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Using Low-Boiling Point Phase Change Contrast Agent Activation Signals for Super Resolution Ultrasound Localization Microscopy

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Abstract*—Ultrasound (US) contrast agents can be used to generate super resolution (SR) images of the vasculature by accumulating the localizations of separable contrast signatures over tens of thousands of frames. This research presents a SR imaging method that localizes the activation signals of octafluoropropane (OFP, C3F8, -37 deg. C boiling point) phase-change contrast agents (PCCAs, or droplets), rather than microbubble signals. The unique activation signature of low-boiling point PCCAs was separated from the tissue background by frequency filtering alone. Plane-wave imaging was used to both activate droplets and image the activation signals. A low-frequency bandwidth (BW) to receive the activation signals and a high-frequency transmit BW enabled the collection of high contrast-to-tissue ratio (CTR) images which were then processed for SR. We demonstrate the application of this technique by localizing activations within a crossed microtube setup in a tissue-mimicking agar phantom kept at 37 deg. C.

***Keywords*—super-resolution, phase-change agent, nanodroplet, contrast agent, microbubble**

I. Introduction

Ultrasound (US) imaging can utilize venously injected contrast agents to increase the visibility of blood vessels, which otherwise demonstrate low contrast from the surrounding tissue. Traditional US contrast agents consist of a gas core encapsulated in a stabilizing shell. Phase change contrast agents (PCCAs), also called droplets, are a specialized variety of US contrast agents that instead have a liquid core. These liquid cores are subsequently vaporized within the body, resulting in local microbubble contrast. This vaporization, or activation, can be achieved by acoustic means (as in the case of acoustic droplet vaporization (ADV) [1], or by other energy input, such as laser [2]. Low-boiling point PCCAs were developed to address concerns regarding the high acoustic pressures required to activate early, sub-micron droplets [3]. Octafluoropropane (OFP, C_3F_8, -37°C boiling point) is one such material used in low-boiling point PCCAs. Although gaseous at room temperature, OFP microbubbles can be condensed to create superheated droplets with diameters in the range of hundreds of nanometers. When compared to decafluorobutane (DFB, C_4F_{10}, -2°C boiling point), another commonly used low-boiling point perfluorocarbon for US contrast agents, OFP yields PCCAs that are less stable and thus easier to activate acoustically.

The activation of low-boiling point PCCAs is of interest for contrast imaging for more than just the resulting microbubble. PCCA activation produces a unique ultrasound signal independent of the acoustic scheme used to activate. When a droplet vaporizes, its encapsulating shell necessarily expands to accommodate the increased volume of the gas phase. This expansion involves an initial over-expansion and ringing behavior as the shell eventually settles on the final bubble diameter. The PCCA activation ringing generates its own US signal at a frequency dependent on the size of the contrast agent, stability, and sensitivity to US, not the frequency of the US used to activate the droplet. Typically, the frequency content of the PCCA activation signal is low (100 kHz - 2.5 MHz). The difference in the transmit US frequency and the PCCA activation signal frequency has inspired several PCCA-specific dual-frequency techniques, such as vaporization detection imaging (VDI) [4] and dual frequency vaporization

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detection [5]. In both techniques, a high-frequency (>2.5 MHz) transmit bandwidth (BW) is used in combination with a low-frequency (<2.5 MHz) receive BW. Tissue and background respond at the fundamental transmit frequency while the PCCA activation signals occur at lower, spectrally-isolated frequencies. This results in high contrast-to-tissue ratios (CTRs). However, due to the low-frequency content of the activation signals, resulting images are hindered by low resolution.

High-resolution vascular images can also be generated by super-resolution (SR) techniques, which are able to overcome the typical diffraction-limited resolution for a given imaging scheme. Recently, two techniques combining SR and PCCAs have been published. The first, known as acoustic wave sparsely activated localization microscopy (AWSALM), consists of an imaging scheme that first activates DFB droplets with a focused pulse, images the resulting microbubbles, and subsequently destroys the microbubbles, making way for more activations [6]. This method provides a close acoustic analog to FPALM [7] and STORM [8], SR techniques in optics, which exploit blinking signals. In general, ultrasound localization microscopy (ULM) describes the process of accumulating precise localizations of these contrast signals to create super-resolved US images. The second technique to combine SR and PCCAs is an extension of the first. Fast-AWSALM is the faster version of regular AWSALM [9]. The speed increase is gained from using OFP droplets instead of DFB droplets, which can be activated by plane wave US, rather than focused US. This research also utilizes OFP droplets to generate SR images. Rather than localizing the resulting microbubble signals, which still need to be separated from the background tissue signal by an additional spatio-temporal filtering step, we localize the activation signal itself, which is separated from the background tissue signal simply by the dual-frequency imaging scheme. The localization step takes advantage of the high CTR of the activation imaging and overcomes the low resolution native to this imaging scheme.

II. Methods

OFP PCCAs were created by condensing microbubbles similar to previously described condensation methods [9]. First, a 3mL vial (1.5 mL lipid solution with OFP headspace) was agitated using a Vialmix (Lantheus Medical Imaging, N. Billerica, MA, USA) to generate lipid-shelled OFP microbubbles. Condensation of these microbubbles into PCCAs was achieved by simultaneously lowering temperature with a chiller and raising pressure by a pressurized nitrogen line.

The PCCAs were injected into two 200 μm cellulose tubes (Baxter Healthcare Corporation, Deerfield, IL, USA) embedded in an agar phantom in a cross-tube configuration (depth = 1 - 1.5 cm). The agar phantom was submerged in a water bath maintained at 36 - 37°C by an ISOTEMP 2013S recirculating water bath (Fisher Scientific, Waltham, MA, USA). A PHD 2000 programmable double-infusion syringe pump (Harvard Apparatus, Holliston, MA, USA) was used to infuse both tubes simultaneously with OFP PCCAs at a constant rate of 50 μL/min.

An ATL L11-5 linear array connected to a Vantage 256 (Verasonics, Kirkland, WA, USA) was used to transmit 5 MHz plane-waves at a frame rate of 10 Hz. Low-pass filtering (cutoff frequency = 2.5 MHz) was applied on the radio-frequency (RF) data. One-frame differential imaging, spectral analysis, and matched filtering of the resulting reconstructed images yielded activation localizations that were then accumulated to generate the SR image.

III. Results

The unique acoustic signal localized to create SR ultrasound images can be visualized in Fig 1A. The ringing behaviour of the expansion of the contrast agent shell is manifested by repeated, decaying signal in depth. Fig. 1B and 1C provide resulting B-mode and SR images, respectively. The full-width half-max (FWHM) measurement of the tube (Fig. 1D) for a well-populated region was 250 μm, which is approximately 1.2 fold better than that achieved with traditional grayscale imaging

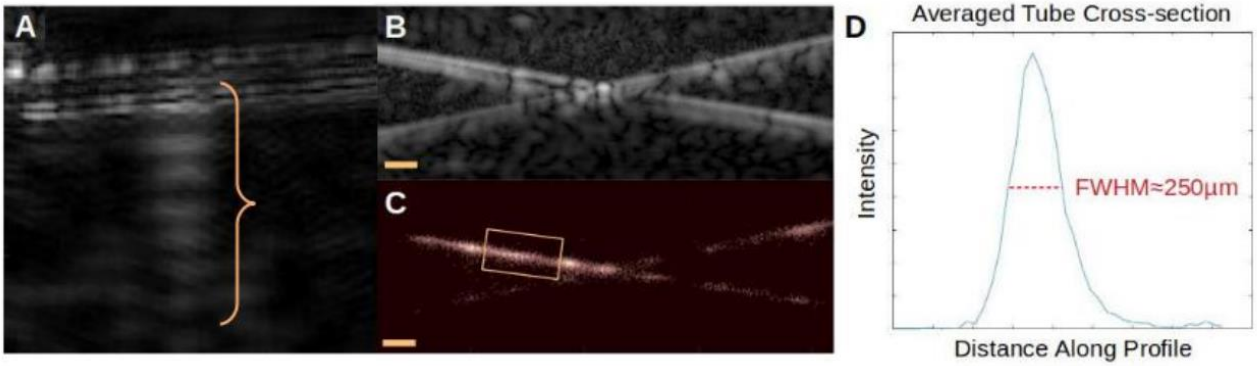

Fig. 1: Phase-change contrast agent (PCCA) activation signal imaging results. 1A, an example of the exponentially decaying sinusoid activation signal in a water phantom (orange brace); 1B, B-mode image of air-filled cross tubes at 5 MHz; 1C, super-resolved image of same imaging plane as Fig. 1B, localizing PCCA activations; 1D, tube cross-section profile averaged over boxed region in Fig. 1C. Scale bars are 2mm.

with the same 5 MHz transducer. The contrast-to-tissue ratio of the SR image is 15.3 dB.

IV. Conclusion

This result provides proof that the localization of PCCA activation signals can be used to generate SR images, even at shallow depths (at least 1.5 cm) through tissue-mimicking attenuating material. The OFP droplets are activatable by plane-wave insonification, and the resulting activation signals are imaged using the same plane-wave. This means a simple single-pulse plane-wave imaging scheme can be used to acquire the droplet activation dual-frequency images. Although the 10 Hz frame rate presented here does not represent high frame-rate imaging, plane-wave imaging could theoretically be conducted at much higher frame rates. The microtubes presented here were not fully populated in the final SR image and the FWHM measured on the only well-populated section of a tube (250 μm) was larger than the 200 μm expected. This may be due to the BW limitations of the L11-5 transducer, which empirical measurement of the <2.5 MHz frequency content was between -10 and -20 dB as compared to the greatest frequency content of the transducer (approximately 5 MHz). The low sensitivity to the desired low-frequency receive BW resulted in lower CTR images that may have contributed to the limited and increased uncertainty in the localizations.

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