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Quantitative Analysis of Vascular Heterogeneity in Breast Lesions Using Contrast-Enhanced 3-D Harmonic and Subharmonic Ultrasound Imaging

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Abstract

Ability to visualize breast lesion vascularity and quantify the vascular heterogeneity using contrast-enhanced 3-D harmonic (HI) and subharmonic (SHI) ultrasound imaging was investigated in a clinical population. Patients ($n = 134$) identified with breast lesions on mammography were scanned using power Doppler imaging, contrast-enhanced 3-D HI, and 3-D SHI on a modified Logiq 9 scanner (GE Healthcare). A region of interest corresponding to ultrasound contrast agent flow was identified in 4D View (GE Medical Systems) and mapped to

raw slice data to generate a map of time-intensity curves for the lesion volume. Time points corresponding to baseline, peak intensity, and washout of ultrasound contrast agent were identified and used to generate and compare vascular heterogeneity plots for malignant and benign lesions. Vascularity was observed with power Doppler imaging in 84 lesions (63 benign and 21 malignant). The 3-D HI showed flow in 8 lesions (5 benign and 3 malignant), whereas 3-D SHI visualized flow in 68 lesions (49 benign and 19 malignant). Analysis of vascular heterogeneity in the 3-D SHI volumes found benign lesions having a significant difference in vascularity between central and peripheral sections (1.71 ± 0.96 vs. 1.13 ± 0.79 dB, $p < 0.001$, respectively), whereas malignant lesions showed no difference (1.66 ± 1.39 vs. 1.24 ± 1.14 dB, $p = 0.24$), indicative of more vascular coverage. These preliminary results suggest quantitative evaluation of vascular heterogeneity in breast lesions using contrast-enhanced 3-D SHI is feasible and able to detect variations in vascularity between central and peripheral sections for benign and malignant lesions.

I. Introduction

Breast cancer ranks as the second most common cancer in the world and the most frequent type of cancer among women (25% of all cancers) [1]. The American Cancer Society estimated 232,340 new cases of invasive breast cancer, as well as an estimated 64,640 additional cases of *in situ* breast cancer and 39,620 breast cancer deaths among women in the United States in 2013 [2]. Mammography is used as the primary imaging modality for screening breast cancer. 65% to 90% of suspicious lesions identified by mammography are found to be benign after breast biopsy [3], [4]. Given the high false-positive rate, there is a need for an additional imaging modality to improve the ability to characterize breast lesions and reduce the number of unnecessary biopsies. Breast ultrasound (US) has been extensively studied with promising results [5]. The ability to easily differentiate between cystic and solid lesions (as compared with mammography) and produce high-resolution images in real-time give US imaging an edge over other breast imaging modalities [4], [6], [7]. Furthermore, US is non-ionizing, relatively inexpensive, and readily available compared with MRI and CT.

Studies have shown that angiogenesis plays an important role in tumor growth and progression [8]–[10]. The ability to visualize and quantify tumor vascularity could potentially serve as an important biomarker for characterization of breast lesions. Early studies using Doppler imaging techniques (power and color) to provide improved breast lesion characterization produced mixed results, due to low sensitivity to slow blood flow (<1 mm/s) and small vessels (<100 μ m) [11]–[14]. The ability to visualize this macro- and micro-vasculature has been proven to be possible using microbubble-based US contrast agents (UCAs) [14]–[17]. These UCAs are gas-filled microbubbles (diameter range: 1–8 μ m) that are able to traverse the lungs and capillaries. Microbubbles are very different in terms of their density and compressibility relative to the surrounding blood and tissue. This results in large acoustic impedance differences making these microbubbles very efficient scatterers of the US signal, in turn providing vascular enhancement on the US image. However, the majority of studies for imaging lesions used Doppler imaging in combination with UCA. Although this has been able to show an improvement in visualization of vasculature, the overall effectiveness of quantitative comparison between malignant and benign lesions is still not conclusive [7], [15]. This is in part due to lack of clear delineation

between the UCA (vascular flow) and surrounding tissue signals. Also, most studies to date have been performed in 2-D. Given that the angiogenic process in malignant lesions tends to be chaotic and tortuous, it is unlikely that the visualization of such vascular networks can be fully appreciated using a single imaging plane. A few studies have looked at the potential of contrast-enhanced 3-D US. One such study specifically evaluated the 3-D US score in evaluating breast tumor angiogenesis and its correlation with biological factors [18]. Another study evaluated the diagnostic potential of 3-D contrast-enhanced power Doppler for imaging breast lesions compared with conventional 2-D and 3-D US imaging [19]. Both studies showed promising results with regard to the use and advantages of contrast-enhanced 3-D US.

With sufficient acoustic excitation and correct transmit frequency (f_0), UCAs are able to elicit non-linear oscillations, producing a wide range of frequency components from the subharmonic ($f_0/2$) to higher harmonics ($n \cdot f_0$). Selectively receiving a specific harmonic may allow for improved UCA detection by substantially reducing linear echoes produced by tissue. One such UCA imaging mode that has now been implemented on most commercial US scanners is the second harmonic or harmonic imaging (HI) mode which suffers from low (up to 7 dB less than SHI *in vivo* [20]) UCA signal-to-tissue ratio because of second harmonic generation in the surrounding tissue [21]. However, generation of the subharmonic frequency component is UCA specific. Imaging at this frequency (i.e., $f_0/2$) therefore allows for improved UCA signal-to-tissue ratios by providing near complete tissue suppression. Our group as well as others have validated the feasibility of SHI [15], [21]–[27]. Three-dimensional imaging can visualize whole lesion volumes, which in turn may provide a more accurate representation of the vascular morphology required to characterize breast lesions [28]. The purpose of our study was to use contrast-enhanced 3-D HI and 3-D SHI to visualize the vascular networks of breast lesions and analyze the homogeneity of said vascularity.

II. Materials and Methods

The Institutional Review Boards of Thomas Jefferson University and University of California–San Diego approved this study. Patients who were identified with breast lesions using mammography and/or regular grayscale US and who were subsequently scheduled for a biopsy (or aspiration in case of cyst) as part of their clinical standard of care were approached to participate in the study. Patients were enrolled in the study after providing written informed consent. All US imaging was performed on a Logiq 9 scanner (GE Healthcare, Milwaukee, WI) equipped with a 4D10L probe modified to perform 3-D HI and 3-D SHI. *In vitro* and *in vivo* testing of the scanner modifications were conducted before this study [20]. For 3-D HI, the transmit frequency was set at 5.0 MHz (2-cycle transmit pulses) and receiving was at 10.0 MHz (bandpass filtered). Similarly, for 3-D SHI, transmit and receive frequencies were 5.8 (4-cycle transmit pulses) and 2.9 MHz (equalization filtered), respectively. Pulse inversion was implemented for both the contrast modes. The UCA used for this study was Definity (Lantheus Medical Imaging, Billerica, MA) [29]. The US transmit and receive parameters for 3-D HI and 3-D SHI were optimized for use with Definity in previous studies [20], [30]. Ultrasound scanning at each site was performed by an experienced sonographer.

For each patient, the lesion was localized using standard 2-D grayscale US and size measurements were recorded. Baseline 2-D grayscale and 2-D power Doppler imaging (PDI) cine clips were acquired, manually sweeping across the extent of the lesion. For the contrast studies, Definity was administered via a peripheral vein, typically the antecubital vein, through a 20-gauge catheter/needle. Contrast agent dosage was selected based on previous imaging experience by our group for each of the contrast modes [15], [30]. The 3-D HI volume acquisition was started simultaneously with the start of bolus administration of the UCA (0.25 mL followed by 10 mL saline flush) and continued until sufficient washout of UCA was observed (typically around 60 s). After a 30-min wait period, a second bolus injection of UCA (weight based dose of 20 $\mu\text{L}/\text{kg}$ up to a maximum of 1.25 mL) was administered and 3-D SHI volumes were acquired. For each patient, before the start of volume acquisition, imaging parameters such as gain (dB), acoustic power (%), depth (cm), focal position, volume angle (deg), and image quality were optimized for visualizing the extent of the lesion with good UCA signal-to-tissue contrast. Imaging parameters were adjusted independently for both 3-D imaging modes to minimize tissue signals, while simultaneously maintaining sufficient acoustic output to get a signal response from the UCAs. Image quality is a preset configurable option on the Logiq9 scanner in 4-D imaging mode that directly relates to the number of pixels used for the rendered image. Higher quality equals more pixels, but lower volume acquisition rate. The mechanical index at maximum acoustic output was 0.36 and 0.33 for 3-D HI and 3-D SHI, respectively. By default the volume angle was set to 19 degrees. At that imaging angle for smaller lesions (less than 1 cm^3) volume acquisition rates were around 3 H z. For larger lesions (greater than 3 cm^3) the acquisition rate dropped to around 1.5 H z because 3-D volume acquisition rates are directly linked to the size of the lesion/imaging area. In total, two still images of the lesion size measurements (along the sagittal and transverse planes), two cine clips of the 2-D baseline grayscale and PDI sweeps across the lesion and two volume files containing the contrast-enhanced 3-D HI and 3-D SHI were saved for each patient. These files were transferred from the scanner to a desktop computer for offline analysis.

A. Image Processing and Data Analysis

All image processing was performed in Matlab (2012a, The MathWorks Inc., Natick, MA, USA). Initially, a radiologist and US physicist (in consensus) analyzed the contrast-enhanced 3-D HI and 3-D SHI volumes (all planes included) to identify lesions with UCA flow using 4D View (GE Medical Systems, Zipf, Austria). The 4D View software allows visualization of the 3-D HI and 3-D SHI data offline. For each case it is possible to view the coronal, sagittal, and transverse planes as well as the overall 3-D rendered volume. This data are stored as a cine loop. It is also possible to analyze these data through the slices of the volume and identify planes with UCA flow (if present). If any single plane showed UCA activity, a ROI was selected corresponding to the visualized vascularity in 4D View. If none of the planes in the volume showed flow, then no ROI was designed. Both, the 3-D rendered image as well the individual slice information was used to identify UCA flow. This ROI was projected across the entire lesion volume. The ROI was then mapped on to the raw volumetric 3-D slice data extracted in Matlab. Time intensity curves (TICs) were generated for all slices within the ROI of the imaged volume (not just a single plane). These TICs were used to build a map of UCA flow (over the entire image acquisition time) within the lesion

volume. Our group has previously investigated the advantages and importance of 4D imaging over a single plane [30]. To standardize the TIC maps, all volumes were terminated after the 60-s time point.

The TIC map was used to develop an averaged volumetric background template based on the first 3 volumes (range; 1–3 s) of data. The volumetric background template was subtracted from the entire volume sequence to reduce any residual background tissue signals. During acquisition of volume data, the patient was asked to breathe normally; this in turn induced some motion artifacts. Basic smoothing was performed using a moving average filter (span of 5 time points) to reduce any breathing artifacts. Other filter lengths (2–10 time points) were tested, but a 5-point filter was long enough to smooth the small breathing artifacts without compromising the behavior of the UCA flow signal. The background filtered TIC map was then used to identify key time points corresponding to UCA flow kinetics; specifically, baseline (T_B : time point corresponding to 10% of peak UCA intensity), peak UCA intensity (T_P : time point of peak UCA intensity), and washout (T_W : time point of return to baseline UCA intensity). To analyze the vascular presence throughout the lesion volume, the normalized change in mean UCA intensity within the ROI (polyline) from T_B to T_P for each TIC in the volume was measured as a distribution across the entire volume. Subsequently, the lesion volume was split into peripheral (defined as the outer third of the entire tumor area including 2 mm around the lesion boundary) and central sections to facilitate assessment of vascular heterogeneity. Because the lesion was not always centered in the volume, separation was performed manually. During PDI acquisitions, a sweep of the entire lesion volume was performed, collecting information as individual 2-D PDI across the lesion volume. Vascularity was identified based on this information. Because this information is saved as a continuous 2-D sweep without any standardization of the sweep rate across patients, vascular heterogeneity was not evaluated in this mode. Moreover, we do not expect that 3-D PDI would perform any better, due to its lower line density relative to 2-D PDI.

B. Statistical Analysis

To identify any basic difference in the vascular characteristics between malignant and benign lesions, the average UCA intensity (single value) at T_P of all slices in the volume was evaluated and compared between malignant and benign cases using an unpaired *t*-test. Differences in the mean age of the patients between malignant and benign cases were also analyzed using a *t*-test. To evaluate the vascular heterogeneity across the lesion, the mean change in UCA intensity from baseline to peak for the peripheral and central sections was compared using an unpaired *t*-test. This was done for both malignant and benign lesions. All statistical analyses were performed using SPSS 20 (IBM Corporation, Armonk, NY) with *p*-values below 0.05 treated as statistically significant.

III. Results

A total of 138 patients were included in the study, and image data were available for 134 subjects because there was a technical problem saving the data in 4 cases (which were dropped from further analysis). The overall mean age of the women who participated in this study was 52 ± 13.3 years. Biopsy of lesions found 99 benign and 35 malignant cases. There

was a significant difference between the mean ages of the malignant and benign cases 58 ± 11.5 vs. 50 ± 13.3 years, respectively ($p < 0.001$). In terms of specific lesion types, invasive ductal carcinomas (23/35) made up the majority of the malignant cases, whereas fibroadenoma (30/99) was the most prevalent classification of the benign lesions; Table I.

Vascularity was observed in 84 cases (63 benign and 21 malignant) with PDI, whereas UCA flow was observed in 8 lesions (5 benign and 3 malignant) for 3-D HI and 68 lesions (49 benign and 19 malignant) with 3-D SHI. Vascular activity was better appreciated in 3-D SHI than in 3-D HI (based on qualitative assessment made by an US physicist and radiologist in consensus), due to the suppression of surrounding tissue signals (Fig. 1). Given the low number of cases identified with UCA flow in 3-D HI mode, no further image processing and analysis was performed on this group. Table I shows the breakdown of malignant and benign lesions in terms of cases identified with UCA flow using 3-D SHI as well as vascularity visualized in PDI mode.

The ability to view and manipulate a 3-D volume with 6 degrees of freedom was beneficial in identifying lesions with UCA flow. In certain cases UCA flow was not observed in the central (default) imaging plane, but after individually assessing the surrounding planes and/or being able to rotate the 3-D volume, UCA flow was apparent. This brings to light the heterogeneous nature of vascular growth within breast lesions as well as the importance of imaging lesions in 3-D space. Volumetric TIC maps based on the UCA flow were constructed after selection of an ROI corresponding to the vascular activity. Fig. 2(a) shows a TIC volume generated from an invasive ductal carcinoma. The wash-in and wash-out of the UCA are clearly visible. Breathing motion artifacts were suppressed to a large extent by the smoothing filter as seen in Fig. 2(b). The amount of inherent tissue suppression in 3-D SHI varied on a case-by-case basis and was dependent on the lesion depth, breast density (reported as part of the patient's clinical mammography examination), and heterogeneity. In some cases, there was near complete tissue suppression, whereas in others surrounding tissue signals were visibly higher. Background filtering was employed, using a volumetric background template to markedly reduce the residual surrounding tissue signal [Fig. 2(b)]. Fig. 2(c) shows suppression of tissue signals on volumetric slice data. Overall, the mean change in UCA intensity across the entire lesion volume between benign and malignant lesion groups was not significantly different (1.32 ± 0.89 vs. 1.38 ± 1.23 dB, $p = 0.73$).

Instead, vascular heterogeneity plots were developed (based on the relative change of UCA intensity from T_B to T_P for each slice within the lesion volume) to better quantify the variation in vascular activity. Fig. 3(a) depicts the vascular heterogeneity plot of a benign case (a fibroadenoma) across the peripheral and central sections. The presence of vascularity in the central sections is increased compared with the peripheral sections. The vascular heterogeneity of an invasive ductal carcinoma is shown in Fig. 3(b). In this case, vascular activity is seen across the entire lesion volume. Although a slight increase in vascular activity is seen in a few of the central slices compared with the general peripheral regions, this difference is not comparable to the variations in the benign case [Figs. 4(a) and 4(b)]. Overall, for benign lesions, the central sections of the lesion showed significantly increased vascular activity relative to the peripheral sections (1.71 ± 0.96 vs. 1.13 ± 0.79 dB, $p < 0.001$). For malignant lesions, however, no significant differences were observed in the

vascular activity between the central and peripheral sections (1.66 ± 1.39 vs. 1.24 ± 1.14 dB, $p = 0.24$); Fig. 4.

IV. Discussion

In this ongoing study, the ability to quantify vascular heterogeneity in breast lesions using contrast-enhanced 3-D HI and 3-D SHI was evaluated in 134 subjects. Both 3-D HI and 3-D SHI modes were able to successfully image and acquire volumetric data. However, 3-D SHI volumes proved to be more sensitive to visualizing UCA flow in the lesions showing UCA activity in 68 lesions compared with 8 in 3-D HI. The numbers for 3-D SHI compared reasonably with lesions exhibiting signs of vascular activity on PDI ($n = 84$). Based on *in vitro* and *in vivo* testing of our setup [20], we found that at equivalent acoustic output levels, HI resulted in similar received signal levels from the UCA to SHI. However, *in vitro* we also found HI showed a dramatic decrease in signal intensity with depth due to increased scattering and attenuation at higher frequencies compared with SHI, which showed increased penetration. When translated into clinical studies, the increased numbers of scattering components possibly induced a higher level of signal attenuation and therefore weaker response from the UCA.

Development of TIC volumes was based on the identification and selection of an ROI corresponding to UCA flow using 4D View. Subsequently, quantitative analysis of vascular heterogeneity in benign cases showed a uniform distribution of vascularity in the central sections of the lesion, possibly from a large central vessel, and minimal activity in the peripheral regions ($p < 0.001$). However, for malignant lesions a wider distribution of vascular activity both in central and peripheral regions was observed, resulting in no significant differences ($p = 0.24$). Early work involving angiography of breast carcinomas demonstrated chaotic vascular patterns including abnormal feeding vessels [31], [32]. In a separate study of endothelial cell proliferation (an essential precursor to angiogenesis) in breast lesions, Fox *et al.* showed a predominant presence at the periphery for tumors [33]. Work done by Weidner *et al.* [34] involving the microvessel density in invasive breast cancer concluded that the tumors were frequently heterogeneous in their microvessel density and that the areas of high neovascularization could occur anywhere in the tumor. Recently, Chen *et al.* showed significant difference in peripheral vessel characteristics between benign and malignant breast lesions using 3-D CEUs [35]. A recurring theme in all these studies is the extremely heterogeneous nature of the vasculature in malignant breast lesions. Although we have been able to successfully quantify the vascular heterogeneity in benign and malignant lesions using contrast-enhanced 3-D SHI, the clinical relevance of this result is yet to be determined. Although optimization based on individual patients will make cross comparison harder, it is more important to achieve the best possible data set in terms of diagnostic value for each patient. The end goal of this clinical study is to be able to provide additional information as an adjunct to mammography to deliver a better diagnostic assessment reflecting the clinical reality.

Although image variability is bound to arise due to biological differences in each subject 3-D imaging allows for acquisition of entire lesion volume, which should minimize the variations that might have occurred if imaged in a single plane only. In terms of image

acquisition, some motion artifacts are introduced from the US transducer being handheld and patient breathing. No direct motion compensation has been implemented for this data set. The raw volume slicedata are saved at a low resolution (usually around 40×50 pixels per slice) to allow system memory for real-time 3-D rendering of images. The 3-D volume acquisition rate is linked directly to the size of the imaging area. For larger lesions, a low volume acquisition rate (1.6 to 1.7 Hz) prevents ideal acquisition parameters for imaging UCA flow. Finally, although biopsy was performed on all lesions to determine malignancy, vascular heterogeneity was not measured on pathology and thus no vascularity reference standard was available.

V. Conclusion

In conclusion, we present our preliminary findings as part of an ongoing study for characterization of breast lesions using contrast-enhanced 3-D HI and 3-D SHI. Quantitative analysis of vascular heterogeneity is shown to be feasible using contrast-enhanced 3-D SHI with significant differences in the vascularity between central and peripheral sections of benign lesions ($p < 0.001$). Malignant lesions exhibited an increased spread of vascularity throughout the entire lesion volume with no significant difference in the central and peripheral sections ($p = 0.24$) concurrent with their chaotic and variable vascular nature.

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References

1. Ferlay, J.; Soerjomataram, I.; Ervik, M.; Forman, D.; Bray, F.; Dikshit, R.; Elser, S.; Mathers, C.; Rebelo, M.; Parkin, DM. Cancer Incidence and Mortality Worldwide: IARC CancerBase No. 11 [Online]. Available: http://globocan.iarc.fr/Pages/fact_sheets_cancer.aspx
2. DeSantis C, Ma J, Bryan L, Jemal A. Breast cancer statistics, 2013. CA Cancer J Clin. 2014; 64(1): 52–62. [PubMed: 24114568]
3. Kopans, DB. Breast Imaging. Vol. 1. Philadelphia, PA: Lippincott Williams & Wilkins; 2007.
4. Zonderland HM, Coerkamp EG, Hermans J, van de Vijver MJ, van Voorthuisen AE. Diagnosis of breast cancer: Contribution of US as an adjunct to mammography. Radiology. 1999; 213(2):413–422. [PubMed: 10551221]
5. Stavros, AT. Breast Ultrasound. Philadelphia, PA: Lippincott Williams & Wilkins; 2004.
6. Stavros AT, Thickman D, Rapp C, Dennis M, Parker S, Sisney G. Solid breast nodules: Use of sonography to distinguish between benign and malignant lesions. Radiology. 1995; 196(1):123–134. [PubMed: 7784555]
7. Taylor KJ, Merritt C, Piccoli C, Schmidt R, Rouse G, Fornage B, Rubin E, Georgian-Smith D, Winsberg F, Goldberg B, Mendelson E. Ultrasound as a complement to mammography and breast examination to characterize breast masses. Ultrasound Med Biol. 2002; 28(1):19–26. [PubMed: 11879948]
8. Folkman J. What is the evidence that tumors are angiogenesis dependent? J Natl Cancer Inst. 1990; 82(1):4–6. [PubMed: 1688381]
9. Weidner N, Folkman J, Pozza F, Bevilacqua P, Allred EN, Moore DH, Meli S, Gasparini G. Tumor angiogenesis: A new significant and independent prognostic indicator in early-stage breast carcinoma. J Natl Cancer Inst. 1992; 84(24):1875–1887. [PubMed: 1281237]

10. Gasparini G, Harris AL. Clinical importance of the determination of tumor angiogenesis in breast carcinoma: Much more than a new prognostic tool. *J Clin Oncol*. 1995; 13(3):765–782. [PubMed: 7533829]
11. Adler DD, Carson PL, Rubin JM, Quinn-Reid D. Doppler ultrasound color flow imaging in the study of breast cancer: Preliminary findings. *Ultrasound Med Biol*. 1990; 16(6):553–559. [PubMed: 2238263]
12. Carson PL, Fowlkes JB, Roubidoux MA, Moskalik AP, Govil A, Normolle D, LeCarpentier G, Nattakom S, Helvie M, Rubin JM. 3-D color Doppler image quantification of breast masses. *Ultrasound Med Biol*. 1998; 24(7):945–952. [PubMed: 9809628]
13. Sehgal CM, Arger P, Rowling S, Conant E, Reynolds C, Patton J. Quantitative vascularity of breast masses by Doppler imaging: Regional variations and diagnostic implications. *J Ultrasound Med*. 2000; 19(7):427–440. [PubMed: 10898296]
14. Ferrara KW, Merritt CR, Burns PN, Foster FS, Mattrey RF, Wickline SA. Evaluation of tumor angiogenesis with US: Imaging, Doppler, and contrast agents. *Acad Radiol*. 2000; 7(10):824–839. [PubMed: 11048880]
15. Forsberg F, Piccoli CW, Merton DA, Palazzo JJ, Hall AL. Breast lesions: Imaging with contrast-enhanced subharmonic US—Initial experience. *Radiology*. 2007; 244(3):718–726. [PubMed: 17690324]
16. Burns P, Powers J, Simpson DH, Brezina A, Kolin A, Chin C, Uhlendorf V, Fritzsche T. Harmonic power mode Doppler using microbubble contrast agents: An improved method for small vessel flow imaging. *Proc Ultrasonics Symp*. 1994:1547–1550.
17. Chaudhari MH, Forsberg F, Voodarla A, Saikali F, Goonewardene S, Needleman L, Finkel GC, Goldberg BB. Breast tumor vascularity identified by contrast enhanced ultrasound and pathology: Initial results. *Ultrasonics*. 2000; 38(1–8):105–109. [PubMed: 10829638]
18. Jia WR, Chai WM, Tang L, Wang Y, Fei XC, Han BS, Chen M. Three-dimensional contrast enhanced ultrasound score and dynamic contrast-enhanced magnetic resonance imaging score in evaluating breast tumor angiogenesis: Correlation with biological factors. *Eur J Radiol*. 2014; 83(7):1098–1105. [PubMed: 24794865]
19. Forsberg F, Goldberg BB, Merritt CR, Parker L, Maitino AJ, Palazzo JJ, Merton DA, Schultz SM, Needleman L. Diagnosing breast lesions with contrast-enhanced 3-dimensional power Doppler imaging. *J Ultrasound Med*. 2004; 23(2):173–182. [PubMed: 14992354]
20. Eisenbrey JR, Sridharan A, Machado P, Zhao H, Halldorsdottir VG, Dave JK, Liu JB, Park S, Dianis S, Wallace K, Thomenius KE, Forsberg F. Three-dimensional subharmonic ultrasound imaging in vitro and in vivo. *Acad Radiol*. 2012; 19(6):732–739. [PubMed: 22464198]
21. Forsberg F, Shi W, Goldberg B. Subharmonic imaging of contrast agents. *Ultrasonics*. 2000; 38(1–8):93–98. [PubMed: 10829636]
22. Bhagavatheeshwaran G, Shi WT, Forsberg F, Shankar P. Subharmonic signal generation from contrast agents in simulated neovessels. *Ultrasound Med Biol*. 2004; 30(2):199–203. [PubMed: 14998672]
23. Eisenbrey JR, Dave JK, Merton DA, Palazzo JP, Hall AL, Forsberg F. Parametric imaging using subharmonic signals from ultrasound contrast agents in patients with breast lesions. *J Ultrasound Med*. 2011; 30(1):85–92. [PubMed: 21193708]
24. Shi WT, Forsberg F, Hall AL, Chiao RY, Liu JB, Miller S, Thomenius KE, Wheatley MA, Goldberg BB. Subharmonic imaging with microbubble contrast agents: Initial results. *Ultrason Imaging*. 1999; 21(2):79–94. [PubMed: 10485563]
25. Shankar PM, Krishna P, Newhouse V. Subharmonic back-scattering from ultrasound contrast agents. *J Acoust Soc Am*. 1999; 106(4):2104–2110. pt 1. [PubMed: 10530033]
26. Goertz DE, Frijlink ME, Tempel D, Bhagwandas V, Gisolf A, Krams R, de Jong N, van der Steen AF. Subharmonic contrast intravascular ultrasound for vasa vasorum imaging. *Ultrasound Med Biol*. 2007; 33(12):1859–1872. [PubMed: 17683850]
27. Chomas J, Dayton P, May D, Ferrara K. Nondestructive subharmonic imaging. *IEEE Trans Ultrason Ferroelectr Freq Control*. 2002; 49(7):883–892. [PubMed: 12152942]

28. Gessner RC, Aylward SR, Dayton PA. Mapping microvasculature with acoustic angiography yields quantifiable differences between healthy and tumor-bearing tissue volumes in a rodent model. *Radiology*. 2012; 264(3):733–740. [PubMed: 22771882]
29. Goertz DE, de Jong N, van der Steen A. Attenuation and size distribution measurements of Definity and manipulated Definity populations. *Ultrasound Med Biol*. 2007; 33(9):1376–1388. [PubMed: 17521801]
30. Sridharan A, Eisenbrey JR, Liu JB, Machado P, Halldorsdottir VG, Dave JK, Zhao H, He Y, Park S, Dianis S, Wallace K, Thomenius KE, Forsberg F. Perfusion estimation using contrast-enhanced 3-dimensional subharmonic ultrasound imaging: An in vivo study. *Invest Radiol*. 2013; 48(9): 654–660. [PubMed: 23695085]
31. Watt AC, Ackerman LV, Shetty PC, Burke M, Flynn M, Grodzinsky C, Fine G, Wilderman S. Differentiation between benign and malignant disease of the breast using digital subtraction angiography of the breast. *Cancer*. 1985; 56(6):1287–1292. [PubMed: 3896454]
32. Feldman F. Angiography of cancer of the breast. *Cancer*. 1969; 23(4):803–808. [PubMed: 5775969]
33. Fox SB, Gatter KC, Bicknell R, Going JJ, Stanton P, Cooke TG, Harris AL. Relationship of endothelial cell proliferation to tumor vascularity in human breast cancer. *Cancer Res*. 1993; 53(18):4161–4163. [PubMed: 7689928]
34. Weidner N, Semple JP, Welch WR, Folkman J. Tumor angiogenesis and metastasis—Correlation in invasive breast carcinoma. *N Engl J Med*. 1991; 324(1):1–8. [PubMed: 1701519]
35. Chen M, Wang WP, Jia WR, Tang L, Wang Y, Zhan WW, Fei XC. Three-dimensional contrast-enhanced sonography in the assessment of breast tumor angiogenesis correlation with microvessel density and vascular endothelial growth factor expression. *J Ultrasound Med*. 2014; 33(5):835–846. [PubMed: 24764339]

Biographies

Anush Sridharan was born in Remera, Rwanda, in 1987. He received his B.E. degree in electrical and electronics engineering from Sri Venkateswara College of Engineering, Chennai, India, in 2009 and M.Sc. degree in electrical and computer engineering from Drexel University, Philadelphia, PA, in 2011. His master's research involved the study of atherosclerotic plaque using contrast enhanced intravascular ultrasound imaging. As a doctoral candidate in the Electrical and Computer Engineering program at Drexel University, his research is focused on the development of three-dimensional imaging techniques for the visualization of breast lesion vascularity using contrast-enhanced ultrasound imaging. His research work is based out of Thomas Jefferson University, Philadelphia, PA. Anush is a student member of IEEE and the American Institute of Ultrasound in Medicine. He is also an avid runner.

John R. Eisenbrey was born in Christiana, DE. He earned his B.Eng. degree in mechanical engineering and B.S. degree in management at the University of Delaware in Newark, DE. He then completed his masters' and Ph.D. degrees in biomedical engineering at Drexel University, Philadelphia, PA, focusing on ultrasound contrast media development. He is currently employed as a research assistant professor in Thomas Jefferson University's Department of Radiology, Ultrasound Division. His research interests focus on contrast-enhanced ultrasound imaging, the use of contrast agents for pressure estimation, and ultrasound-triggered drug delivery. John is currently a member of IEEE, the American Institute of Ultrasound in Medicine, the International Contrast Ultrasound Society, and the American Association for the Advancement of Science.

Priscilla Machado was born in Sao Paulo, Brazil, on August 12, 1978. She received her medical degree from Faculdade de Medicina do ABC, Santo Andre, Brazil in 2003, and did her residence in Radiology at Santa Casa de Sao Paulo, Sao Paulo, Brazil, finishing in 2007. After that, she worked in private practice for 3 years. In 2010, she started her research fellowship in the Department of Radiology at Thomas Jefferson University, where she stayed for four and a half years; her research focused on ultrasound contrast agents, subharmonic imaging, quantitative contrast measurements and novel clinical imaging modes. She went back to work in private practice in 2014.

Haydee Ojeda-Fournier's photograph and biography were unavailable at time of publication.

Annina Wilkes is a Radiologist at Thomas Jefferson University Hospital, specializing in breast imaging and ultrasound. Dr. Wilkes is a graduate of Tufts University, Temple University Medical School, and completed her fellowship training in ultrasound and breast imaging at Thomas Jefferson University Hospital. She has been on staff at Jefferson for 25 years.

Her clinical activities include interpretation of mammograms, breast ultrasound, and MRI as well as performing percutaneous breast biopsies and cyst aspirations. The Thomas Jefferson Breast Imaging Center performs more than 40 000 studies per year. Clinical activities also include interpretation of diagnostic ultrasound, with a focus on women's imaging.

Her academic interests include teaching and research. Her recent research has been in the fields of breast ultrasound and digital mammography.

Dr. Wilkes enjoys full participation in community education forums and educating women about ways to improve their health. She frequently conducts seminars about the importance of screening mammography. Dr. Wilkes enjoys travel and has been an international visiting professor in radiology in many countries including South Africa, Uganda, Egypt, Italy, France, Belgium, Honduras, and Mexico.

Dr. Wilkes lives in Philadelphia, PA, with her husband of 32 years and is the proud parent of two daughters.

Alexander Sevrakov was born in Moscow, Russia, in 1974. He received an M.D. degree from the Sechenov Moscow Medical Academy (former Medical Faculty of the Moscow Imperial University and now the First Moscow State Medical University) in 1998.

From 1999 to 2005, he worked on cardiac CT imaging as a Sr. Research Specialist at the University of Illinois at Chicago. He began his residency training in radiology at Washington University, Mallinckrodt Institute of Radiology in St. Louis in 2005, followed

by clinical fellowships in abdominal and breast imaging in 2009 and 2010, respectively. In 2011, he joined the faculty of the Department of Radiology at Thomas Jefferson University specializing in breast imaging, and since 2013 has been the Director of Women's Diagnostic Center at Jefferson's Methodist Hospital. His clinical and research interests are in the areas of screening and diagnosis of breast cancer. He is a member of the American College of Radiology, the Radiologic Society of North America, and the Society of Breast Imaging.

Robert F. Mattrey was born in 1948. He received his B.S. and M.S. degrees in electrical engineering and his M.D. degree from the State University of New York at Buffalo in 1971, 1973, and 1978, respectively. He completed his diagnostic radiology residency and body imaging fellowship at the University of California, San Diego in 1981 and 1982; he joined the faculty in 1982 and has remained at UCSD. He became a Professor of Radiology in 1988.

He has served UCSD and the scientific communities in many capacities, including as President of the Association of University Radiologist in 1998 and the President of the Radiology Research Alliance in 2002. He also served as the Vice-Chair and Director of Research at UCSD from 1996 to 2014. Dr. Mattrey has won and been given many prestigious awards including the Gold Medal from the Association of University Radiologists, the Life-time Achievement Award from the Society of Abdominal Radiology, and has been inducted in the Academy of Radiology Research of Distinguished Investigators.

Dr. Mattrey is a clinician/scientist with research expertise in contrast media, instrumentation, drug/gene delivery, and nanotechnology, with special interest in ultrasound. He has translated contrast media for CT, MRI, and ultrasound imaging. His current interest is in molecular imaging in general and ultrasound molecular imaging and therapy in particular.

Kirk Wallace was born in Cherry Point, NC, in 1970. He received the B.A. degree in physics and mathematics from Ithaca College, Ithaca, NY, in 1992 and the M.A. degree in physics from Washington University in St. Louis, St. Louis, MO, in 1994. Kirk worked at HP Medical Products Group, Andover, MA, in 1995 and 1996 on applied echocardiographic research topics. In 2001, he received his Ph.D. degree in physics from Washington University in St. Louis with research focused on myocardial tissue characterization, targeted contrast agents, and nonlinear propagation of diffracting finite-amplitude ultrasound fields. From 2002 to 2005, he held an appointment as research faculty in the Department of Physics at Washington University in St. Louis. From 2006 to 2010, he was a senior scientist within the Cardiovascular Division of the Washington University School of Medicine and researcher within the Siteman Center of Cancer Nanotechnology Excellence. In 2010, Kirk moved to upstate NY and transitioned from academia to industry, joining the ultrasound research team at GE Global Research in Niskayuna, NY, where his research interests include diagnostic and therapeutic applications of ultrasound. Dr. Wallace is a member of the Institute of Electrical and Electronic Engineers (IEEE), the Acoustical Society of America (ASA), and the Grand National Curling Club (GNC), where he is an avid curler during the winter months.

Carl Chalek received the Sc.B. degree in chemistry from Brown University, Providence, RI, in 1973, the Ph.D. degree from the Massachusetts Institute of Technology, Cambridge, MA, in 1977, and the M.S. degree in computer science from Union College, Schenectady, NY, in 1988.

He began his professional career as a chemist at the General Electric Corporate Research and Development Center in Niskayuna, NY, working on lighting applications. In 1984, his focus changed to laboratory automation, and since 1988, he has been working on applications of computer science in medical diagnostic ultrasound imaging.

Kai E. Thomenius (M'66) was born a long time ago in Helsinki, Finland. His academic background is in electrical engineering with a minor in physiology with B.S., M.S., and Ph.D. degrees from Rutgers University in 1968, 1970, and 1978, respectively.

Dr. Thomenius is currently a Visiting Scientist at the Institute of Medical Engineering and Science at MIT, Cambridge, MA. Until his recent retirement, he was a Chief Technologist in Diagnostics & Biomedical Technologies at General Electric Global Research in Niskayuna, NY, USA. Previously, he has held senior R&D roles at ATL Ultrasound Inc. (now part of Philips), Interspec Inc., and Elscint Inc., as well as other companies. In addition, he has been an Adjunct Professor in the Electrical, Computer, and Systems Engineering Department at Rensselaer Polytechnic Institute.

Dr. Thomenius' long-term interests are in beam formation and miniaturization of scanners, propagation of acoustic waves in inhomogeneous media, delivery of drugs and DNA to cells, and gaining physiological information from echoes that arise from ultrasound beams. Dr. Thomenius is a Fellow of the American Institute of Ultrasound in Medicine. He is on the Editorial Board of *Ultrasonic Imaging*. He is a frequent reviewer of manuscripts for *IEEE Transactions on Ultrasonics, Ferroelectrics, and Frequency Control*; *Ultrasound in Medicine and Biology*; the *Journal of Applied Physics*; *Ultrasonic Imaging*; *Ultrasonics*, and other journals, as well as serving on the technical program committees for several ultrasound- related symposia. Similarly, Dr. Thomenius is a reviewer of grant proposals for the National Institutes of Health (NIH) and other agencies.

Flemming Forsberg was born in Copenhagen, Denmark, on October 20, 1961. He received an M.Sc. degree in electronic engineering and a Ph.D. degree in biomedical engineering from the Technical University of Denmark, Lyngby, Denmark, in 1987 and 1990, respectively.

From 1987 to 1990, he worked on Doppler ultrasound signal processing at the Electronics Institute, Technical University of Denmark, before becoming a postdoctoral research fellow at King's College, London, England from 1990 to 1991. After a year in private industry, developing an intravascular imaging system, he went to the department of Radiology, Thomas Jefferson University, Philadelphia, PA, in 1992 as an Ultrasound Physicist. Dr. Forsberg is currently a Professor of Radiology, and his research focuses on ultrasound contrast agents, subharmonic imaging, quantitative contrast measurements, and novel clinical imaging modes.

Dr. Forsberg is a senior member of the Institute of Electrical and Electronic Engineers (IEEE), and a fellow of the American Institute of Ultrasound in Medicine (AIUM) as well as the American Institute for Medical and Biological Engineering (AIMBE). He is a member of the Editorial Boards of *Ultrasound in Medicine and Biology* and *Ultrasonic Imaging* as well as a Deputy Editor for Basic Science of the *Journal of Ultrasound in Medicine*.

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Fig. 1. Contrast-enhanced 3-D HI and 3-D SHI at peak UCA intensity in the same lesion. Regions of UCA flow are visible (yellow arrows) on the axial planes and on the rendered image (red arrows).

Fig. 2.

(a) Volumetric TIC of an invasive ductal carcinoma, (b) TIC volume showing background tissue suppression after background filtering and smoothing. Key time points corresponding to baseline (T_B), peak intensity (T_P), and wash-out (T_W) of UCA flow are identified on the TIC volume, and (c) suppression of surrounding tissue signal is seen after background filtering on the volumetric slicedata.

Fig. 3. Vascular heterogeneity plots of (a) benign (fibroadenoma) and (b) malignant (invasive ductal carcinoma) lesions across the peripheral and central sections of the lesion volume.

Fig. 4. Overall comparison of vascularity between the central and peripheral sections for benign and malignant cases.

TABLE I

Breakdown of Lesions by Type Showing Vascularity in Power Doppler (PDI) and UCA Flow in 3-D SHI.

	Lesion type	No. of cases	Flow in 3-D SHI	Flow in PDI
Benign	Fibrosis and simple cysts	16	7	5
	Hyperplasia	11	7	11
	Adenosis	5	4	3
	Fibroadenomas	30	16	21
	Intraductal papillomas	7	6	6
	Fat necrosis and oil cysts	1		
	Mastitis	1	1	
	Fibroepithelial lesion	2	1	1
	Other	15	2	7
	Mixed	11	5	9
Malignant	Ductal carcinoma <i>in situ</i>	5	2	3
	Invasive (or infiltrating) ductal carcinoma	23	13	14
	Invasive (or infiltrating) lobular carcinoma	2	2	
	Papillary carcinoma	1	1	1
	Mixed carcinoma (both invasive ductal and lobular)	4	1	3
	Total	134	68	84