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Optimizing Acoustic Activation of Phase Change Contrast Agents with the Activation Pressure Matching Method: A Review

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Abstract

Sub-micron phase-change contrast agents consist of a liquid perfluorocarbon core that can be vaporized by ultrasound (acoustic droplet vaporization) to generate contrast with excellent spatial and temporal control. When these agents, commonly referred to as nanodroplets, are formulated with cores of low boiling-point perfluorocarbons such as decafluorobutane and octafluoropropane, they can be activated with low-mechanical index imaging pulses for diagnostic applications. Since the utilization of minimum mechanical index is often desirable to avoid unnecessary biological effects, enabling consistent activation of these agents in an acoustic field is a challenge because the energy that must be delivered to achieve the vaporization threshold increases with depth due to attenuation. A novel vaporization approach called Activation Pressure Matching has been developed to deliver the same pressure throughout a field of view in order to produce uniform nanodroplet vaporization and to limit the amount of energy that is delivered. In this manuscript, we discuss the application of this method with a Versasonics V1 Research Ultrasound System to modulate the output pressure from an ATL L11–5 transducer. Vaporization-pulse spacing optimization can be used in addition to matching the activation pressure through depth, and we demonstrate the feasibility of this approach both *in vivo* and *in vitro*. The use of optimized vaporization parameters increases the amount of time a single bolus of nanodroplets can generate useful contrast and provides consistent image enhancement *in vivo*. Therefore, APM is a useful technique for those wishing to maximize the efficacy of phase change contrast agent while minimizing delivered acoustic energy.

Index Terms

Acoustic droplet vaporization; activation pressure matching; phase change contrast agents; optimized droplet activation; perfluorocarbon; nanodroplet

Conflict of Interest

Paul Dayton declares that he is a co-inventor on a patent which describes the formulation of low-boiling point PCCAs and that he is also a co-founder of Nanosonic Bioreagents, LLC, a company which has licensed this patent and manufactures perfluorocarbon nanodroplets for commercial sale.

I. Introduction

Phase-Change contrast agents (PCCAs) are a unique variant of ultrasound contrast agents that can have a longer in vivo circulation time than conventional microbubbles and can be formulated to have a distribution in the hundred-nanometer range [1] [5]. This small size might allow them to extravasate from the intravascular into the extravascular space in regions of leaky endothelium such as that caused by tumor-associated angiogenesis. Low-boiling point PCCAs have superheated perfluorocarbon (PFC) cores that remain stable in vasculature due to increased Laplace pressure and nucleation requirements of the pure perfluorocarbon [2], [4] [7]. However, the liquid core of these droplets can be vaporized or “activated” with ultrasound to form echogenic microbubbles in a process referred to as Acoustic Droplet Vaporization (ADV). Historically, droplets have been used for therapeutic applications such as cavitation enhancement and thermal ablation [8] [13], vessel occlusion [9], [14], [15], aberration correction [9], [16], drug delivery [17] [19], and more recently, MR-guided tumor ablation [20], [21]. ADV can also generate contrast at desired locations on demand and the vaporization-generated microbubbles have been evaluated [22] [24] for diagnostic applications such as perfusion, molecular imaging, and tumor imaging [1], [25] [28]. Much work has been done on the physics of ADV [29] [34] and its dependence on acoustic parameters. Recently, droplets have been examined as temperature probes [35]. For these purposes, high stability is desired so PFCs with boiling-points close to body temperature such as perfluoropentane (PFP) and perfluorohexane (PFH) are used. Droplet vaporization occurs once a pressure threshold is reached and micron-size droplets composed of PFCs such as PFP and PFH require vaporization pressures that are above the FDA limit of 1.9 for diagnostic imaging [2]. However, the use of low boiling point PFCs such as decafluorobutane (DFB) and octafluoropropane (OFP) allows for PCCAs to be used with low-mechanical index (MI) imaging pulses that are under the FDA limit [2], [4], [27], [36].

Creating uniform contrast throughout the target organ is important for obtaining accurate measurements of targeting or perfusion and can be challenging due to the energy-sensitive activation thresholds of these agents; therefore, optimal activation parameters should be used. It is difficult to create the custom pulse sequences necessary for effective PCCA vaporization using commercial ultrasound systems because of their lack of programmability. Commercial systems have been used for droplet vaporization [27], but this approach relies on conventional B-mode pulses for vaporization so there is little spatial control of activation. Furthermore, since multiple frames are acquired to generate maximum intensity projections, generated microbubbles are interrogated multiple times with high intensity pulses which can lead to bubble destruction or cavitation. However, programmable systems such as the Verasonics Research Ultrasound System (Verasonics, Kirkland, WA, USA) can be used to create unique pulse sequences for droplet vaporization [1], [37]. In this manuscript, we describe a pulse optimization approach for generation of uniform PCCA vaporization in a region of interest (ROI).

II. Materials and Methods

A Verasonics V1 system driving a 128-element ATL L11–5 linear array transducer has been chosen as the example for the optimization of activation parameters discussed here. The

imaging scheme that was used to capture contrast generated by droplet activation consisted of a plane-wave pulse inversion approach where all 128 elements were fired at the same time to deliver a single-cycle, 4.5 MHz unfocused pulse, followed by a second unfocused pulse that was 180° out of phase. The receive data from both transmissions were added to produce a single pulse inversion frame. The transmit beam was steered between $\pm 18^\circ$ to acquire data from 7 different angles and the resulting frames were averaged and reconstructed using Verasonics reconstruction algorithms to form one pulse inversion image. The transducer was driven with 10 V and produced a free-field Peak-Negative-Pressure (PNP) of 459 kPa. Activation was accomplished by using the entire aperture to deliver a series of 5 MHz, 5 cycle, focused pulses to different locations within a predetermined ROI. One frame was captured using the plane-wave pulse inversion pulse sequence both before and after activation in order to monitor contrast generation.

A condensation procedure that has previously been described was used to make droplets for all *in vitro* and *in vivo* experiments from lipid-shelled microbubbles [2], which had the following formulation: the lipids 1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC) and 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-methoxy(polyethylene-glycol)-2000 (DSPE-PEG2000) in a 9:1 M ratio and a total lipid concentration of 1.0 mg/mL were dissolved in a solution of phosphate-buffered saline (PBS), propylene glycol, and glycerol (16:3:1). 1.5 mL of the solution was added to a 3 mL glass vial and the head space was gas-exchanged with either DFB or OFP. Microbubbles (1–5 μm) were produced by using an agitation technique. The microbubble solution was cooled to temperatures ranging between -10°C and -12°C and condensed by pressurizing the vial with room air.

For all *in vitro* experiments, a 300 μL volume of the stock droplet solution was injected into a water bath at room temperature (22°C), and a series of focused activation pulses were delivered to produce vaporization. Peak rarefactional pressures of 3.75 MPa and 1.5 MPa were chosen because they are above the activation thresholds for DFB and OFP [2] [4], [38], respectively, for the *in vitro* experiments which were conducted at room temperature (22°C).

III. Activation Pressure Matching

Since the L11–5 has an elevational lens, the free-field pressure for a single applied voltage greatly varies with depth, increasing as the elevational focus is approached (Fig. 1a). As a result, the amount of vaporization, or the number of droplets that are activated, varies with depth in a water bath, as can be seen in the size difference of the generated bubble clouds (Fig. 1b). Furthermore, diffraction of focused pulses and a changing F-number, since the aperture size is being kept constant, can cause pressure variations in the field of view and may be additional factors that contribute to the change of vaporization levels with respect to depth. Puett *et al.* described a method for obtaining uniform contrast generation by optimizing the spacing between the activation pulses so that individual bubble clouds form a larger region that is full of contrast [37]. Although this method accomplishes the goal of uniform droplet vaporization in an ROI, it results in over-activation in the deeper regions of the field of view since the pressure distribution is non-uniform through depth. Consequently,

the ROI is insonified with more energy than is necessary which may be undesirable for diagnostic purposes.

The rationale for Activation Pressure Matching (APM) is to deliver pulses with the same rarefactional pressure to all activation locations in order to limit the delivered energy. This technique not only overcomes the changes in pressure due to the elevational lens, but also due to diffraction, a changing F-number, and attenuation in tissue. By varying the output voltage, the appropriate acoustic pressure can be delivered at different depths. However, the Verasonics system requires hundreds of microseconds to switch between voltages, thus a complete activation sequence may take several milliseconds. All of the activation pulses should be delivered as quickly as possible to ensure uniform vaporization within a single plane, as respiratory motion may alter the imaging plane during in vivo imaging. The generated contrast must also be imaged quickly to ensure accurate measurement since the bubbles produced by the early activation pulses will begin to be cleared from the imaging plane. Fortunately, the Verasonics offers a tool for modulating the amplitude of the output waveform: Pulse-Width Modulation (PWM). The system uses a tri-state pulser to generate a large variety of waveforms (Fig. 2a–b), so in addition to changing the voltage, the amplitude of the generated waveform can be adjusted by modifying the number of clock cycles the output is at +volts, ground, and volts. As can be seen in Fig. 2a–b, the square wave for a PWM value of 0.6 maintains the high or low voltage value for less time compared to the wave for a value of 1 which produces an output pulse having a lower pressure amplitude. Therefore, a consistent pressure through depth can be achieved by selecting an appropriate voltage and adjusting the PWM parameter. Because this approach does not require multiple voltage changes, different pressures can be quickly delivered. An example of the relationship between output pressure and PWM value for a given voltage is illustrated in Fig. 2c; the output pressure increases as the PWM value increases, with a value of 1 signifying no PWM.

In order to determine the correct voltage and PWM values for a desired activation pressure at a specific depth, the system should be calibrated with a hydrophone. We utilized a needle hydrophone (HNA-0400, Onda, Sunnyvale, CA, USA) to calibrate the L11–5 transducer for a range of PWM values at various voltages and depths. MATLAB (MathWorks, Natick, MA, USA) can then be readily used to calculate an appropriate voltage-PWM combination that resulted in the desired pressure for each activation depth. An example of this process is shown in Fig. 3. The target pressure is set to 1.5 MPa, and by using 6 V and the PWM values shown in the solid gray line, the output pressure can be set to within the target $\pm 10\%$ to account for measurement error from the hydrophone. Furthermore, as shown in Fig. 3, the output pressure at 6 V without PWM is enough to cause vaporization at 0.9 cm but is too high in deeper regions and would result in over-activation. When APM is used, the bubble clouds produced by the activation pulses are of similar size as a result of the consistent activation pressure throughout the field of view (Fig. 4a). Conversely, when a single voltage is used without PWM, the bubble clouds are vastly different in size, indicating that the activation pressure for the deeper regions was excessive since vaporization was achieved with a smaller pressure in shallower areas (Fig. 4b). The size of the bubble clouds produced by the activation pulses was calculated using a method described previously by Puett et al. [37], where the number of pixels with an intensity higher than 1% of the cloud's brightest pixel were counted, and this number was multiplied by the pixel size to get an area

measurement (Fig. 4c–d). Each box in the box plot represents the area for the 5 bubble clouds at the different depths (rows). The size of the bubble clouds is very similar between the different depths when APM is used (Fig 4c); cloud size between the different depths is not significant for any two depths. In contrast, when a single voltage is used for activation, there is a clear positive trend as the elevational focus is approached, and the area of the bubble clouds was significant between rows in all but 3 cases (Fig 4d).

IV. Optimization of activation pulse spacing

In order to produce a uniform area of activation in the ROI, the spacing between the activation pulses must be adjusted. Puett et al. described a procedure for optimizing the spacing that consisted of activating a group of vertical and horizontal lines, and varying the spacing between them until the full-width-half-maximum (FWHM) of the intensity profile overlapped [37]. Since APM was not used, the size of the bubble clouds produced by droplet vaporization varied with depth. Therefore, individual lateral and axial spacings had to be derived for different regions in the field of view. In this example, the same procedure described by Puett et al. is used, but since APM produces same-sized bubble clouds regardless of depth, only one set of lateral and axial spacing needed to be calculated (Fig. 5). Contrast lines separated by different distances were created by activating droplets in a water bath and intensity profile were generated by averaging the uncompressed image data along either the lateral dimension, for calculating the optimal axial spacing, or axially, for calculating the optimal lateral spacing. As discussed above, the optimal spacing is defined by the overlap of the FWHM of the intensity profiles. However, too much overlap is not desirable since any interaction that may cause cavitation or bubble destruction between an activation pulse and a pre-existing bubble cloud. Therefore, the distance between the right FWHM point on the left activation line intensity profile and the left FWHM point on the right intensity profile should be as close as possible to zero, so the spacing that has the lowest distance value between these two points is the optimal spacing. When the 2 intensity profile cannot be differentiated, or the FWHM points are not visible, because the spacing is too small, the distance value will be given by subtracting twice the mean FWHM of the largest spacing (1 mm and 0.75 mm for the axial and lateral cases, respectively) from the FWHM of the intensity profile. For example, the FWHM distance value for 0.5 mm in Fig 5a will be twice the mean FWHM of the intensity profile for the 1mm spacing (0.95 mm), subtracted from the FWHM of the 0.5 mm intensity profile (0.475 mm), which produces a value -0.475 mm. Three trials were averaged for each spacing, and the FWHM distances for the axial spacings 1, 0.75, and 0.5 mm were 0.515 ± 0.13 , 0.16 ± 0.034 , and -0.475 ± 0.11 mm, respectively, and the values for the lateral spacings 0.75, 0.5, 0.25 mm were 0.4 ± 0.089 , 0.22 ± 0.056 , and -0.36 ± 0.08 mm, respectively. Therefore, the optimal spacing for the L11–5 is around 0.75 mm and 0.5 mm in the axial and lateral directions, respectively. Using APM along with the optimized spacing, a uniform region of vaporization can be generated (Fig. 6). As previously discussed, uniform activation is possible without APM using the methods described by Puett et al. [37]. However, that approach requires over-activation of droplets around the elevational focus in order to vaporize regions located further away from the transducer. APM creates uniform vaporization without excessive vaporization or energy delivery in the near-field.

V. *In Vivo* Demonstration of apm

APM can be easily translated to *in vivo* applications. The previously described procedure can be applied *in vivo* with attenuation compensation. Here, we demonstrate the effectiveness of APM in rat kidneys. As with *in vitro* activation, an appropriate PWM value must be selected for each depth for a specific voltage. However, the target pressure will not be flat through depth but rather, it will increase so that the same pressure is delivered to all the activation locations after it is attenuated by tissue (Fig. 7). In order to calculate the required initial pressure before attenuation, the free-field pressure at each depth is derated using the following model: 3.5 mm of superficial tissue ($\alpha = 0.6$ dB/MHz/cm) followed by kidney tissue ($\alpha = 1$ dB/MHz/cm), assuming a kidney frequency dependence of 1.1 ($\alpha = \alpha_0 f^{1.1}$) [39], [40]. Similar to the *in vitro* case, APM can yield a pressure through depth that is within $\pm 10\%$ of the target pressure by selecting the appropriate attenuation model and PWM values for the different depths while using the same voltage (8 V). Additionally, not using APM would result in over-activation in the shallower regions since 8 V produces a pressure that is higher than the target without PWM.

Using the attenuation-corrected APM method with the optimized activation pulse spacing discussed earlier, OFP droplets were activated in a rat kidney (Fig. 8). A custom MATLAB script was used to manually segment the kidney and calculate the locations of the activation pulses and their appropriate PWM values. Activation locations start with the deepest lines first in order to avoid attenuation of the subsequent pulses by the generated contrast, as indicated by the red dashed lines. APM generates uniform vaporization inside the kidney, indicating that the pressure delivered to each activation location was similar, and the spacing between locations was appropriate.

VI. *In Vivo* benefit of apm

Efficient activation of PCCAs is important not only for depositing the minimum amount of energy necessary to cause PCCA activation, but also for improving the contrast enhancement generated over time in order to reduce contrast agent dose. In a previous study using un-optimized vaporization parameters, the circulation half-life of DFB and OFP droplets in rats was estimated at 11 and 3.5 minutes, respectively [1]. In this previous study, droplets injected in a bolus were activated using the same parameters over a period of 19 minutes and the generated contrast enhancement at all time points was fitted to a mono-exponential to obtain the circulation half-life. In this study, the same procedure is followed except that APM was used for vaporization at the DFB threshold (3.5 MPa). Briefly, a bolus consisting of 60 μL droplets (around 1×10^9 droplets) diluted in 60 μL of saline was injected and a series of activation pulses was delivered every 3 minutes for 19 minutes, starting 1 minute after injection. Optimized spacing was not used in order to reduce over-activation of OFP, as it is much more volatile than DFB. As expected, OFP yielded a higher contrast enhancement with more droplets activated for a given pulse (Fig. 9). However, the decreased stability of OFP leads to spontaneous vaporization which reduces the concentration of agents in droplet form and decreases the circulation time [1]. It should be clarified that what is being referred to as “circulation time” in this study is the time for which detectable contrast can be generated, not the time the droplets remain in the vasculature. Fig. 9 shows that the contrast

enhancement, in dB, relative to a baseline image taken prior to the injection of PCCAs. The Contrast enhancement for OFP is almost three times that of DFB (a) at the one-minute time point, and OFP is cleared much faster than DFB (b). The contrast enhancement at each time-point is normalized to the one-minute time point and fitted to a mono-exponential decay model in order to calculate the circulation half-life. The half-life for DFB and OFP was 15.97 ± 3.0 and 6.92 ± 0.7 min, respectively. In both cases, the resulting value obtained by using APM was substantially higher than previously reported. The droplets are circulating the same amount of time, but using APM makes the vaporization at all depths more efficient enabling the use of the injected droplets for a much longer time. This part of the study demonstrates the benefit of using optimized activation parameters for *in vivo* applications.

VII. Conclusion

The purpose of this manuscript is to present a novel approach for optimizing activation parameters for PCCA vaporization. Activation Pressure Matching, the method described in this study, relies on using PWM to regulate the output pressure so that all activation locations are sonified with the same amount of pressure. The use of this approach allows for efficient droplet vaporization and the generation of uniform contrast in a region of interest without delivery of excessive pressure. In addition to modulating the output pressure for different depths, optimal spacing between activation points can be found in order to consistently vaporize droplets in the target. APM can be applied *in vivo* by using an appropriate attenuation model. In this study a rat kidney model was used to demonstrate that by modulating the initial output pressure, uniform vaporization is achieved in the tissue after attenuation compensation. When APM was used to activate OFP and DFB droplets over time, it was found that the circulation time of both compounds was much longer than indicated by previous experiments that used un-optimized activation parameters because higher amounts of detectable contrast are generated.

This study has presented a new method for activating droplets that can be a valuable tool for PCCA applications for the treatment and assessment of disease. For example, if droplets are targeted to a disease marker, it is imperative that the vaporization pressure is consistent throughout the tissue since variations in activation pressure will cause spatial inhomogeneities in vaporization. This is an issue because it would be challenging to determine if differences in contrast are a result of inconsistent pressures or different levels of targeting throughout the tissue.

Inconsistent activation pressure would also be a problem for a perfusion imaging approach using PCCAs. Instead of measuring the wash-in of contrast into an imaging plane, as is done in microbubble destruction reperfusion techniques, the contrast generated by droplet activation can be monitored as it washes out of the imaging plane to obtain a measure of perfusion. Because droplets provide excellent temporal and spatial control of contrast generation, different regions of a target can be activated at different times in order to see any differences in perfusion in different tissues. A previous study found that the perfusion rate in kidneys depends on the concentration of microbubbles that is used [41]. Therefore, if different vaporization pressures are used, the perfusion rates might be different due to a disparity in droplet activation and not due to any physiological factors.

Additionally, when investigating bio-effects caused by droplet vaporization, it is essential to produce uniform vaporization in order to reliably correlate droplet vaporization with therapeutic effects or tissue damage. Also, it is reasonable to assume that limiting the amount of energy delivered into the tissue to the minimum needed to achieve the desired result will induce the least amount of bioeffects. These needs suggest that APM will be a desirable tool in not only imaging but also therapeutic applications.

APM can vaporize droplets efficiently to produce uniform contrast generation in rat kidneys. However, APM does not take into account phenomena such as aberration which is a significant factor in human ultrasound imaging. As a result of aberration, pulses become defocused by changes in density and speed of sound between different tissues. De-focusing of activation pulses can result in uneven vaporization of PCCAs and may require the tissue to be insonified with increased energy to overcome the loss of pressure due to weaker focusing. Therefore, a simple attenuation model may not be appropriate for use in humans where the volume of tissue that is interrogated is much larger than in a rat or mouse. Interestingly, using droplets for aberration correction has been proposed [9], so it is possible that preliminary pulses can be delivered to form microbubbles and monitor vaporization differences in order to evaluate aberration effects and further calibrate APM for efficient activation. Additionally, APM depends heavily on using the correct attenuation model and therefore, a different derating scheme must be used for each type of tissue (various types of tumors and body organs) in order to efficiently obtain uniform droplet activation.

APM is a novel approach to PCCA activation that has various advantages over prior methods; mainly, the ability to deliver the same amount of pressure to all activation sites in order to minimize the energy that is delivered and produce uniform activation throughout the target. Furthermore, APM can serve as an important tool for the accurate assessment of disease progression and response to therapy.

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Biographies

Juan D. Rojas (S'14) obtained his B.S. degree in biomedical engineering from the University of North Carolina in 2012. He joined the Dayton lab in 2013 and is pursuing a Ph.D. from the University of North Carolina and North Carolina State University Joint Department of Biomedical Engineering. His research includes the development of technologies for the use of phase change contrast agents to assess disease progression and evaluate response to therapy.

Paul A. Dayton received his B.S. degree in physics from Villanova University in 1995, his M.E. degree in electrical engineering from the University of Virginia in 1998, and his Ph.D. degree in biomedical engineering in 2001, also from the University of Virginia. He pursued postdoctoral research and was later research faculty at the University of California, Davis. Much of Dr. Dayton's training was under the mentorship of Dr. Katherine Ferrara; his initial studies involved high-speed optical and acoustical analysis of individual contrast agent microbubbles. In 2007, Dr. Dayton moved to the Joint Department of Biomedical Engineering at UNC Chapel Hill and NC State University, Raleigh, where he is now Professor and Associate Department Chair. Dr. Dayton is currently Associate Director for Education for the Biomedical Imaging Research Center, and his research interests involve ultrasound contrast imaging, ultrasound-mediated therapies, and medical devices. Dr. Dayton is a member of the technical program committee for the IEEE UFFC Society, and a member of the editorial boards for the journals *IEEE Transactions on Ultrasonics, Ferroelectrics, and Frequency Control*; *Molecular Imaging*; and *Bubble Science, Engineering, and Technology*.

Fig. 1.

Several activation pulses were delivered to the locations indicated by the green markers in order to activate OFP droplets in a water bath. Output pressure varies with depth in a water bath for a single voltage as a result of the elevational lens on the L11-5 (a). This change in pressure leads to different levels of droplet activation (b). The pressure increases as the 1.4 cm elevational focus is approached, causing the bubble clouds produced by vaporization to be larger.

Fig. 2.

The Verasonics system uses PWM to modulate the amplitude of the output waveform by changing the time the output is at each of the three voltage states (+V, -V, ground). As can be seen in (a), the width of each square cycle is narrower for the PWM of 0.6 (gray line) than for the PWM value of 1 (black line), and this difference produces waveforms of different amplitudes (b). (c) demonstrates that decreasing the PWM value from 1 (no PWM) produces a waveform with a lower peak negative pressure. Therefore, a range in pressures can be achieved using only 1 peak-to-peak voltage.

Fig. 3.

In this example of the APM process, a target activation pressure of 1.5 MPa (solid black) is chosen, and the PWM values (solid gray) are applied to 6 V to obtain an output pressure (dashed black) that is within $\pm 10\%$ (light dashed) of the target pressure for depths ranging from 0.9 to 1.7 cm.

Fig. 4.

Difference between using APM (a) and a single voltage without PWM (b). The bubble clouds on (a) are similar in size, indicating that the vaporization pressure was uniform for all activation areas. Not using PWM results in a range of activation pressures that vary with depth, which increases contrast generation as the pulses approach the elevational focus. The area of the activation clouds was measured for each depth (rows) and APM produced bubble clouds of the same size (c), while the size of the clouds increased with depth when a single voltage was used (d).

Fig. 5.

Intensity profile (rows a and c) were generated by averaging imaged data from activation lines (rows b and d) separated by a range of distances between 0.25 and 1 mm. The optimal spacing between activation lines is characterized by the overlap of the FWHM of the intensity profile of adjacent lines and so 0.75 mm and 0.5 mm are the best axial and lateral spacing.

Fig. 6.
APM and optimized spacing creates a region of uniform contrast.

Fig. 7.

Pressure map with distance after accounting for attenuation using a rat kidney attenuation model. The target pressure (solid black) of 1.5 MPa increases with depth since the initial pressure will be derated by the tissue. Using APM with the PWM values shown in the solid gray curve, the pressure delivered to each depth is within $\pm 10\%$ of the target pressure (dashed black line). The output generated without APM (dotted black) is higher than the target and would result in the delivery of higher pressure than is necessary for droplet vaporization.

Fig. 8.

OFP droplet activation in a rat kidney using APM and optimized spacing. The left image shows the contrast image of the kidney before droplet vaporization, and the image on the right is an image of the post-activation contrast that was generated by the activation pulses (center). The green dots on the center image represent the activation locations. Consistent activation throughout the kidney was achieved by sending activation pulses with the same peak rarefactional pressure regardless of depth.

Fig. 9.

Contrast enhancement over the baseline case for each time point (a), and normalized to the one-minute time-point (b). The plot on the left shows that the contrast generated when OFP droplets were activated using APM is much greater than that of DFB at the one-minute time point. Conversely, OFP has a circulation time that is less than that of DFB, as can be seen on the right plot. A mono-exponential (Ae^{-bt}) was fit to the normalized contrast enhancement data (solid line) and used to compute the circulation half-life for each type of droplet. The dashed lines represent the 95% confidence intervals of the fit. The half-life was 15.97 ± 2.96 min for DFB and 6.92 ± 0.65 min for OFP. Both values are much higher than previously reported.