator-dilution analysis. The removal of baseline subtraction streamlines imaging workflow, eliminates the problems associated with registration of pre- and post-injection images, and enables real-time linear CEUS perfusion imaging. Future work will include applying this method to contrast data gathered using destruction-reperfusion imaging.

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