Published in final edited form as:

IEEE Trans Ultrason Ferroelectr Freq Control. 2019 April ; 66(4): 692–700. doi:10.1109/TUFFC.2019.2895248.

Leveraging the Imaging Transmit Pulse to Manipulate Phase-Change Nanodroplets for Contrast-Enhanced Ultrasound

Yiying I. Zhu,

School of Electrical and Computer Engineering, Georgia Institute of Technology, Atlanta, GA 30332 USA.

Heechul Yoon [Student Member, IEEE],

School of Electrical and Computer Engineering, Georgia Institute of Technology, Atlanta, GA 30332 USA.

Andrew X. Zhao, and

Wallace H. Coulter Department of Biomedical Engineering, Georgia Institute of Technology and Emory University School of Medicine, Atlanta, GA 30332 USA.

Stanislav Y. Emelianov [Senior Member, IEEE]

School of Electrical and Computer Engineering, Georgia Institute of Technology, Atlanta, GA 30332 USA, and with Wallace H. Coulter Department of Biomedical Engineering, Georgia Institute of Technology and Emory University School of Medicine, Atlanta, GA 30332 USA

Abstract

Phase-change perfluorohexane nanodroplets (PFHnDs) are a new class of recondensable sub-micrometer-sized contrast agents that have potential for contrast-enhanced and super-resolution ultrasound imaging with an ability to reach extravascular targets. The PFHnDs can be optically triggered to undergo vaporization, resulting in spatially stationary, temporally transient microbubbles. The vaporized PFHnDs are hyperechoic in ultrasound imaging for several to hundreds of milliseconds before recondensing to their native, hypoechoic, liquid nanodroplet state. The decay of echogenicity, i.e., the dynamic behavior of the ultrasound signal from optically triggered PFHnDs in ultrasound imaging, can be captured using high frame rate ultrasound imaging. We explore the possibility to manipulate the echogenicity dynamics of optically triggered PFHnDs in ultrasound imaging by changing the phase of the ultrasound imaging pulse. Specifically, the ultrasound imaging system was programmed to transmit two imaging pulses with inverse polarities. We show that the imaging pulse phase can affect the amplitude and the temporal behavior of PFHnD echogenicity in ultrasound imaging. The results of this study demonstrate that the ultrasound echogenicity is significantly increased (about 78% improvement) and the hyperechoic timespan of optically triggered PFHnDs is significantly longer (about 4 times) if the nanodroplets are imaged by an ultrasound pulse starting with rarefactional pressure versus a pulse starting with compressional pressure. Our finding has direct and significant implications for contrast-enhanced ultrasound imaging of droplets in applications such as super-resolution imaging and molecular imaging where detection of individual or low-concentration PFHnDs is required.

Index Terms

Laser triggered perfluorohexane phase-change nanodroplets; perfluorocarbon nanodroplet recondensation dynamics; optical droplet vaporization; ultrafast ultrasound imaging; ultrasound transmit pulse phase

I. INTRODUCTION

CONTRAST agents play an important role in medical ultrasound, in terms of both diagnostic imaging and therapy [1–3]. Microbubbles are ideal contrast agents for ultrasound imaging, and have been used for vascular imaging [4, 5], drug delivery [6, 7], and cavitation-based therapies [8–11] (e.g. temporal blood-brain barrier opening [12]). However, their micrometer size (between 1 and 10 μm in diameter [13]) does not allow for extravasation; microbubbles rarely reach targets outside of vascular compartments. An emerging new class of sub-micrometer-size triggerable particles, referred to as phase-change perfluorocarbon nanodroplets (PFCnDs) [14], is actively being investigated to address this limitation. These stable liquid droplets feature a liquid core that can vaporize in response to a trigger, either an ultrasound wave (acoustic droplet vaporization [15]) or a laser pulse (optical droplet vaporization [16]), to form gaseous bubbles. In their droplet form, the sub-micrometer-sized PFCnDs can be delivered into extravascular space, and once delivered and activated, the PFCnDs can be converted to microbubbles serving as ultrasound contrast agents. Indeed, the vaporized PFCnDs, activated either optically or acoustically, exhibit high ultrasound contrast. However, the ultrasound waves used to acoustically vaporize droplets can interfere with the subsequent ultrasound imaging pulses, whereas optical activation does not suffer from this interference, because laser activation and ultrasound imaging processes are independent, which allows for interference-free reliable ultrasound contrast induced by vaporized PFCnDs [17, 18]. Moreover, optically triggered PFCnDs can provide additional contrast via photoacoustic imaging during optical activation.

Recent PFCnD studies have utilized a high boiling point (56°C) perfluorohexane (PFH) core, which imparts distinctive properties from previously investigated PFCnDs with lower boiling point cores [19–21]. As illustrated in Fig. 1, the perfluorohexane nanodroplets (PFHnDs), under a short laser pulse irradiation, vaporize and sustain in a gaseous form for a short period (sub-second range: both microseconds [20] and milliseconds [22] have been reported) before stochastic recondensation back to liquid form – this is a unique property of high boiling point perfluorocarbon droplets. In contrast, nanodroplets made out of low boiling point perfluorocarbon will permanently remain as gas bubbles after vaporization. More importantly, the process of PFHnD vaporization followed by recondensation is repeatable over multiple laser pulses. This phenomenon results in temporal variations of PFHnD originated ultrasound echogenicity, i.e. echogenicity is enhanced upon vaporization of PFHnDs and then it vanishes upon their condensation to liquid phase [23]. Generally, ultrafast plane-wave ultrasound imaging may be capable of capturing such transient vaporization-recondensation dynamics [24]. Recent studies have demonstrated that the ‘blinking’ ultrasound contrast from multiple optical activations can be used to obtain background-free ultrasound imaging [23], and enable super-resolution imaging [19]. In both

of these techniques, a major limitation is the short timespan during which PFHnDs are detectable in ultrafast ultrasound imaging. The transient detection time of vaporized PFHnDs by ultrasound limits imaging contrast and spatial resolution. Indeed, extending the ultrasound hyperechogenicity time is desirable because it will increase the detectable time of PFHnDs in ultrasound imaging thus leading to improved PFHnD detection efficiency as well as imaging contrast and spatial resolution by allowing for more averaging [25]. Therefore, prolonging the ultrasound hyperechogenicity time of the transient vaporized PFHnD bubbles without sacrificing any favorable properties of the nanodroplets would be ideal. There are investigations reporting on various factors that may contribute to the PFHnD bubble lifespan, such as laser fluence [26] and the particle size [20]. However, increasing the size of PFHnDs to prolong bubble timespan may impede extravasation of PFHnDs [27].

In this paper, we report that altering the phase of the ultrasound imaging pulses can change the amplitude and duration of echogenicity induced by phase-change nanodroplets. This discovery leads to a new method to leverage the ultrasound imaging pulse to not only image but also manipulate the hyperechogenicity dynamics of optically triggered PFHnDs. Specifically, we programmed the imaging system to transmit imaging pulses of inverse polarities to compare their imaging capabilities on PFHnDs. The results of this study suggest that the ultrasound echogenicity and the hyperechogenicity timespan of optically triggered PFHnDs are significantly increased if the particles are imaged by an ultrasound pulse starting with a rarefactional phase compared to an ultrasound pulse starting with a compressional phase.

II. MATERIALS AND METHODS

A. Synthesis of PFHnDs

The PFHnDs consisted of a perfluorohexane core, a stabilizing lipid shell and an encapsulated dye, and were synthesized following a protocol adapted from a previous study [28]. Briefly, 0.5 mg of Epolight 3072 dye (Epolin, Inc., Newark, NJ, USA), which exhibits a peak optical absorption centered at 1054 nm wavelength, was combined with a lipid mixture with a total mass of 1.15 mg in chloroform. The lipid was composed of 18:0 PEG2000 PE (1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[methoxy(polyethylene glycol)-2000]; Avanti Polar Lipids, Inc., Alabaster, AL, USA) and 18:0 PC (DSPC) (1,2-distearoyl-sn-glycero-3-phosphocholine; Avanti Polar Lipids, Inc., Alabaster, AL, USA) at a 9:1 molar ratio. Then, chloroform was removed using a rotary evaporator leaving a cake of lipid and dye. Next the cake was suspended in 1 mL of phosphate-buffered saline (PBS) using a bath sonicator (Branson 1800, Richmond, VA, USA). To this solution, 50 μL of perfluorohexane (FluoroMed, L.P., Round Rock, TX, USA) was added. Subsequently, droplets were formed by adding all the materials to a 7 mL scintillation vial, placing the vial in a water bath, and sonicating the mixture using a probe sonicator (Q500, QSonica LLC, Newtown, CT, USA). The resulting emulsion was centrifuged at 200 relative centrifugal force (RCF) for 2 minutes. The pellet was discarded to remove excess dye aggregates and micrometer-sized droplets. The supernatant was then centrifuged at 3,000 RCF for 5 minutes to pellet droplets and the resulting supernatant of excess lipids and any remaining dye was discarded. The pellet was suspended in 1 mL of PBS. The droplet concentration was

approximately 10^8 nanodroplets/mL. The synthesized droplets were sized at $295 \text{ nm} \pm 120 \text{ nm}$ (Zetasizer Nano ZS, Malvern Instruments Ltd., Malvern, UK).

B. Tissue Mimicking Phantoms Containing PFHnDs

Two tissue mimicking polyacrylamide phantoms were fabricated (Fig. 2). One phantom was optically and acoustically transparent, and contained uniformly distributed PFHnDs throughout the volume – this phantom was denoted as the transparent phantom (Fig. 2a). Another phantom contained uniformly dispersed acoustic scatters in the background and both PFHnDs and acoustic scatters within a “GT” shaped inclusion positioned in the center – this phantom was denoted as the GT phantom (Fig. 2b).

To construct the transparent tissue mimicking gel phantom, 150 mL of degassed water was mixed with 50 mL of 40% acrylamide (Thermo Fisher Scientific, Waltham, MA, USA). To this solution, 100 μL of stock as-prepared PFHnDs were added followed by 2 mL of 10% (w/v) ammonium persulfate solution (Sigma-Aldrich, St. Louis, MO, USA). Next, 625 μL of TEMED (N, N, N', N'-Tetramethylethylenediamine, Sigma-Aldrich, St. Louis, MO, USA) was added to crosslink the acrylamide. The entire solution was quickly poured into a rectangular $58 \times 58 \times 78 \text{ mm}$ mold and allowed to polymerize for 10 minutes.

For the GT phantom, 0.25% (m/v) 25 μm silica gel (Sigma-Aldrich, MO, USA) was added into a solution (150 mL of degassed water and 50 mL of 40% acrylamide) before crosslinking. The solution was then split into two parts: 5 mL was used to prepare an inclusion and the remaining 195 mL was used to prepare the background. After PFHnDs were added into the 5 mL solution for the inclusion, two parts of the inclusion were prepared first by polymerizing the solution inside a tall containers with cross-sections resembling either “G” or “T” letter. These two parts were then removed from the containers and positioned vertically in a rectangular mold. Finally, the background solution was poured into the mold and polymerized forming the phantom with a “GT” inclusion.

C. Research Ultrasound Imaging System and Programming of Imaging Transmit Pulses

Each phantom was imaged from the top (Fig. 2a) using an ultrasound linear array transducer L11-4v (Verasonics Inc., Kirkland, WA, USA) integrated with an optical fiber bundle as described previously [25]. The imaging probe was acoustically coupled with the phantom using clear ultrasound gel. The optical fiber bundle, connected to a pulsed laser system (Phocus Mobile, Opotek Inc., Carlsbad, CA, USA) operating at 1,064 nm wavelength, was used to irradiate the phantom with 5-ns laser pulses at a pulse-repetition frequency of 10 Hz. The estimated fluence at the output of the fiber bundles, measured by a laser power meter (Nova II, Ophir-Spiricon, LLC, North Logan, UT, USA), was 60 mJ/cm^2 ($\pm 5\%$ allowance).

To image each phantom, the ultrasound transducer was driven at 7 MHz center frequency using a programmable research ultrasound imaging system (Vantage 256™, Verasonics Inc., Kirkland, WA, USA). Two ultrasound imaging transmit pulses with opposite phases were used for ultrafast plane wave imaging. One, denoted as P-pulse, had an initial compressional phase followed by a rarefactional phase. The other one, referred as N-pulse, had an initial rarefactional phase followed by a compressional phase. The ultrasound pulses were measured using a calibrated hydrophone (0.2 mm needle hydrophone, Precision Acoustics

Ltd, Dorchester, UK). To compare the effect of P-pulse and N-pulse, two independent sets of ultrasound B-mode data were collected for the same imaging cross-section: one imaged with P-pulse and the other with N-pulse.

Immediately following each laser pulse, one photoacoustic image was captured first. Then, 50 ultrafast ultrasound B-mode images were acquired at a pulse repetition frequency of 2 kHz over a period of 25 milliseconds by 0-degree planar ultrasound waves. Additional background ultrasound images were acquired before laser activation. The full imaging window was 38.8 mm wide, and 25 mm deep.

For the transparent phantom, a total of five lateral-axial cross-sections, about 8 mm apart, were imaged, as shown in Fig. 2a. Each cross-section was irradiated with 20 laser pulses for one ultrasound pulse type (Fig 1). Then another 20 optical activations were applied for the other ultrasound pulse type. The order of ultrasound imaging pulse type was randomly assigned. Different cross-sections were imaged to obtain more measurements for statistical analysis. For each cross-section, only the data from the last 10 laser pulses were used for analysis. This ensured that all PFHnDs had already been previously optically activated by at least the first 10 laser pulses and possibly by laser pulses from other cross-sections. Therefore, each laser activation followed by ultrasound imaging could be assumed to be independent of other activations because of the repeatable vaporization-recondensation dynamic nature of PFHnDs.

For the GT phantom, 3D volumetric image sets were acquired by scanning the GT phantom along the elevational direction at a step size of 0.5 mm, as shown in Fig. 2b. Therefore, the C-scan (elevational-lateral cross-section) should exhibit the GT pattern. At each scanning step, the ultrasound imaging (either P-pulse or N-pulse) was performed after laser activation the same way as described earlier for the transparent phantom.

D. Image Acquisition and Quantitative Analysis

For the transparent phantom, the PFHnD B-mode images were first subtracted from a background frame and then thresholded to detect the hyperechoic areas produced by the vaporized PFHnDs, creating a spatial mask used to analyze PFHnD-induced hyperechogenicity behavior over time. The total area of the mask (i.e. hyperechoic area) was used to quantitatively assess the hyperechogenicity persistence induced by vaporized PFHnD. The change of total hyperechoic area over time was characterized by a least-square fitting to a function

$$s(t) = a \cdot t^{-b} + c \quad (1)$$

to analyze the recondensation dynamics of PFHnD, where $s(t)$ is the total hyperechoic area as a function of time t and a , b , c are fitting parameters. We defined the timespan from when the PFHnDs were optically activated to when only 10% of the initial hyperechoic area remains (i.e. when the majority of the initial hyperechoic area disappears) as characteristic decay time, τ_{decay} .

$$\tau_{\text{decay}} = t \Big|_{s(t) = s(t_0) \times 10\%} \quad (2)$$

where t_0 is the time when the first ultrasound image was captured after laser activation, i.e. $t_0 = 0.5$ ms in our 2 kHz imaging. The characteristic decay time indicates the complete loss of hyperechogenicity for the majority of PFHnDs. In addition, the signal-to-noise ratio (SNR) was calculated by dividing the maximum signal from hyperechoic signal in B-mode images by average background noise signal. In both SNR and characteristic decay time, the data from last ten laser pulses were used for statistical analysis for each imaging-section.

For the GT phantom, C-scan 2D images were extracted from 3D image sets at 10 mm away from the surface of the transducer corresponding to the geometric focus of the light delivery system. Then, differential ultrasound images were processed to detect activated PFHnDs as described previously [24]. Briefly, ultrasound images were subtracted from their adjacent preceding frames to suppress stationary signals and enhance sudden appearing/disappearing signals from vaporized PFHnDs. Additionally, maximum intensity projection (MIP) along axial direction was performed for both differential ultrasound and photoacoustic images. The SNRs were quantified by the ratio of the average signals from the region inside the GT inclusion over the average signals from the left most region outside the GT inclusion. Note that the different choices of SNR quantification (maximum vs. average PFHnD signal over noise) for the two phantoms stemmed from both the PFHnD distribution and the light illumination. The PFHnDs were uniformly distributed inside the transparent phantom and were not confined to a specific region while in the GT phantom, PFHnDs were only distributed in a confined region, the GT inclusion. Therefore, defining a region of interest for averaging was feasible for the GT phantom only. Moreover, the transparent phantom was imaged in the lateral-axial plane where the light illumination is not uniform, while the GT phantom was 3D scanned to reconstruct the elevational-lateral plane where laser fluence should not vary spatially. Thus, maximum signal from PFHnDs was used in SNR for the transparent phantom whereas average signal within the GT region was used for the GT phantom.

III. RESULTS

The measured ultrasound imaging pulse waveforms used in imaging experiments are shown in Fig. 3. The P-pulse (Fig. 3a) starts with a compressional phase while the N-pulse (Fig. 3b) starts with a rarefactional phase. The two types of imaging transmit pulses have similar magnitudes but their phases are inverses of each other.

Each imaging cross-section of the transparent phantom was irradiated by multiple laser pulses. Each laser pulse was followed immediately by a photoacoustic image acquisition (Fig. 4a) and subsequently by a series of B-mode images (Fig. 4b) acquired using either P-pulse or N-pulse. All images were cropped to display the activated PFHnDs located in the region of interest extending from -10.4 mm to 10.4 mm laterally and from 6.6 mm to 18.1 mm axially, with the reference origin being 0 mm at the lateral center of the imaging transducer and 0 mm depth corresponding to the surface of the imaging transducer.

For each imaging cross-section, a comparable number of PFHnDs were activated by all the laser pulses of the same optical energy. For example, two photoacoustic images obtained right before P-pulse and N-pulse ultrasound images are very similar (Fig. 4a). The average photoacoustic signals from the region of interest for each image cross-sections and each ultrasound imaging pulse are shown in Table I. However, the ultrafast ultrasound images acquired using P-pulse versus N-pulse (Fig. 4b) reveal that ultrasound imaging transmit pulse imposed different effects on the ultrasound echogenicity induced by the vaporized PFHnDs.

Immediately after PFHnDs were activated by the laser pulse, the first ultrasound frame at 0.5 millisecond with N-pulse exhibits higher ultrasound intensity than that with P-pulse (Fig. 4b). The results of quantitative analysis of PFHnD SNRs in ultrasound images normalized to the mean SNR from P-pulse imaging for each imaging plane are shown in Fig. 5. The results from five different imaging cross-sections demonstrate an approximate 5 dB increase (i.e., approximately 78% improvement) in SNR when N-pulse was used for imaging. Note that the background signal here came purely from noise as the phantom was acoustically transparent. With the background noise signal level being the same for two different imaging pulses, the SNR improvement indicates that imaging with N-pulse results in higher signal intensity and, therefore, PFHnDs produce increased echogenicity in ultrasound images.

The decay of PFHnD induced hyperechogenicity process, captured by measuring the echogenic area in ultrasound images over time, was analyzed for both imaging schemes (P-pulse and N-pulse). The decrease and ultimate disappearance of ultrasound signal indicate that vaporized PFHnDs eventually recondense to their original droplet form. It is qualitatively clear (Fig. 4b) that the hyperechogenicity from vaporized PFHnDs dissipate slower when N-pulse is used for ultrasound imaging compared to P-pulse. For the same imaging cross-section as in Fig. 4, the normalized change in hyperechoic area is plotted in Fig. 6. To compare quantitatively the relative hyperechoic area variation over time, the hyperechoic area detected in images acquired using P-pulse or N-pulse were normalized respectively to the hyperechoic area detected in the first ultrasound imaging frame, which indicates the initial hyperechoic area detected from vaporized PFHnDs. The quantitative results showed that characteristic decay time with N-pulse imaging was 8.7 milliseconds compared to P-pulse yielding 1.9 milliseconds (Fig. 6). A comparison of characteristic decay time resulting from P-pulse vs. N-pulse from five different imaging cross-sections is shown in Fig. 7. These quantitative results show that there is a roughly 4 times longer decay time with N-pulse than with P-pulse.

As a demonstration of P-pulse and N-pulse imaging capability, we applied P-pulse vs. N-pulse to image the acoustically scattering phantom with a “GT” inclusion containing PFHnDs. The C-scan images at 10 mm depth are shown in Fig. 8. Signals from acoustic scatters distributed across the phantom were present in the first frame of raw ultrasound images (Fig. 8a). Table II summarizes the SNR measurements for the first frame of differential ultrasound images (US) shown in Fig. 8b, the maximum intensity projection (MIP) for the first frames of differential ultrasound imaging (US MIP) shown in Fig. 8c, and the MIP photoacoustic image (PA MIP) shown in Fig. 8d. In both US images (Fig. 8b) and US MIP images (Fig. 8c), N-pulse imaging results in higher contrast than P-pulse

imaging. In contrast, PA MIP images (Fig. 8d) show similar PFHnD activation before P-pulse imaging and N-pulse imaging. Note that the “ghost” artifacts are caused by grating lobes due to angular undersampling in both photoacoustic imaging and one-angle ultrasound plane-wave imaging [29].

IV. DISCUSSION

Our study revealed that imaging transmit pulses with different phases can lead to a significant difference in both ultrasound hyperechoic amplitude and echogenicity dynamics for optically vaporized PFHnDs. Using an imaging pulse starting with a rarefactional phase (i.e. N-pulse) to image optically triggered PFHnDs distributed in an tissue-mimicking gel phantom, we observed an increased echogenicity and an extended detectable timespan of corresponding hyperechoic signals, while compressional phase first imaging pulse (i.e. P-pulse) promoted the disappearances of PFHnD induced echogenicity.

A. Ultrasound Imaging of PFHnDs

We have demonstrated an approach to achieve better contrast-enhanced ultrasound imaging for phase-change PFHnDs in an acoustically scattering phantom, by leveraging the phase of the imaging transmit pulse. More than 10 dB SNR increase was observed in contrast-enhanced imaging using N-pulse compared to P-pulse (Table II). Note that a direct comparison of imaging contrast between photoacoustic imaging and ultrasound imaging would not be appropriate here because we used phantoms with no optical absorbers thus there was no background photoacoustic signals. In other words, the higher SNR in photoacoustic imaging than differential ultrasound imaging in the study does not provide evidence of photoacoustic imaging prevailing over ultrasound imaging. However, photoacoustic imaging provided confirmation of repeatable and reliable PFHnD vaporization.

One of the main interests for investigating PFCnDs is that their vaporized gaseous form is an analog of a microbubbles, a widely-used contrast agent for ultrasound. It is reasonable and desirable to replicate and expand the capabilities of microbubbles to PFCnDs. For example, contrast harmonic imaging (i.e. pulse inversion imaging) enhances bubble contrast by distinguishing microbubbles from the background [30]. It may also be possible to use coded ultrasound pulses to create spatio-temporal signature of vaporized PFHnDs and to identify resulting bubbles.

B. Ultrasound Echogenicity Dynamics of PFHnDs

The discovery that the ultrasound imaging transmit pulse alone, without any modification of the existing components (such as PFHnD synthesis or laser activation process), can induce significantly different PFHnD echogenicity behaviors presenting in ultrasound imaging unlocks the potential to manipulate and control PFHnD related dynamics while simultaneously imaging. The results presented in this work suggest that it is possible to customize PFHnD related dynamics by designing corresponding ultrasound imaging sequences. For example, super-resolution imaging benefits from a longer PFHnD detectable time to capture more ultrasound imaging frames for averaging, which will result in improved

imaging resolution [25]. On the other hand, other applications utilizing droplet vaporization such as blood-brain barrier opening may require faster recondensation so that the same droplet can be vaporized more times to induce sufficient opening [31]. Several groups have reported different observations on recondensable droplet dynamic behaviors under different conditions: some reported that the microbubble triggered optically persist for tens to hundreds of milliseconds [19, 20] [25] while some reported the bubble life being a few microseconds [32]. Subsequent work may investigate other ultrasound imaging parameters that may affect PFHnD related dynamic behaviors, such as ultrasound center frequency, number of pulse cycles, acoustic pressure amplitude and imaging pulse repetition frequency. For example, by using a versatile imaging platform [33], which allows users to create custom imaging sequences containing various imaging parameters, would enable a thorough understanding of PFHnD-related phenomena. This parametric study may provide a comprehensive imaging guideline and therefore offer opportunities to develop new clinical applications.

The fact that we were able to extend the detectable time of vaporized PFHnDs has a significant impact on ultrasound imaging of optically triggered nanodroplets. First of all, the longer echogenicity lifespan can lead to a significant improvement in molecular imaging and super-resolution imaging, where identification of individual PFHnDs is required. Although a population of activated PFHnDs disappear in ultra-sound imaging stochastically, an individual PFHnD detected by ultrafast ultrasound imaging can only be localized if it loses echogenicity at a different time point (sampled by an ultra-sound imaging frame rate) compared to neighboring PFHnDs. Increasing the hyperechoic periods of all activated PFHnDs will increase the probability of ultrasound imaging to detect individual PFHnDs. Moreover, in ultrasound super-resolution imaging, an increased number of ultrasound imaging frames for vaporized PFHnDs will enhance imaging contrast and elevate spatial resolution by allowing for more averaging to suppress outliers/noise [25]. In addition, with increased decay time, PFHnD related molecular imaging and super-resolution imaging may become more feasible in a clinical setting using existing ultrasound imaging systems with imaging frame rate lower than ultrafast imaging [34].

Furthermore, PFHnD induced hyperechoic dynamics, i.e. characteristic decay time, can be potentially used to as a signature to differentiate PFHnD populations. Studies have shown that gaseous bubble related dynamics vary based on the environment [35]. It implies that the vaporized PFHnD droplets may behave differently in different microenvironment, e.g. in a blood flow versus inside tissue. It raises the possibility to capture a distribution of distinct PFHnD dynamics to map the microenvironment, which can potentially be used for noninvasively probing the tumor microenvironment *in vivo*. At that time, this imaging tool will bring an entirely new prospect in tumor diagnosis and personalized cancer treatment planning, which is currently suffering from intratumor heterogeneity within a single tumor [36]. It will not only lead to a better understanding of disease progression, but also result in improved treatment outcomes via better selection of therapeutic agents and enhanced monitoring of treatment responses.

C. Mechanism of PFHnD Echogenicity and Dynamics Change

It has been demonstrated here that the optically triggered PFHnDs exhibit clear differences in the intensity and the decay of ultrasound echogenicity between P-pulse and N-pulse ultrasound imaging. Bubbles are known to respond nonlinearly to a driving ultrasound pulse [37]. There have been extensive investigations in contrast enhanced ultrasound imaging with microbubble contrast agents based on different bubble responses to pulses of inverted polarities, e.g. pulse-inversion imaging [38]. However, for phase-changing contrast agents such as PFHnDs, the theoretical mechanism yielding the phenomenon reported here has not been investigated. Our observations of the different responses induced by the different imaging transmit pulses indicate that vaporized PFHnDs interact with ultrasound beyond linearly scattering, analogous to nonlinear response of gas microbubbles. When a vaporized PFHnD experiences ultrasound wave, the rarefactional pressure contributes to the expansion of the cavity while compressional pressure compresses the cavity [39]. In our study, initial rarefactional phase and higher peak rarefactional pressure of N-pulse compared to P-pulse likely lead to a larger size cavity, which resulted in higher echogenicity and slower recondensation. However, other factors may contribute to PFHnD dynamics including heat transfer after optical vaporization, recondensation of phase-change droplets, non-linear interactions of liquid/gaseous form of PFHnDs with ultrasound, and the environment the PFHnDs reside in. There are more to understand and exploit for those phase-change nanodroplets in future investigations.

V. CONCLUSION

In this study, we demonstrated that for laser-activated perfluorohexane nanodroplets embedded in tissue mimicking gel phantoms, ultrafast ultrasound imaging using a transmit pulse with an initial rarefactional phase provides a significantly higher ultrasound echogenicity and induces a significantly slower decay of hyperechoic signals than with an initial compressional phase pulse.

This finding that inverting the phase of an imaging transmit pulse alone can affect the PFHnD related hyperechoic dynamics paves the way to leverage the imaging transmit pulse in manipulating phase-change droplets for contrast-enhanced ultrasound imaging. Extended detectable time of vaporized PFHnDs in ultrasound imaging can improve detection efficiency as well as contrast and spatial resolution by allowing for more averaging. It also implies that encoded ultrasound imaging pulses can manipulate nanodroplet related dynamics, which can potentially be used for tumor heterogeneous microenvironment mapping.

ACKNOWLEDGMENT

The authors would like to thank Kristina A. Hallam and Steven K. Yarmoska at Georgia Institute of Technology and Emory University School of Medicine for their help with acoustic calibration of the ultrasound imaging system and synthesis of droplets, correspondingly. The authors would also like to thank Khosro Shahbazi at South Dakota School of Mines and Technology for the discussion on mechanism of PFHnD echogenicity and dynamics change.

This work was supported by the BCRF-17-043 grant from Breast Cancer Research Foundation and National Institutes of Health under grants EB008101, CA158598 and CA149740. Asterisk indicates the corresponding author.

REFERENCES

- [1]. Ignee A, Atkinson NS, Schuessler G, and Dietrich CF, "Ultrasound contrast agents," *Endosc. Ultrasound*, vol. 5, no. 6, pp. 355–362, 12, 2016. [PubMed: 27824024]
- [2]. Li H, Gan S, Feng ST, Zhou G, Dong Z, Zheng R, and Li ZP, "Nano-sized Ultrasound Contrast Agents for Cancer Therapy and Theranostics," *Curr. Pharm. Des.*, vol. 23, no. 35, pp. 5403–5412, 2017. [PubMed: 28699518]
- [3]. Zhu YI, Miller DL, Dou C, Lu X, and Kripfgans OD, "Quantitative assessment of damage during MCET: a parametric study in a rodent model," *J Ther Ultrasound*, vol. 3, pp. 18, 2015. [PubMed: 26478815]
- [4]. Song P, Trzasko JD, Manduca A, Huang R, Kadirvel R, Kallmes DF, and Chen S, "Improved Super-Resolution Ultrasound Microvessel Imaging With Spatiotemporal Nonlocal Means Filtering and Bipartite Graph-Based Microbubble Tracking," *IEEE Trans. Ultrason., Ferroelectr., Freq. Control*, vol. 65, no. 2, pp. 149–167, 2, 2018. [PubMed: 29389649]
- [5]. Brandt AH, "Evaluation of new ultrasound techniques for clinical imaging in selected liver and vascular applications," *Dan. Med. J.*, vol. 65, no. 3, 3, 2018.
- [6]. Wu SY, Fix SM, Arena CB, Chen CC, Zheng W, Olumolade OO, Papadopoulou V, Novell A, Dayton PA, and Konofagou EE, "Focused ultrasound-facilitated brain drug delivery using optimized nanodroplets: vaporization efficiency dictates large molecular delivery," *Phys. Med. Biol.*, vol. 63, no. 3, pp. 035002, 1 22, 2018. [PubMed: 29260735]
- [7]. Jain A, Tiwari A, Verma A, and Jain SK, "Ultrasound-based triggered drug delivery to tumors," *Drug. Deliv. Transl. Res.*, vol. 8, no. 1, pp. 150–164, 2, 2018. [PubMed: 29204925]
- [8]. Mehrad H, Farhoudi M, and Paknezhad A, "Inertial Cavitation Effect of Catheter-Based Q-Switched Nd:Yag Laser Enhance Extracorporeal Pulsed-Focused Ultrasound Thrombolysis," *Atherosclerosis*, vol. 263, pp. E164–E164, 8, 2017.
- [9]. Goyal A, Yu FTH, Tenwalde MG, Chen XC, Althouse A, Villanueva FS, and Pacella JJ, "Inertial Cavitation Ultrasound with Microbubbles Improves Reperfusion Efficacy When Combined with Tissue Plasminogen Activator in an in Vitro Model of Microvascular Obstruction," *Ultrasound Med. Biol.*, vol. 43, no. 7, pp. 1391–1400, 7, 2017. [PubMed: 28395964]
- [10]. Miller DL, Lu X, Dou C, Zhu YI, Fabiilli ML, Owens GE, and Kripfgans OD, "Multiple ultrasound cavitation-enabled treatments for myocardial reduction," *J. Ther. Ultrasound*, vol. 5, pp. 29, 2017. [PubMed: 29152303]
- [11]. Zhu YI, Miller DL, Dou C, and Kripfgans OD, "Characterization of macrolesions induced by myocardial cavitation-enabled therapy," *IEEE Trans. Biomed. Eng.*, vol. 62, no. 2, pp. 717–727, 2, 2015. [PubMed: 25347871]
- [12]. Chu PC, Chai WY, Tsai CH, Kang ST, Yeh CK, and Liu HL, "Focused Ultrasound-Induced Blood-Brain Barrier Opening: Association with Mechanical Index and Cavitation Index Analyzed by Dynamic Contrast-Enhanced Magnetic-Resonance Imaging," *Sci. Rep.*, vol. 6, 9 15, 2016.
- [13]. Abdalkader R, Kawakami S, Unga J, Higuchi Y, Suzuki R, Maruyama K, Yamashita F, and Hashida M, "The development of mechanically formed stable nanobubbles intended for sonoporation-mediated gene transfection," *Drug Deliv.*, vol. 24, no. 1, pp. 320–327, 11, 2017. [PubMed: 28165819]
- [14]. Sheeran PS, Yoo K, Williams R, Yin M, Foster FS, and Burns PN, "More Than Bubbles: Creating Phase-Shift Droplets from Commercially Available Ultrasound Contrast Agents," *Ultrasound Med. Biol.*, vol. 43, no. 2, pp. 531–540, 2, 2017. [PubMed: 27727022]
- [15]. Wilson CG, Martin-Saavedra FM, Padilla F, Fabiilli ML, Zhang M, Baez AM, Bonkowski CJ, Kripfgans OD, Voellmy R, Vilaboa N, Fowlkes JB, and Franceschi RT, "Patterning expression of regenerative growth factors using high intensity focused ultrasound," *Tissue Eng. Part C Methods*, vol. 20, no. 10, pp. 769–79, 10, 2014. [PubMed: 24460731]
- [16]. Hannah A, Luke G, Wilson K, Homan K, and Emelianov S, "Indocyanine green-loaded photoacoustic nanodroplets: dual contrast nanoconstructs for enhanced photoacoustic and ultrasound imaging," *ACS Nano*, vol. 8, no. 1, pp. 250–9, 1 28, 2014. [PubMed: 24303934]

- [17]. Sheeran PS, Matsunaga TO, and Dayton PA, "Phase change events of volatile liquid perfluorocarbon contrast agents produce unique acoustic signatures," *Phys. Med. Biol.*, vol. 59, no. 2, pp. 379–401, 1 20, 2014. [PubMed: 24351961]
- [18]. Strohm E, Rui M, Gorelikov I, Matsuura N, and Kolios M, "Vaporization of perfluorocarbon droplets using optical irradiation," *Biomed. Opt. Express*, vol. 2, no. 6, pp. 1432–1442, 6 1, 2011. [PubMed: 21698007]
- [19]. Luke GP, Hannah AS, and Emelianov SY, "Super-Resolution Ultra-sound Imaging in Vivo with Transient Laser-Activated Nanodroplets," *Nano Lett.*, vol. 16, no. 4, pp. 2556–9, 4 13, 2016. [PubMed: 27035761]
- [20]. Yu J, Chen XC, Villanueva FS, and Kim K, "Vaporization and recondensation dynamics of indocyanine green-loaded perfluoropentane droplets irradiated by a short pulse laser," *Appl. Phys. Lett.*, vol. 109, no. 24, 12 12, 2016.
- [21]. Li DS, Yoon SJ, Pelivanov I, Frenz M, O'Donnell M, and Pozzo LD, "Polypyrrole-Coated Perfluorocarbon Nanoemulsions as a Sono-Photoacoustic Contrast Agent," *Nano Lett.*, vol. 17, no. 10, pp. 6184–6194, 10, 2017. [PubMed: 28926276]
- [22]. Yoon H, Yarmoska SK, Hannah AS, Yoon C, Hallam KA, and Emelianov SY, "Contrast-enhanced ultrasound imaging in vivo with laser-activated nanodroplets," *Med. Phys.*, vol. 44, no. 7, pp. 3444–3449, 7, 2017. [PubMed: 28391597]
- [23]. Hannah AS, Luke GP, and Emelianov SY, "Blinking Phase-Change Nanocapsules Enable Background-Free Ultrasound Imaging," *Theranostics*, vol. 6, no. 11, pp. 1866–76, 2016. [PubMed: 27570556]
- [24]. Yoon H, and Emelianov S, "Combined multi-wavelength photoacoustic and plane-wave ultrasound imaging for probing dynamic phase-change contrast agents," *IEEE Trans. Biomed. Eng.*, 6 20, 2018.
- [25]. Yoon H, Hallam KA, Yoon C, and Emelianov SY, "Super-resolution imaging with ultrafast ultrasound imaging of optically triggered perfluorohexane nanodroplets," *IEEE Trans. Ultrason., Ferroelectr., Freq. Control*, pp. 1–1, 2018.
- [26]. Lajoinie G, Gelderblom E, Chlon C, Bohmer M, Steenbergen W, de Jong N, Manohar S, and Versluis M, "Ultrafast vapourization dynamics of laser-activated polymeric microcapsules," *Nat. Commun.*, vol. 5, pp. 3671, 4 22, 2014. [PubMed: 24752357]
- [27]. Sheeran PS, Wong VP, Luo S, McFarland RJ, Ross WD, Feingold S, Matsunaga TO, and Dayton PA, "Decafluorobutane as a Phase-Change Contrast Agent for Low-Energy Extravascular Ultrasonic Imaging," *Ultrasound Med. Biol.*, vol. 37, no. 9, pp. 1518–1530, 9, 2011. [PubMed: 21775049]
- [28]. Yarmoska S, Yoon H, and Emelianov S, "Altering lipid shell composition enables the tunability of perfluorocarbon nanodroplets," in *2017 IEEE International Ultrasonics Symposium (IUS)*, 2017.
- [29]. Denarie B, Tangen TA, Ekroll IK, Rolim N, Torp H, Bjastad T, and Lovstakken L, "Coherent Plane Wave Compounding for Very High Frame Rate Ultrasonography of Rapidly Moving Targets," *IEEE Trans. Med. Imag.*, vol. 32, no. 7, pp. 1265–1276, 7, 2013.
- [30]. de Jong N, Frinking PJ, Bouakaz A, and Ten Cate FJ, "Detection procedures of ultrasound contrast agents," *Ultrasonics*, vol. 38, no. 1–8, pp. 87–92, 3, 2000. [PubMed: 10829635]
- [31]. Hallam KA, Donnelly EM, Karpouk AB, Hartman RK, and Emelianov SY, "Laser-activated perfluorocarbon nanodroplets: a new tool for blood brain barrier opening," *Biomed. Opt. Express*, vol. 9, no. 9, pp. 4527–4538, 9 1, 2018. [PubMed: 30615730]
- [32]. Wang Y, Zaytsev ME, Lajoinie G, The HL, Eijkel JCT, van den Berg A, Versluis M, Weckhuysen BM, Zhang X, Zandvliet HJW, and Lohse D, "Giant and explosive plasmonic bubbles by delayed nucleation," *Proc. Natl. Acad. Sci. U. S. A.*, vol. 115, no. 30, pp. 7676–7681, 7 24, 2018. [PubMed: 29997175]
- [33]. Yoon H, Zhu YI, Yarmoska SK, and Emelianov SY, "Design and Demonstration of a Configurable Imaging Platform for Combined Laser, Ultrasound, and Elasticity Imaging," *IEEE Trans. Med. Imag.*, 12 27, 2018.
- [34]. Szabo TL, and Lewin PA, "Ultrasound transducer selection in clinical imaging practice," *J. Ultrasound Med.*, vol. 32, no. 4, pp. 573–82, 4, 2013. [PubMed: 23525382]

- [35]. Dayton PA, Chomas JE, Lum AF, Allen JS, Lindner JR, Simon SI, and Ferrara KW, "Optical and acoustical dynamics of microbubble contrast agents inside neutrophils," *Biophys. J.*, vol. 80, no. 3, pp. 1547–56, 3, 2001. [PubMed: 11222315]
- [36]. Heindl A, Nawaz S, and Yuan Y, "Mapping spatial heterogeneity in the tumor microenvironment: a new era for digital pathology," *Lab. Invest.*, vol. 95, no. 4, pp. 377–84, 4, 2015. [PubMed: 25599534]
- [37]. Burns PN, Powers JE, and Fritzsche T, "Harmonic Imaging - New Imaging and Doppler Method for Contrast-Enhanced Ultrasound," *Radiology*, vol. 185, pp. 142–142, 11, 1992.
- [38]. Simpson DH, Chin CT, and Burns PN, "Pulse inversion Doppler: A new method for detecting nonlinear echoes from microbubble contrast agents," *IEEE Trans. Ultrason., Ferroelectr., Freq. Control*, vol. 46, no. 2, pp. 372–382, 3, 1999. [PubMed: 18238434]
- [39]. de Jong N, Emmer M, van Wamel A, and Versluis M, "Ultrasonic characterization of ultrasound contrast agents," *Med. Biol. Eng. Comput.*, vol. 47, no. 8, pp. 861–873, 8, 2009. [PubMed: 19468770]

Fig. 1.

Vaporization and recondensation of optically triggered PFHnDs. When laser-activated, PFHnDs immediately exhibit hyperechogenicity in ultrasound imaging, which then disappears as the PFHnDs recondense to native, weakly scattering liquid droplets. This repeatable vaporization-recondensation dynamic exhibits echogenicity variations in ultrasound imaging. Ultrafast ultrasound imaging is exploited to capture the fast change of ultrasound echogenicity induced by PFHnD dynamics.

Fig. 2.

Diagrams of experimental setups and imaging of: (a) the optically and acoustically transparent polyacrylamide gel phantom where the red rectangle indicates the optical focus of the light delivery system within the imaging plane; (b) the acoustically scattering polyacrylamide gel phantom containing GT-shaped PFHnD inclusion.

Fig. 3. Waveforms of ultrasound imaging transmit pulses: (a) initial compressional phase followed by rarefactional phase, denoted as P-pulse, (b) initial rarefactional phase followed by compressional phase, denoted as N-pulse.

Fig. 4.

Representative images of one imaging cross-section from the transparent PFHnD phantom, cropped to highlight the region of interest (ROI), measuring 20.8 mm laterally and 11.5 mm axially, where PFHnDs were primarily vaporized. The ROI was located 6.6 mm away from the transducer. (a) Photoacoustic images appear to be very similar indicating consistent activation of PFHnDs. (b) Ultrasound images over time for P-pulse vs. N-pulse exhibit difference in ultrasound echogenicity induced by vaporized PFHnDs (scale bar = 5 mm).

Fig. 5. Normalized SNR of vaporized PFHnDs in ultrasound images from five different imaging cross-sections for P-pulse vs. N-pulse, error bars are standard deviation (N=10).

Fig. 6. Normalized hyperechoic area induced by vaporized PFHnDs over time acquired by P-pulse vs. N-pulse imaging, respectively. The same cross-section was imaged. The threshold shown (10%) was used to evaluate the characteristic decay time.

Fig. 7. Characteristic decay time from 5 different imaging cross-sections for P-pulse vs. N-pulse, error bars represent standard deviation (N=10).

Fig. 8.

C-scan images at 10 mm depth for the PFHnD GT phantom imaged using P-pulse (top) vs. N-pulse (bottom) imaging transmit pulses, respectively: (a) the first frame of ultrafast ultrasound imaging; (b) the first frame of differential ultrasound imaging (Δ US); maximum intensity projection obtained from (c) the first frames of differential ultrasound imaging (Δ US MIP) and (d) the photoacoustic images (PA MIP).

TABLE I**AVERAGE PHOTOACOUSTIC SIGNALS**

Imaging cross-section #	Imaged by N-pulse (x 10^7 arb. unit)	Imaged by P-pulse (x 10^7 arb. unit)
1	1.47 ± 0.07	1.46 ± 0.08
2	1.50 ± 0.07	1.54 ± 0.08
3	1.51 ± 0.07	1.51 ± 0.07
4	1.41 ± 0.07	1.35 ± 0.07
5	1.34 ± 0.07	1.40 ± 0.08

Average photoacoustic signals for the transparent phantom from the region of interest for five different imaging cross-sections for P-pulse vs. N-pulse, plus/minus values indicate standard deviation (N=10).

TABLE II

SNR FOR GT PHANTOM IMAGING

Ultrasound imaging pulse type	US (dB)	US MIP (dB)	PA MIP (dB)
N-pulse	32.1	25.5	18.2
P-pulse	19.3	13.0	17.7

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript