Morphological Reconstruction Improves Microvessel Mapping in Super-Resolution Ultrasound

Scott Schoen, Jr.®, Graduate Student Member, IEEE, Zhigen Zhao, Student Member, IEEE, Ashley Alva, Student Member, IEEE, Chengwu Huang®, Shigao Chen®, Senior Member, IEEE, and Costas Arvanitis®, Member, IEEE

Abstract—Generation of super-resolution (SR) ultrasound (US) images, created from the successive localization of individual microbubbles in the circulation, has enabled the visualization of microvascular structure and flow at a level of detail that was not possible previously. Despite rapid progress, tradeoffs between spatial and temporal resolution may challenge the translation of this promising technology to the clinic. To temper these tradeoffs, we propose a method based on morphological image reconstruction. This method can extract from ultrafast contrast-enhanced US (CEUS) images hundreds of microbubble peaks per image (312-by-180 pixels) with intensity values varying by an order of magnitude. Specifically, it offers a fourfold increase in the number of peaks detected per frame, requires on the order of 100 ms for processing, and is robust to additive electronic noise (down to 3.6-dB CNR in CEUS images). By integrating this method to an SR framework, we demonstrate a sixfold improvement in spatial resolution, when compared with CEUS, in imaging chicken embryo microvessels. This method that is computationally efficient and, thus, scalable to large data sets may augment the abilities of SR-US in imaging microvascular structure and function.

Index Terms—Acoustic cavitation, super-resolution (SR), ultrasound (US) imaging.

I. INTRODUCTION

MICROBUBBLE ultrasound contrast agents (USCAs) exploit the way in which sound waves are reflected due to acoustic impedance differences between these microscale gas pockets and blood or tissue. As the acoustic impedance of gas is typically thousands of times lower than blood or tissue, gas microbubbles are ideal point scatterers for ultrasound (US), offering sufficient SNR to detect a single microbubble [1]. Moreover, their nonlinear response provides the means to isolate their echoes from the tissue. As such, USCAs are routinely used in the clinics for vascular imaging using B-mode (a pulse echo technique to localize linear microbubble echoes) or nonlinear imaging, such as pulse inversion (a coherence-based phase inversion technique to localize nonlinear microbubble echoes) [2]–[4]. Despite the improved ability of contrast-enhanced US (CEUS) to quantify tissue perfusion using USCAs, there is an inherent tradeoff between the resolution and penetration depth that for clinically relevant frequencies provides an effective lower bound to the imaging resolution (e.g., for a \( f_0 = 4 \) MHz pulse the limit is \( \sim c/f_0 = 385 \, \mu \text{m} \)).

By expanding concepts originally developed in superlocalization optical microscopy [5], [6], it was shown that US in combination with microbubbles [7]–[10] or phase-changing nanodroplets [11]–[13] can alleviate tradeoffs between resolution and penetration depth. Based on these methods, microbubbles that act as (moving) sono-activatable point sources are localized one by one with a precision far beyond the diffraction limit by identifying the centroid or peak intensity of each isolated peak region from each frame. Superimposing all the localized points to form density maps of USCA positions allows to resolve the microvascular “fingerprint” of organs with subwavelength resolution [10], [14]. Resolution eightfold below the diffraction limit has been consistently reported across several studies in healthy and diseased rodents using research or clinical US scanners [10], [14]–[16] (see also Suppl. Table S-1). Such super-resolution US (SR-US) techniques have a theoretically achievable resolution on the order of a few micrometers for clinical US frequencies [17]. However, the improved spatial resolution of SR-US comes at the cost of poor temporal resolution, defined as the total time required to acquire the data and generate the final image [18]. While high frame rates (up to several kilohertz) [19] have enabled reduced image acquisition times...
for SR-US with microbubbles from minutes [8], [10], [20] to a few seconds [7], [21]–[23], the processing time to generate the final image (i.e., peak extraction and final image formation) can take from several minutes to several hours to complete [18]. Moreover, due to: 1) the stringent selection and acceptance criteria (e.g., a single bubble detection threshold); 2) the proportionally fewer bubbles in microvessels, which are the vessels of particular interest in SR-US [24]; and 3) the potentially lower SNR of the USCAs in these vessels (due to higher damping by the vessel wall), which might render them below detection thresholds [8], [9], [25], only a small fraction of the bubbles that fulfill all the selection criteria are used for imaging sub-diffraction vessels. Collectively, these constraints limit our ability to overcome tradeoffs between temporal and spatial resolution with minimum penalty on image quality.

Optimizing each processing step in the SR framework (i.e., detection of microbubbles from the surrounding tissue, isolation of individual microbubble signals, and localization of the microbubble at a precision beyond the diffraction limit) could potentially address these tradeoffs without compromising the image quality (i.e., how well a microvessel is resolved). For instance, increasing the number of bubbles identified per frame could either reduce the number of frames required for the final SR-US image or lead to improved contrast-to-noise ratio (CNR) (for a given number of frames) and accuracy, as more USCAs will be detected from a given number of frames. To date, up to tens of bubbles within a single frame have been detected (about 1 bubble per thousand pixels) using high SNR data sets [22], [23] (see Suppl. Table S-1). Improving the number of bubbles detected in each frame, without introducing additional computational burden, could have palpable effects on temporal resolution.

Here, we propose to use grayscale morphological reconstruction (MR) to isolate and localize up to several hundred bubbles per frame with different signal intensities. We first analyze the ability of MR to super-localize points and characterize the effects of algorithm parameters and added noise on the generated SR images. With use of reference optical microscopy data, we estimate the accuracy of the localizations and the performance of the technique compared with the theoretical maximum performance. Finally, we measure the computational efficiency of the algorithm.

II. METHODS

A. Data Acquisition

Data were collected as reported in [22] and as recounted briefly here. The CEUS images were taken from the choroidal-lantoic membrane (CAM) of chicken embryos. This model is attractive due to the long microbubble recirculation times, small bulk tissue motions, and the ability to directly compare the US images with high-resolution optical microscopy images of the vasculature. A bolus injection of microbubbles (Lumason, Bracco Diagnostics Inc., Monroe Township, NJ) at $1.8 \times 10^7$ microbubbles per milliliter and imaging acquisitions were performed at the microbubble concentration plateau after the injection.

CEUS images were obtained with a Verasonics Vantage 256 ultrasound system (Verasonics Inc., Kirkland, WA, USA) with a 25-MHz linear array transducer (L35-16vX, Verasonics Inc.). Ultrafast plane wave imaging (15 angles, $-7^\circ$ to $7^\circ$) was performed at 500 compounded frames per second, with 5-V transmit excitation. At each location, five successive acquisitions of 720 frames each (total acquisition length 3600 frames over 7.2 s) were captured. The IQ data were stored and postprocessed with custom MATLAB scripts on a standard desktop computer (four cores at 2.8 GHz, 16-GB RAM).

B. Raw Data Filtering

To distinguish better the bubble signal from tissue scattering, an SVD filter was applied to each stack of raw CEUS images [Fig. 1(a)]. Singular values larger than 10% of the first (maximum) singular value (typically the first two to five values) were set to 0 [Fig. 1(b)]. Additionally, the smallest singular value was seen to contain largely noise and was also removed. Filtered frames were interpolated from the original pixel size (60 $\mu$m by 30 $\mu$m; diffraction limits in the axial and transverse directions) up to 12 times (5 $\mu$m by 2.5 $\mu$m). To ensure the intensity varied smoothly and improve isolation by the MR algorithm, the frames were then smoothed with a 2-D Gaussian filter with size 30 $\mu$m.

C. Morphological Reconstruction

To identify local intensity maxima whose absolute intensities may vary widely (i.e., that would be missed by simple thresholding), each filtered, smoothed, interpolated frame was multiplied by a factor $(1 - h)$ where $h \sim 0.05$, to obtain marker image, and the original map is used as the mask image [Fig. 1(c1)]. The optimal value of the morphological offset $h$ depends on the data; data with low background signal permit a smaller threshold and effectively higher sensitivity, which potentially may allow more bubble peaks to be found in each frame. Then, a grayscale MR was performed with the marker and mask images (via MATLAB’s imreconstruct); this process may be considered as repeated dilations of the marker image until it fills the mask as in Fig. 1(c2). Finally, this reconstruction (the masked dilated image) was subtracted from the original smoothed map. The effect of this procedure is to segment the image into regions containing regional maxima (termed “h-domes” by Vincent [26]; see the Appendix), which now have comparable amplitudes despite their disparate intensity values in the raw image [Fig. 1(c3)]. Finally, peaks in the reconstructed frame with amplitudes within 10% of the maximum intensity of that frame were retained. To compare with a simple thresholding method, the SVD filtered images were also binarized (such that image regions with less than some threshold fraction of the peak intensity, nominally 90% but varied to adjust the sensitivity [8], were set to 0) to form the segmented peak regions. Representative peak region images, with and without MR, are shown in Suppl. Fig. S-1.
Finally, to super-localize the bubble within each segmented region, the orientation of each region was computed, and a local 2-D convolution of the region was performed with a Gaussian approximation of the observed point spread function at the specific location (standard deviation of 30 μm in each direction) [27]. While a uniform PSF was used here, a position-dependent one could be used since the locations of the peak regions are known. The peak of each of these convolutions was taken as the super-localized (SL) bubble location [Fig. 1(c3)]. SR images were formed by summing uniform amplitude Gaussians (standard deviations of λ/8) at each SL bubble location [Fig. 1(d) and (e)].

The CNR for the images at a given location \( r \) was computed as

\[
\text{CNR}(r) = \frac{I(r) - \mu_{bg}}{\sigma_{bg}}
\]

where \( I \) is the image intensity, and \( \mu_{bg} \) and \( \sigma_{bg} \) are the mean and standard deviation, respectively, of the intensity in a manually specified background region. To evaluate the performance of MR super-localizations in the presence of noise, following application of the SVD filter, we added Gaussian-weighted random noise with amplitude equal to some fraction of each individual frame’s mean intensity.

**D. Evaluation of Localization Accuracy**

To determine the accuracy and quality of the peaks found via MR, the final SR image (i.e., the intensity field due to the summed Gaussian distributions at each SR peak location) was registered with an optical microscopy image of the vasculature (via MATLAB’s `imregister`). From the registered optical data, a binary mask was created as a reference standard [Fig. 1(f)], such that the SR points in the acoustic image within the mask are considered true positives, that is, they fall within the vasculature and may be considered microbubble localizations. To create the binary mask, the intensity of the optical microscopy image was adjusted to account for nonuniform illumination (with MATLAB’s default `imadjust`). Then, an empirically determined normalized intensity threshold (here 0.4) was used to binarize the image. Finally, to ensure the remaining regions represented vessels and not noise from the optical data, an area opening algorithm (`imopen`) was used to remove binary regions whose total size was less than 400 pixels (0.4 μm²).

As the registration is imperfect, a tolerance distance \( \delta \) was defined such that if the SL points were less than \( \delta \) from the vessel mask, they were considered successful localizations. Finally, knowledge of the mask area and size of the imaging wavelength were used to estimate the upper bound on the number of bubbles that could be localized (i.e., the area of the vessel mask divided by the size of a square wavelength). Finally, to confirm that peaks isolated by MR were due to flowing scatterers, we considered a simple nearest-neighbor pairing of localized peaks between frames to obtain estimation of the flow velocity; that is, \( v_i = (r_{i+1}^n - r_i^n) / \Delta t \), where \( r_i^n \) is the position of peak \( i \) in frame \( n \), and \( \Delta t = 2 \text{ ms} \) is the time between the acquired frames.

**III. RESULTS**

**A. Integration of MR into SR Framework**

To evaluate the integration of MR peak-finding algorithm in the SR-US framework, we applied it to the ultrafast CEUS vascular imaging data sets of healthy CAM chicken embryos. Fig. 2(a) shows the SR-image-based built from the peaks obtained via MR with \( h = 0.050 \). The image resolution is significantly better than that obtained from a maximum intensity projection of the CEUS stack [Fig. 2(b)] and is comparable to that of the optical image [Fig. 2(c)]. The intensity profiles shown in Fig. 2(d) for the indicated lines in Fig. 2(a) show that bifurcations with separations as small as 45 μm may be imaged (profile 1), and sub-vessel detail for vessels as small as 20 μm are identifiable (profile 4). These details, which are not visible in the CEUS image (gray lines in Fig. 1(d)) and fall below the Rayleigh criterion [28] for resolvability for the
and mask formation. However, given the imperfect registration within the vessel, including the uncertainties due to registration errors, different tolerances (i.e., vessel true location) were considered. The peaks found per frame via MR were approximately two to three times as many; for an offset $h = 0.075, 38.8 \pm 4.6$; for $h = 0.050, 49.1 \pm 4.8$; and for $h = 0.025, 66.2 \pm 5.3$. The increased sensitivity ($h = 0.025$) had slightly lower accuracy than the non-MR case; for instance, $h = 0.025$ had a lower bound ($\delta = 0 \mu m$) of 69.7% localized within the vessel, including the uncertainties due to registration and mask formation. However, given the imperfect registration aperture and field of view (approximately 55 $\mu m$), are resolved with high contrast in the SR image. Thus, the peaks identified with MR may generate SR images with resolution significantly better than that of the raw CEUS images and comparable to that of optical microscopy.

B. Sensitivity Versus Accuracy of SL Peaks

After demonstrating that MR can be used for peak detection in the SR-US imaging framework, we assessed its robustness and accuracy. As the co-registered optical data are available, we consider these data as a ground truth and compared the SL peaks obtained from the MR process with a binary mask created from the vascular map. The location of each SL point was compared with the binary mask. Peaks that fell within the vessel region (i.e., at pixels where the mask had value 1) were labeled as within the vessel (i.e., real peaks) and outside the vessel otherwise (i.e., faulty peaks). To account for image registration errors, different tolerances (i.e., vessel true location) were considered.

Fig. 3 demonstrates that without MR, 20.5 ± 3.4 peaks were detected in each frame, with 89.6% located within the vessel mask. For a tolerance of 20 $\mu m$, 95.7% were within the vessel, and for 50 $\mu m$, 98.6% were labeled as within the vessel. The peaks found per frame via MR were approximately two to three times as many; for an offset $h = 0.075, 38.8 \pm 4.6$; for $h = 0.050, 49.1 \pm 4.8$; and for $h = 0.025, 66.2 \pm 5.3$. The increased sensitivity ($h = 0.025$) had slightly lower accuracy than the non-MR case; for instance, $h = 0.025$ had a lower bound ($\delta = 0 \mu m$) of 69.7% localized within the vessel, including the uncertainties due to registration and mask formation. However, given the imperfect registration (see Suppl. Fig. S-2), even a small tolerance of 20 $\mu m$ labeled 86% of these localizations as within the vessel. The black markers in Fig. 3 also indicate that lowering the intensity threshold for the non-MR case reduces the number of peaks found, as a lower cutoff causes nearby high-intensity peak regions to merge.

Finally, since the area of the vasculature is known, an approximate upper bound on the number of separable point sources per frame that might be identified by the diffraction-limited system can be established. If each source is imaged as a brightness peak with an area of 1 square wavelength, then the total area of the vasculature in Fig. 2 divided by the PSF area yields a theoretical maximum of 667 bubbles. Given that MR identifies up to 62 peaks per frame, nearly 10% of this upper bound was achieved.

The peak regions had a mean size of approximately 0.2 square wavelengths, approximately 1/5th that of the PSF, consistent with the assumption that the identified peaks are due to subwavelength scatterers, as the area of the peak region is governed by the spatial distribution of the peak, rather than by its amplitude or depth within in the raw image (see Suppl. Figs. S-3 and S-4). Finally, velocity estimations from the pairing of peaks between frames yield flow rates and directions consistent with more sophisticated algorithms [23], suggesting the localizations are indeed flowing contrast agents (see Suppl. Fig. S-5).

Collectively our findings indicate the potential abilities of the MR algorithm for accurate (number of bubbles within the vessel) and precise (fraction of bubbles within the vessel) microbubble peak detection allowing to assemble SR images with resolution comparable to optical imaging.

C. MR Improves Localization in Small Vessels

To assess the abilities of MR to identify USCA peaks within small vessels, which are also the vessels of interest in SR-US and have low incidence rate, we evaluated the number of peaks found in different vessels as a function of time (Fig. 4). In the smallest vessels ($d_1 = 33 \mu m$ and $d_2 = 49 \mu m$),...
no peaks were detected via thresholding (i.e., without MR and for intensity thresholds 0.9, 0.7, 0.5; the dashed curves shown are for a threshold of 0.9). However, tens to hundreds of peaks were identified via MR, with more peaks isolated for lower offsets (higher sensitivity). Indeed, over the entire image region, 80.4%, 75.1%, and 68.1% of all peaks had amplitude less than 0.5 for offsets of \( h = 0.025 \), 0.050, and 0.075, respectively. While lowering this threshold further may detect some of the lower amplitude peaks in the small regions, doing so merges adjacent, high-amplitude peak regions, and thus lowers the accuracy and number of total peaks (Fig. 2). For larger vessels, thresholding identified more bubbles, though still the number of localizations was significantly fewer than the number identified with MR. Together, these data emphasize the potential of MR to identify USCA peaks within small vessels.

D. Higher Sensitivity Maintains Contrast

To ensure that the additional peaks detected were not spurious localizations, we evaluated the contrast in the resulting SR images (Fig. 5). While for the smallest offset (\( h = 0.025 \)), the CNR was lower than the contrast in the non-MR images (i.e., the image formed with only thresholded peaks), likely due to some spurious localizations contributing to background noise, for larger offsets (\( h = 0.050 \)), the CNR was quite high and exceeded that of the non-MR images [Fig. 5(f)]. This is because the MR was able to identify more peaks in the smaller vessels [yellow arrows, Fig. 5(e)] resulting in stronger signal when compared with the same positions in the non-MR images.

To demonstrate the robustness of the resultant images to measurement noise, we evaluated the CNR for images formed with \( h = 0.05 \), over the same vessel and background locations shown in Fig. 5. Fig. 6 demonstrates that while the addition of noise to the CEUS frames decreases the CNR of the output SR image, the contrast between vessels is consistent across all locations and remains positive [Fig. 6(b)]. However, without MR, some smaller vessels have poor contrast [as in Fig. 5(e)], which give vanishing CNRs and subsequently wide variability in the image contrast between different size vessels. Lower thresholds would exacerbate this effect, as fewer peaks would be isolated (Fig. 3), reducing the signal intensity in larger vessels, while locating few additional peaks in smaller vessels (Fig. 4). The addition of noise resulted in a mean CNR in the maximum intensity projection of the raw image stack (i.e., with no processing) which was 3.6 ± 10 dB over the same locations shown in Fig. 6.
E. MR Is Computationally Efficient

Finally, we measured the computation time required per frame of the MR peak-finding algorithm as a function of the interpolation of a 1 mm by 1 mm region from the CAM data set shown in Fig. 7(a) (times do not include SVD filtering, which required 6.6 s for each 720 frame stack). Smaller offset cases required slightly longer processing times (e.g., 18.9 ± 4 ms versus 16.6 ± 2.8 ms per frame at 4× interpolation for $h = 0.025$ and $h = 0.075$, respectively) due to the larger number of points found (Fig. 3) in each frame, which increases the number of convolutions. Peak-finding without MR required slightly less time (10.4 ± 4 ms per frame), but identified only 5.2 peaks per 1000 pixels over the same region, compared with 20 in the MR case. The required computational time per peak was comparable across all offsets, but depended largely on the interpolation [0.6–0.7 ms per peak for 2× interpolation up to 10.5–12.7 ms per peak for 8× interpolation; Fig. 7(b)]. Thus, MR enables a roughly twofold improvement in the temporal resolution (defined as the total acquisition and processing time to generate the SR image) for these data.

IV. DISCUSSION

Imaging the microvasculature noninvasively and beyond the capabilities of conventional US systems has driven the development of a range of SR techniques. This article describes a computationally efficient method for microbubble isolation and localization based on MR and demonstrates that it can be readily integrated to the SR-US framework (Fig. 1). The SL points formed SR images that resolved the vasculature of a chicken embryo with resolution comparable to optical microscopy, and substantially better than a maximum intensity projection (Fig. 2). According to the optical microscopy images of the vasculature, approximately 90% of the localizations corresponded to positions within vessels (Fig. 3 and Suppl. Fig. S-2). Moreover, the number of peaks approached 10% of the maximum possible number of points given the size of the PSF and total vascular area. The CNRs of the SR images obtained with the SL peaks were higher at small vessels when compared with images formed with the thresholded peaks (Fig. 5). Finally, the peak finding routine was quite efficient, requiring, for example, less than 10 ms per frame of a 1 mm by 1 mm region at 2× interpolation (65-by-130 pixels), in which up to 30 peaks were isolated (Fig. 7).

The existing methods for microbubble localization have reported identification of a few to several tens of microbubbles [7], [8], [10], [20], [22], [23], [29], [30]. While the number of localizations depends in part on the vascular density, bubble concentration, and field of view, they are comparable across recent studies (see Suppl. Table S-1). The MR-based localization procedure presented here identified up to 66 ± 5 total intensity peaks per frame for the data set in the region shown in Fig. 2 (75-by-65 pixels native resolution), a threefold increase compared with the thresholded case without MR, while being nearly as accurate (94% within vasculature as determined by optical comparison versus 98% without MR). The improvement over the non-MR case was most pronounced in smaller vessels (less than 50 μm), for which no localizations were found via thresholding, but for which several hundreds were with MR (Fig. 4). While a lower threshold (higher sensitivity) may detect more peaks in such vessels, the likelihood of false positives is also increased and would require more careful processing to remove false localizations. Additionally, while the reconstruction largely retains the shape of the original distribution (as we seek to remove the baseline without altering the shape of the peak itself), the area peak region is threshold-dependent (Suppl. Fig. S-3). Future work should address how peak detection algorithms, such as MR, transform an individual PSF and explore strategies to best fit the PSF of the original or post-MR images.

A significant benefit of the MR algorithm is that it is agnostic to imaging modality and thus amenable to analysis of both B-mode and PI images. Additionally, it may be used in conjunction with other advancements such as spatiotemporal filtering [23] or sparsely activated phase change contrast agents [13] to streamline further the overall SR imaging.
pipeline and reduce the requisite acquisition time. Moreover, the computational efficiency of the algorithm (order of 100 ms per frame for the regions analyzed here) allows relatively efficient determination of multiple points. As the computation for each frame is independent, the algorithm is also fully parallelizable. Additionally, morphological operations are naturally extensible to three dimensions and could thus be used for volumetric imaging data [26], which would obviate the problem of out-of-plane localization errors. Information about the peak region geometries may also be of interest for deep-learning-based methods, for example, to discriminate overlapping bubble signal [31]. More research in this direction is warranted.

The MR method for peak detection described here has a few limitations. First is the choice of parameters. For MR-based peak finding, the offset must be determined empirically for the data set, depending on the SNR of the data, as has been reported for other methods [8]. A range of 0.025–0.075 was seen to give good results here, though larger values may be required for data with higher background signal. Additionally, a relatively high concentration of USCA was used for this study, which enables more localization opportunity and thus more efficient imaging of small diameter vessels. For lower concentrations typically used in SR imaging [18], the improvement over the non-MR case may be less dramatic, as the dynamic range of signal intensities would be lessened. Still higher concentrations may increase the benefit of the MR technique, which provides the most utility when the dynamic range of the images is large.

Finally, given this high USCA concentration used in the current experiments, it cannot be stated with certainty that the SL peaks represent signal from a single bubble. It was beyond the scope of this study to determine exactly when an intensity peak is due to multiple scatterers or to a single one, or indeed if it is due to electronic noise in non-MR images. However, based on our data (Suppl. Fig. S-3), several strategies can be envisioned. Most notably, peak regions with a too small area could be attributed to noise, while peak regions with a too large area could come from overlapping bubbles. However, the area of the peak regions found in the reconstructed images suggests that the scatterers are appreciably smaller than a wavelength (see Suppl. Figs. S-3 and S-4) that they can be used to estimate vascular flow (Suppl. Fig. S-5), and their localizations were predominantly within the vasculature (Fig. 3 and Suppl. Fig. S-2). Moreover, the ability of the presented method to support the localization of a densely spaced group of contrast agents may complicate tracking and velocity calculations. Future work with varying concentrations or simultaneous optical imaging of the bubbles may address this concern, potentially combined with automated selection of algorithm parameters and qualification of peak detection based on the statistical characteristics of the peak.

V. Conclusion

We have presented how the MR algorithm can be integrated to the SR-US framework. Peak extraction via MR enables two- to threefold increase in the number of peaks detected per frame compared with a thresholding technique with comparable accuracy as determined by comparison with optical ground truth. Furthermore, comparison with the vascular density of the model suggests that nearly 10% of the theoretical maximum number of localizations was achieved. The greater sensitivity of the method enables improved detection of peaks in small vessels, while maintaining good image contrast. The method requires on the order of 100 ms per frame for processing. Together, the proposed framework could augment our ability to perform SR-US and may facilitate the development of clinically effective SR-US.

Appendix

After [26], consider discrete distributions and defined on a rectangular, connected domain (i.e., the 2-D intensity profile represented by a grayscale image). Now define as the set of points in whose intensity is larger than some threshold $k$

$$T_k(I) = \{r \in D | I(r) \geq k\}. \quad (2)$$

The reconstruction of $I$ (called the mask image) from $J$ (called the marker image) is denoted as $\rho_I(J)$ and is defined as

$$\rho_I(J)(r) = \max\{k | r \in \rho_I(J)(r)\}. \quad (3)$$

An equivalent but perhaps more intuitive definition uses the dilation operation $\delta$. Given a structuring element $S(r')$ where $r' \in D' \supseteq D$, the dilation is defined as

$$\delta(I) = I \oplus S = \max_{r' \in D'} [I(r) + S(r - r')]. \quad (4)$$

That is, every value of $I$ is replaced with the maximum value of $I + S$ within a neighborhood defined by local support of $S$. Typically, the template $S$ is defined as 0 for the 3-by-3 neighborhood, such that each pixel is replaced with the maximum value of any adjacent pixel. The dilation of $I$ under $J$, written as $\delta_I(J)$, is then simply given as

$$\delta_I(J) = \delta(J) \bigcap I \quad (5)$$

where $\bigcap$ indicates that the point-by-point minimum is taken. This operation dilates $J$, but limits the intensity by the maximum value of $I$ at each position. Successive iterations of this dilation operation are denoted as $\delta_I^n(J) = \delta_I(\cdots \delta_I(J) \cdots)$. Then, the MR of $I$ from $J$ may be expressed as

$$\rho_I(J)(r) = \bigvee_n \delta_I^n(J) \quad (6)$$

where $\bigvee$ denotes the point-by-point maximum. In (6), the operation is repeated until the output stops changing. To extract the peak regions, the grayscale CEUS frame is used as $I$, and the shifted distribution $I - h$ is used as $J$. The image containing peak regions $P$ (and that is 0 elsewhere) is

$$P = I - \rho_I(J). \quad (7)$$

See also Supplementary Material and Suppl. Fig. S-6.

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Chengwu Huang received the B.S. degree in biomedical engineering from Beihang University, Beijing, China, in 2012, and the Ph.D. degree from the Department of Biomedical Engineering, Tsinghua University, Beijing, in 2017. He is currently an Assistant Professor with the Department of Radiology, Mayo Clinic College of Medicine, Rochester, MN, USA. His current research interests include SR ultrasound microvessel imaging, functional ultrasound imaging, ultrafast vascular imaging, and ultrasound elastography.

Shigao Chen (Senior Member, IEEE) received the B.S. and M.S. degrees in biomedical engineering from Tsinghua University, Beijing, China, in 1995 and 1997, respectively, and the Ph.D. degree in biomedical imaging from the Mayo Graduate School, Rochester, MN, USA, in 2002. He is currently a Professor with the Mayo Clinic College of Medicine, Rochester. His research interest is ultrasound viscoelasticity imaging and ultrasound microvessel imaging.

Costas Arvanitis (Member, IEEE) received the Ph.D. degree in medical physics from University College London, London, U.K., in 2008. He held postdoctoral appointment at the Institute of Biomedical Engineering, University of Oxford, Oxford, U.K. Until 2016, he was an Instructor with the Department of Radiology at Brigham and Women’s Hospital and Harvard Medical School, Boston, MA, USA. He is currently an Assistant Professor with the School of Mechanical Engineering, Georgia Tech, Atlanta, GA, USA, and the Department of Biomedical Engineering at Georgia Tech and Emory University, Atlanta. His research interests include linear and nonlinear acoustics, biomedical acoustics, microbubble dynamics (acoustic cavitation) and control, and image-guided therapy in the brain.