Journal of Visualized Experiments

Contrast-Enhanced Subharmonic Aided Pressure Estimation (SHAPE) Using Ultrasound Imaging with a Focus on Identifying Portal Hypertension

--Manuscript Draft--

Article Type:	Invited Methods Article - JoVE Produced Video
Manuscript Number:	JoVE62050R1
Full Title:	Contrast-Enhanced Subharmonic Aided Pressure Estimation (SHAPE) Using Ultrasound Imaging with a Focus on Identifying Portal Hypertension
Corresponding Author:	Flemming Forsberg UNITED STATES
Corresponding Author's Institution:	
Corresponding Author E-Mail:	Flemming.Forsberg@jefferson.edu
Order of Authors:	Flemming Forsberg
	Ipshita Gupta
	Priscilla Machado
	Colette Shaw
	Jonathan Fenkel
	Kirk Wallace
	John Eisenbrey
Additional Information:	
Question	**Response**
Please specify the section of the submitted manuscript.	Bioengineering
Please indicate whether this article will be Standard Access or Open Access.	Standard Access (US$2,400)
Please indicate the **city, state/province, and country** where this article will be **filmed**. Please do not use abbreviations.	Philadelphia, Pennsylvania, United States of America
Please confirm that you have read and agree to the terms and conditions of the author license agreement that applies below:	I agree to the Author License Agreement
Please provide any comments to the journal here.	

TITLE:

Contrast-Enhanced Subharmonic Aided Pressure Estimation (SHAPE) Using Ultrasound Imaging with a Focus on Identifying Portal Hypertension

AUTHORS:

Flemming Forsberg[1], Ipshita Gupta[1,2], Priscilla Machado[1], Colette M. Shaw[1], Jonathan M. Fenkel[3], Kirk Wallace[4], John R. Eisenbrey[1]

[1]Department of Radiology, Thomas Jefferson University, Philadelphia, PA, USA,
[2]School of Biomedical Engineering, Sciences and Health Systems, Drexel University, Philadelphia, PA, USA
[3]Department of Medicine, Division of Gastroenterology and Hepatology, Thomas Jefferson University, Philadelphia, PA, USA
[4]GE Global Research, Niskayuna, NY, USA

Email addresses of co-authors:

Ipshita Gupta	(ipshitagupta89@gmail.com)
Priscilla Machado	(priscilla.machado@jefferson.edu)
Colette M. Shaw	(colette.shaw@jefferson.edu)
Jonathan M. Fenkel	(jonathan.fenkel@jefferson.edu)
Kirk Wallace	(wallacek@ge.com)
John R. Eisenbrey	(john.eisenbrey@jefferson.edu)

Corresponding author:

Flemming Forsberg (flemming.forsberg@jefferson.edu)

KEYWORDS:

Pressure estimation, contrast-enhanced ultrasound, subharmonic imaging, liver, hepatic venous pressure gradient, portal hypertension

SUMMARY:

A protocol for noninvasively estimating ambient pressures utilizing subharmonic ultrasound imaging of infused contrast microbubbles (following appropriate calibration) is described with examples from human patients with chronic liver disease.

ABSTRACT:

Noninvasive, accurate measurement of pressures within the human body has long been an important but elusive clinical goal. Contrast agents for ultrasound imaging are gas-filled, encapsulated microbubbles (diameter < 10 μm) that traverse the entire vasculature and enhance signals by up to 30 dB. These microbubbles also produce nonlinear oscillations at frequencies ranging from the subharmonic (half of the transmit frequency) to higher harmonics. The subharmonic amplitude has an inverse linear relationship with the ambient hydrostatic pressure. Here an ultrasound system capable of performing real-time, subharmonic aided pressure estimation (SHAPE) is presented. During ultrasound contrast agent infusion, an algorithm for

optimizing acoustic outputs is activated. Following this calibration, subharmonic microbubble signals (i.e., SHAPE) have the highest sensitivity to pressure changes and can be used to noninvasively quantify pressure. The utility of the SHAPE procedure for identifying portal hypertension in the liver is the emphasis here, but the technique has applicability across many clinical scenarios.

INTRODUCTION:

A number of different ultrasound contrast agents (UCAs) are approved for clinical use in cardiology (in particular left ventricular opacification) and radiology (in particular adult and pediatric liver lesion characterization) across the world[1]. The sensitivity and specificity of ultrasound imaging can be improved by intravenous (IV) injection of gas-filled microbubbles (diameter < 10 μm) encapsulated by a lipid or protein shell as UCAs that traverse the entire vasculature and enhance signals by up to 30 dB[1]. These UCAs not only enhance the backscattered ultrasound signals, but at sufficient acoustic pressures (> 200 kPa) they also act as nonlinear oscillators. Hence, significant energy components will be produced in the received echoes ranging from subharmonic and harmonic to ultraharmonic frequencies[1,2]. These nonlinear signal components can be extracted from tissue and linear bubble echoes (e.g., using pulse inversion) and used to create contrast -specific imaging modalities such as subharmonic imaging (SHI), which receives at half the transmit frequency (i.e., at $f_0/2$) [3]. Our group has demonstrated in human clinical trials that SHI can detect the blood flow in neovessels and arterioles associated with a variety a tumors and tissues[4-9].

We have advocated the use of UCAs not as vascular tracers, but as sensors for noninvasive pressure estimation in the circulatory system by monitoring subharmonic contrast bubble amplitude variations[10]. This innovative technique, called subharmonic-aided pressure estimation (SHAPE), relies on the inverse linear correlation between the amplitude of the subharmonic signals and hydrostatic pressure (up to 186 mmHg) measured for most commercial UCAs in vitro ($r^2 > 0.90$) as summarized in **Table 1**[10,11]. However, it should be noted that not all UCAs exhibit this behavior. Most notably, it has been shown that subharmonic signals from the UCA SonoVue (known as Lumason in the USA) initially rise with hydrostatic pressure increases, followed by a plateau and a decreasing phase[12]. Nonetheless, SHAPE offers the possibility of allowing pressure gradients in the heart and throughout the cardiovascular system as well as interstitial fluid pressure in tumors to be obtained noninvasively[13-17]. Recently, we implemented a real-time version of the SHAPE algorithm on a commercial ultrasound scanner and provided proof-of-concept that SHAPE can provide in vivo pressure estimates with errors of less than 3 mmHg in the left and right ventricles of patients[16,17].

The most experience with SHAPE has been gained diagnosing portal hypertension with more than 220 subjects enrolled to date and initial findings confirmed in a multi-center trial[13,14]. Portal hypertension is defined as an increase in the pressure gradient between the portal vein and hepatic veins or the inferior vena cava exceeding 5 mmHg, while clinically significant portal hypertension (CSPH) requires a gradient or its equivalent, a hepatic venous pressure gradient (HVPG) ≥ 10 mmHg[18]. CSPH is associated with an increased risk of gastroesophageal varices, ascites, hepatic decompensation, post-operative decompensation, and hepatocellular

carcinoma[18,19]. Patients who develop ascites have a 50% three-year mortality and those who develop spontaneous infection of the ascites fluid carry a 70% one-year mortality. Patients with cirrhosis have a 5-10% yearly incidence of gastroesophageal variceal formation, and a 4-15% yearly incidence of bleeding; each bleeding episode carries up to a 20% risk of death[18,19].

This manuscript describes how to conduct a SHAPE study using commercially available equipment and UCAs with an emphasis on identifying portal hypertension in the liver of patients. The critical calibration procedure required to achieve the highest sensitivity to estimating pressure changes is explained in detail.

PROTOCOL:

The institutional review boards of both Thomas Jefferson University and the Hospital of the University of Pennsylvania approved this protocol. The protocol is compliant with the Health Insurance Portability and Accountability Act. The United States Food and Drug Administration (FDA) issued an Investigational New Drug approval (IND # 124,465 to F. Forsberg) for this protocol. GE Healthcare (Oslo, Norway) provided the UCA used in this research (Sonazoid; **Table 1**). Sonazoid is not approved by the FDA for any clinical applications in the United Sates, which is why an IND was necessary. Other UCAs with FDA approval[1] can be used off-label at the discretion of the treating physician if deemed potentially clinically useful.

NOTE: The full protocol and statistical analysis plan are available at https://clinicaltrials.gov/ct2/show/NCT02489045. Trial registration number: NCT # 02489045.

1. Subject preparation

1.1 Review the subject's known drug allergies or intolerances in particular any known allergy to the UCA being used.

1.2 Exclude subjects with unstable cardiopulmonary conditions or who are generally medically unstable.

1.3 Put the subject on a stretcher in the supine position.

1.4 Place an 18 - 22 gauge cannula in a vein in the subject's right or left arm for the UCA infusion.

1.5 Make sure emergency services (e.g., a crash cart) will be available within the hospital in case of any acute adverse reactions.

NOTE: UCAs are very safe with serious anaphylactoid-type reactions reported at a rate of less than 0.01%.[20]

2. UCA preparation (Specific to Sonazoid)

2.1 Prepare three (3) vials with 48 µL of microbubbles (6 mL) d for each subject by resuspending according to the manufacturer's instructions. The UCA is supplied as a dry powder within 10 mL sealed vials. The headspace of the vials contains perfluorobutane.

2.1.1 Perforate the stopper of the UCA vial with a chemospike.

2.1.2 Remove the protective cap from the syringe port of the chemospike and add 2 mL of sterile water.

2.1.3 With the syringe remaining attached to the chemospike, immediately shake the product for 1 minute to ensure a homogeneous product.

2.1.4 Withdraw the product into the syringe and re-inject the product back into the vial again. This is to avoid dilution of the product due to the dead-space volume in the chemospike.

2.1.5 Remove the syringe from the syringe port and reattach the protective cap. The concentration of the reconstituted UCA is 8 µL microbubbles/mL.

2.1.6 Repeat the reconstitution procedure for the other 2 vials.

3.2 Use saline (0.9% NaCl solution) to fill up the connecting tubes before being connected to a 3-way stopcock. The stopcock will then be connected to the extension tubing leading to the cannula.

3.3 Draw all three (3) vials of suspended UCA into a 10 mL syringe, and place it in a syringe pump at the same level or below the patient, and connect directly to the stopcock.

3.4 After the initial ultrasound imaging and after the stopcock has been opened, infuse the NaCl solution at a rate of 120 mL/hour, and co-infuse Sonazoid at a rate of 0.024 µL per kg body weight per minute (suspension infusion rate of 0.18 mL/kg/hour).

NOTE: This infusion rate was selected based on our group's previous experiences with Sonazoid infusion in portal hypertension subjects undergoing SHAPE[13,14,21]. The exact resuspension procedure and infusion method will vary depending on the UCA used.

3. Initial ultrasound imaging

3.1 Power up an ultrasound scanner (e.g., Logiq E10) and select the C1-6-D curvilinear probe.

3.2 Select an abdominal preset on the ultrasound scanner and use a curvi-linear array (typically with a 1-6 or 2-8 MHz bandwidth) to acquire grayscale images of both the portal and a hepatic vein in the same imaging plane and at similar depths (**Figure 1**). This is generally best achieved via a subcostal approach.

4.3 Optimize the images based on Good Clinical Practice and take care to select the hepatic vein region away from the inferior vena cava to avoid the influence of retrograde flow.

4. SHI and SHAPE imaging

4.1 Activate the SHI contrast imaging mode in dual display mode (i.e., running real-time B-mode and SHI simultaneously) using the **Subharmonic Contrast** touch panel button and activate Contrast mode. Then select **SUBH-AM** on the rotary control.

4.1.1 Perform SHI at a transmit frequency of 2.5 MHz and obtain the received signals at 1.25 MHz.

4.1.2 Use pulse-shaping to maximize the generation of subharmonic microbubble signals, such as a Gaussian windowed binomial filtered square wave with Sonazoid,[21] but this is scanner and UCA dependent[17].

NOTE: The choice of imaging frequency and pulse shape may not be available to end-users.

4.2 Confirm the patency of the portal and the hepatic vein as well as the presence of microbubbles, which can take up to 1-2 minutes from the start of the infusion.

4.3 Activate the SHAPE automated optimization code to optimize SHAPE by compensating for varying depth and attenuation[22,23]. Select **TIC Analysis** on the touch panel followed by **F6** and then the **k** button.

4.4 The SHAPE optimization algorithm will acquire subharmonic data for every acoustic output level. Once data acquisition is complete, position an ROI on the portal vein in the contrast sample window (top left on the TIC Analysis screen).

4.4.1 Plot the average subharmonic data within the ROI as a function of acoustic output and fit a logistic curve to the data. Select the inflection point of this curve (or rather the peak in the derivative curve shown underneath) as the optimized power, as this has been shown to be the point of greatest SHAPE sensitivity[22,23]. One such set of curves is shown in **Figure 2**.

4.5 Adjust the acoustic output power to the value identified in step 4.4.1, which will ensure the maximum change in subharmonic amplitudes a function of ambient pressure (i.e., maximizing the sensitivity of SHAPE).

4.6 Acquire subharmonic data from the microbubbles (i.e., SHAPE) in 5-15 s segments during the infusion of the UCA suspension (**Figure 3**).

5. SHAPE data processing

5.1 Once the optimized SHI cine-loop has been acquired (step 5.6) select "**TIC Analysis**" on the touch panel.

5.1.1 Make sure "**Motion Tracking**" is activated on the touch panel, which adjusts the ROI position for each frame to compensate for any breathing or other motion.

5.1.2 Make sure dB is selected as the unit for the Y-axis on the traces in the analysis window.

5.2 In the contrast sample window (top left on the screen) select identical ROIs (elliptical regions are default) within the hepatic and portal veins. In the analysis window (to the right) the subharmonic signal (in dB) within each vessel is averaged over all the frames in a 0.5 MHz bandwidth around 1.25 MHz.

5.3 Calculate the final SHAPE gradient (in dB) as the difference in the mean subharmonic signal between the hepatic and the portal vein ROIs. Based on current studies, the optimal operating point for identifying CSPH is -0.11 dB and the linear regression equation is HVPG = 0.81 x SHAPE + 9.43.[14] It is important to note that this cutoff and equation are both scanner and UCA dependent.

REPRESENTATIVE RESULTS:

As with all ultrasound imaging examinations, the first consideration for liver SHAPE is to obtain the best possible baseline grayscale images of the target region and to ensure (using Doppler imaging) that there are no intrahepatic portal venous shunts or other vascular abnormalities present. In the case of liver imaging for diagnosing portal hypertension the key is to visualize both the portal vein and a hepatic vein at the same depth to minimize the impact of attenuation (**Figure 1**).

Even though UCA concentration is not considered a critical factor in SHAPE procedures[10,23], it is nonetheless recommended to infuse the UCA to minimize all sources of variability. The UCA should be reconstituted and infused (preferably through a 20 or 22 gauge needle[24]) according to the manufacturer's specific instructions. Once equilibrium enhancement is reached the optimization algorithm should be activated and an ROI in the portal vein selected, which will produce curves such as those shown in **Figure 2.** Once the optimal acoustic output power has been selected calibrated SHI data (i.e., SHAPE) can be acquired.

Examples of SHAPE images in subjects with and without CSPH are presented in **Figure 3**. The main visual difference is the marked subharmonic signal present in the hepatic vein in the subject with CSPH (**Figure 3B**) and absent in the other case (**Figure 3A**). Quantitative, relative pressure estimates can be calculated from the difference between the average subharmonic signals of ROIs placed in the hepatic and portal veins (i.e., the SHAPE gradient). However, in approximately 10% of cases studied so far, the subharmonic signal was too close to the scanner's noise floor and had to be discarded. This could be due to inadequate contrast enhancement. Moreover, there are patients who present with clinical and laboratorial signs of portal hypertension, but who have HVPG values that are normal or zero. This can be attributed to a number of anatomical

and/or vascular variations, such as one subject with a fistula between the portal and hepatic vein leading to no difference between the free and wedged pressures and, thus, an incorrect SHAPE diagnosis (**Figure 4**).

We conducted a first-in-humans, pilot study of SHAPE in 45 patients undergoing transjugular liver biopsy (providing HVPG measurements as the reference standard), which showed significantly higher SHAPE gradients between the portal and hepatic veins in subjects with CSPH (i.e., a HVPG ≥ 10 mmHg) than in those with lower HVPGs (1.37 ± 0.59 dB vs. -1.68 ± 0.27 dB, $p < 0.001$)[13].

Recently, we expanded on the concept of using SHAPE for portal pressure estimation in a large multi-center clinical trial. Results from 178 subjects across two sites using modified Logiq 9 systems established the utility of SHAPE for diagnosing CSPH with a sensitivity of 91% (95% confidence interval: 88-93%) and a specificity of 82% (95% confidence interval: 75-85%)[14]. The overall accuracy was 95% for diagnosing subjects with CSPH (95% confidence interval (CI): 89-99%) and these subjects had a higher SHAPE gradient than participants with lower HVPGs (0.27 ± 2.13 dB vs -5.34 ± 3.29 dB; $p < 0.001$) indicating SHAPE may indeed be a useful tool for the diagnosis of portal hypertension[14]. Similarly, the sensitivity and specificity for diagnosing all subjects with portal hypertension (i.e., HVPG ≥ 5 mmHg) were 71% and 80%, respectively.

FIGURES AND TABLE LEGENDS:

Figure 1: Example of grayscale liver image for SHAPE initiation. Arrows indicate the portal vein (PV) and a hepatic vein (HV).

Figure 2: Calibration curve for SHAPE optimization. (A) Subharmonic amplitude (in dB) as a function of acoustic output power (in %) showing the characteristic S-curve behavior. **(B)** The derivative of the S-curve for output power selection (arrow indicates selected peak and, thus, power).

Figure 3: Dual Imaging with B-mode (black and white) and subharmonic imaging (gold) on the left and right respectively of each image. (A): A patient with normal HVPG values (3 mmHg) with a bright subharmonic signal from the portal vein (PV) and inadequate signal from the hepatic vein (HV). **(B):** A patient with CSPH and a HVPG of 15 mmHg demonstrating considerable subharmonic signals in both portal and hepatic veins.

Figure 4: Unsuccessful SHAPE study in subject with a fistula between the hepatic veins. This anatomical variation resulted in an HVPG of 0 mmHg even though the gradient pressures (referred to as the free and wedged pressures[18,19]) were both 39 mmHg (i.e., a difference of 0 mmHg), while the SHAPE gradient was -15.33 dB.

Table 1: Subharmonic response (and correlation) of commercial UCAs to a pressure increase of approximately 185 mmHg.

DISCUSSION:

Noninvasive, accurate measurement of pressures within the human body has long been an important but elusive clinical goal. The protocol for SHAPE measurements presented here achieves this goal. The most critical component of the SHAPE procedure is the optimization algorithm, since subharmonic data not acquired at the optimal acoustic power output will correlate poorly with hydrostatic pressures[17,22,23]. The initial version of this software implemented on a Logiq 9 scanner was prone to displaying multiple peaks in the derivative of the S-curve (cf., **Figure 2B**) making the correct output power selection difficult[13,14]. However, with improved motion correction on the Logiq E10 scanner this issue has been somewhat mitigated[23]. Moreover, the SHAPE algorithm as currently implemented has a failure rate of approximately 10%, where the subharmonic signal-to-noise ratio is too low for reliable pressure estimates to be calculated[14]. No differences in age, body-mass-index, depth of imaging or liver status have been identified between subjects with successful and with failed SHAPE studies.

In this protocol the UCA highlighted for SHAPE was Sonazoid, but a number of commercial UCAs can be used (cf., **Table 1**)[11,13-16]. The infusion setup and microbubble concentration required for any given UCA used with SHAPE should be adjusted based on the recommendations from the specific manufacturer.

Although this is not typically a user accessible parameter, using pulse-shaping to maximizing the generation of subharmonic microbubble signals is important for a successful SHAPE procedure. For the Logiq family of scanners, a Gaussian windowed binomial filtered square wave with Sonazoid appears optimal[21], but this is scanner and UCA dependent.[17] For the SonixTABLET scanner from BK Ultrasound both a square wave and a chirp pulse can be used (with different UCAs) [17]. Apart from the systems mentioned above, the only other commercial ultrasound scanners currently available with SHI and, thus, SHAPE are from MindRay.

This protocol focused on identifying portal hypertension in patients with chronic liver disease as the clinical application. A major reason is that existing noninvasive techniques, such as using CT, MRI or ultrasound imaging, are indirect and qualitative and results have been quite mixed[19]. Noninvasive ultrasound measures such as elastography for liver stiffness are quantitative techniques that can identify patients at high risk of CSPH; especially when combined with measurement of spleen size and platelet count. Accuracies of 90-94% for the initial diagnosis of CSPH have been reported, but these methods are not sufficiently precise to allow therapeutic reductions in HVPG to be tracked[19]. Improvement in clinical scoring systems, normalization of serum liver function tests, or reduction in ascites and varices qualitatively indicate improvement in portal hypertension[18]. However, unlike SHAPE, none of these measures provides a quantitative measure of the portal pressure. Consequently, the only clinically accepted method for quantifying portal pressures is through the HVPG measured via an invasive pressure catheter.

Likewise, the SHAPE algorithm can provide cardiac pressure estimates with errors of less than 3 mmHg in patients[16]. No quantitative, noninvasive alternatives to SHAPE exist in cardiology. This is nonetheless a challenging application, since absolute, real-time pressure estimates are required[16,17]. Investigations into 3D SHAPE for monitoring interstitial fluid pressure as a measure of breast cancers response to neoadjuvant therapy have shown that at 10% completion

of therapy (i.e., after one chemotherapy cycle) the SHAPE gradient between the tumor and the surrounding normal tissue can differentiate responders from partial/non responders (3.23 ± 1.41 dB vs. -0.88 ± 1.46 dB; p = 0.001)[15]. Other clinical areas, such as noninvasively estimating pressures in the bladder or the brain, are being pursued by researchers around the world demonstrating the wide applicability of the SHAPE technique.

In summary, this SHAPE protocol combines commercially available UCAs, an ultrasound scanner, and calibrated SHI to provide real time, noninvasive quantitative pressure estimates, thus, fulfilling a significant and hitherto unmet clinical need.

ACKNOWLEDGEMENTS:

This work is supported in part by the U.S. Army Medical Research Material Command under W81XWH-08-1-0503, and W81XWH-12-1-0066, by AHA grants no 0655441U and 15SDG25740015 as well as by NIH R21 HL081892, R21 HL130899, R21 HL089175, RC1 DK087365, R01 DK098526, R01 DK118964, R01 CA140338, R01 CA234428, by Lantheus Medical Imaging and by GE Healthcare, Oslo, Norway.

DISCLOSURES:

Drs. Forsberg, Gupta, Wallace and Eisenbrey have a patent pending on the SHAPE technology. Dr. Wallace is an employee of GE.

REFERENCES:

1. Lyshchik, A. (Editor). *Fundamentals of CEUS*. Elsevier. Manitoba, Canada. (2019).
2. Leighton, T. G. *The Acoustic Bubble*. Academic Press: London, England (1994).
3. Forsberg, F., Shi, W. T., Goldberg, B. B. Subharmonic imaging of contrast agents. *Ultrasonics.* **38** (1-8), 93-98 (2000).
4. Forsberg, F., Piccoli, C. W., Merton, D. A., Palazzo, J. P., Hall, A. L. Breast lesions: imaging with contrast-enhanced subharmonic US - initial experience. *Radiology.* **244** (3), 718-726 (2007).
5. Sridharan, A. et al. Characterizing breast lesions using quantitative parametric 3D subharmonic imaging: a multi-center study. *Academic Radiology.* **27** (8), 1065–1074 (2020).
6. Forsberg, F. et al. Subharmonic and endoscopic contrast imaging of pancreatic masses: a pilot study. *Journal of Ultrasound in Medicine.* **37** (1), 123-129 (2018).
7. Delaney, L. J. et al. Characterization of adnexal masses using contrast-enhanced subharmonic imaging: a pilot study. *Journal of Ultrasound in Medicine*. **39** (5), 977-985 (2020).
8. Eisenbrey, J. R. et al. Contrast-enhanced subharmonic and harmonic ultrasound of renal masses undergoing percutaneous cryoablation. *Academic Radiology.* **22** (7), 820-826 (2015).
9. Gupta, I. et al. Transrectal subharmonic ultrasound imaging for prostate cancer detection. *Urology.* **138** (4), 106−112 (2020).
10. Shi, W. T., Forsberg, F., Raichlen, J. S., Needleman, L., Goldberg, B. B. Pressure dependence of subharmonic signals from contrast microbubbles. *Ultrasound in Medicine and Biology*. **25** (2), 275-283 (1999).
11. Halldorsdottir, V. G. et al. Subharmonic contrast microbubble signals for noninvasive pressure estimation under static and dynamic flow conditions. *Ultrasonic Imaging.* **33** (3), 153-164 (2011).

12. Nio, A. Q. X. et al. Optimal control of SonoVue microbubbles to estimate hydrostatic pressure. *IEEE Transactions on Ultrasonics, Ferroelectrics and Frequency Control.* **67** (3), 557–567 (2020).
13. Eisenbrey, J. R. et al. Chronic liver disease: noninvasive subharmonic aided pressure estimation of hepatic venous pressure gradient. *Radiology*. **268** (2), 581-588 (2013).
14. Gupta, I. et al. Diagnosing portal hypertension with noninvasive subharmonic pressure estimates from an ultrasound contrast agent. *Radiology* (2020).
15. Nam, K. et al. Monitoring neoadjuvant chemotherapy for breast cancer by using three-dimensional subharmonic aided pressure estimation and imaging with US contrast agents: preliminary experience. *Radiology*. **285** (1), 53-62 (2017).
16. Dave, J. K. et al. Non-invasive intra-cardiac pressure measurements using subharmonic-aided pressure estimation: proof of concept in humans. *Ultrasound in Medicine and Biology*. **43** (11), 2718-2724 (2017).
17. Esposito, C., Dickie, K., Forsberg, F., Dave, J. K. Developing an interface and investigating optimal parameters for real-time intra-cardiac subharmonic aided pressure estimation. *IEEE Transactions on Ultrasonics, Ferroelectrics and Frequency Control.* doi: 10.1109/TUFFC.2020.3016264 (2020).
18. Bosch, J., Groszmann, R. J., Shah, V. H. Evolution in the understanding of the pathophysiological basis of portal hypertension: How changes in paradigm are leading to successful new treatments. *Journal of Hepatology*. **62** (1 Suppl) S121–S130 (2015).
19. Procopet, B., Berzigotti, A. Diagnosis of cirrhosis and portal hypertension: imaging, non-invasive markers of fibrosis and liver biopsy. *Gastroenterology Report (Oxford Academic)*. 5 (2),79-89 (2017).
20. Dietrich, C. F. et al. Guidelines and good clinical practice recommendations for contrast-enhanced ultrasound (CEUS) in the liver-update 2020 WFUMB in cooperation with EFSUMB, AFSUMB, AIUM, and FLAUS. *Ultrasound in Medicine and Biology.* Jul 22:S0301-5629(20)30200-3 (2020).
21. Gupta, I. et al. Effect of pulse shaping on subharmonic aided pressure estimation in vitro and in vivo. *Journal of Ultrasound in Medicine*. **36** (1), 3-11 (2017).
22. Dave, J. K. et al. On the implementation of an automated acoustic output optimization algorithm for subharmonic aided pressure estimation. *Ultrasonics*. **53** (4), 880-888 (2013).
23. Gupta, I., Eisenbrey, J. R., Machado, P., Stanczak, M., Wallace, K., Forsberg, F. On factors impacting subharmonic- aided pressure estimation (SHAPE). *Ultrasonic Imaging*. **41** (1), 35–48 (2019).
24. Eisenbrey, J. R., Daecher, A., Kramer, M. R., Forsberg, F. Effects of needle and catheter size on commercially available ultrasound contrast agents. *Journal of Ultrasound in Medicine*. **34** (11), 1961-1968 (2015).

Fig 1

Click here to access/download;Figure;Fig 1 final.psd

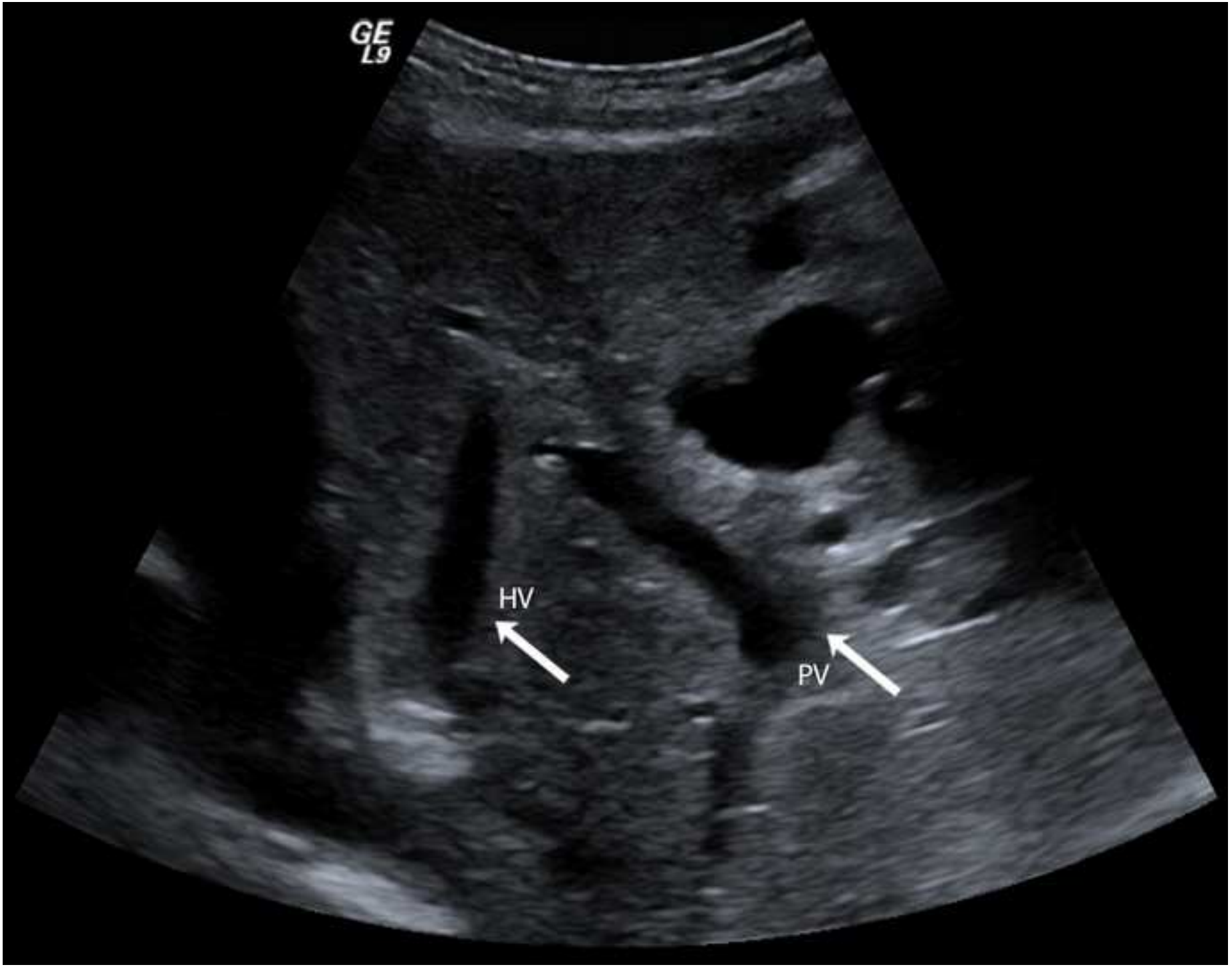
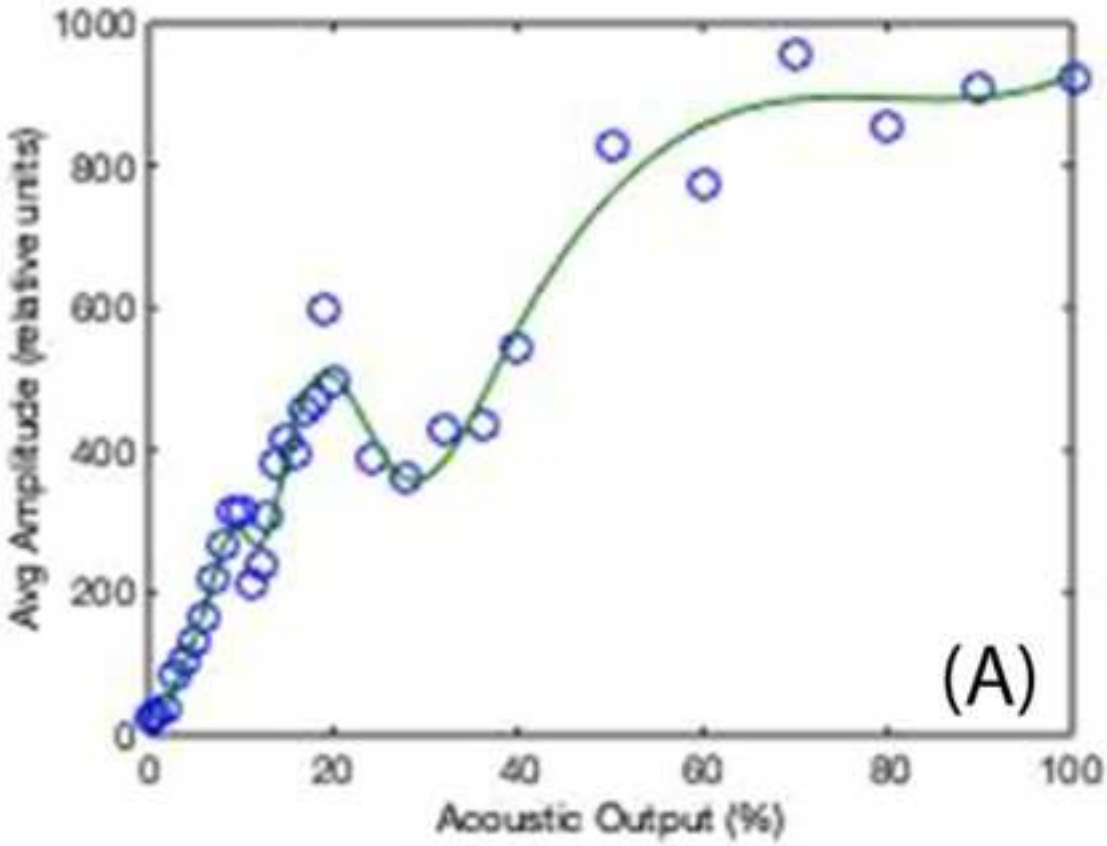


Fig 2

Click here to access/download;Figure;Fig 2 final.psd



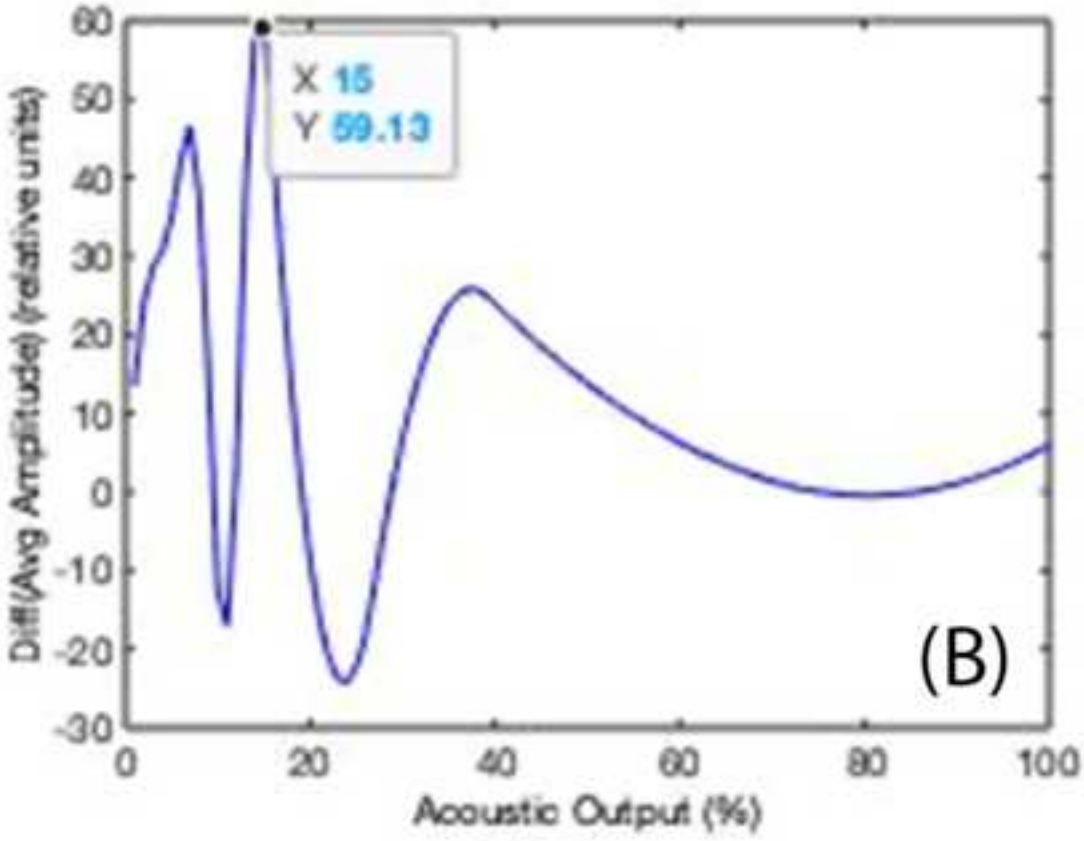
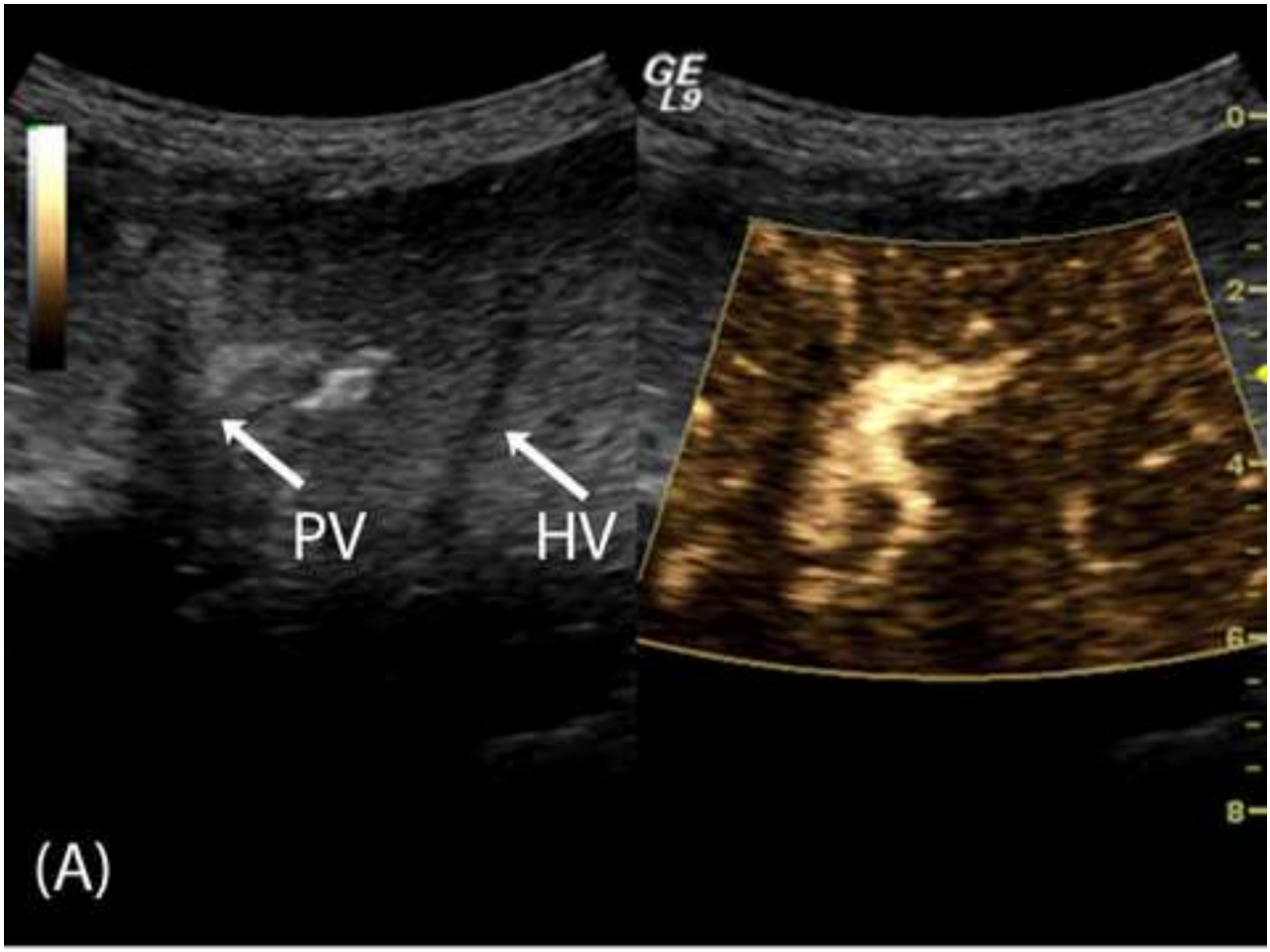


Fig 3

Click here to access/download;Figure;Fig 3 final.psd



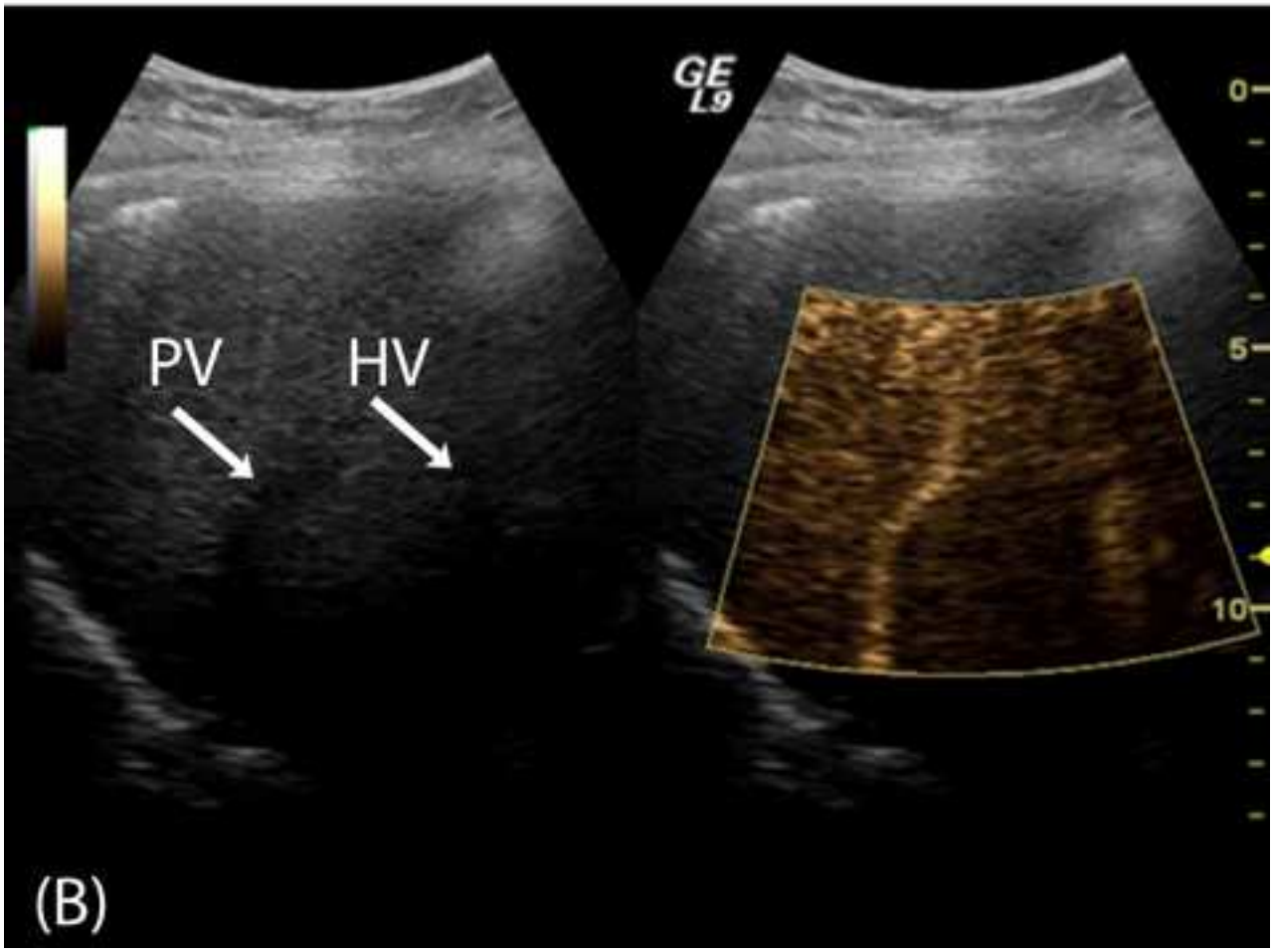
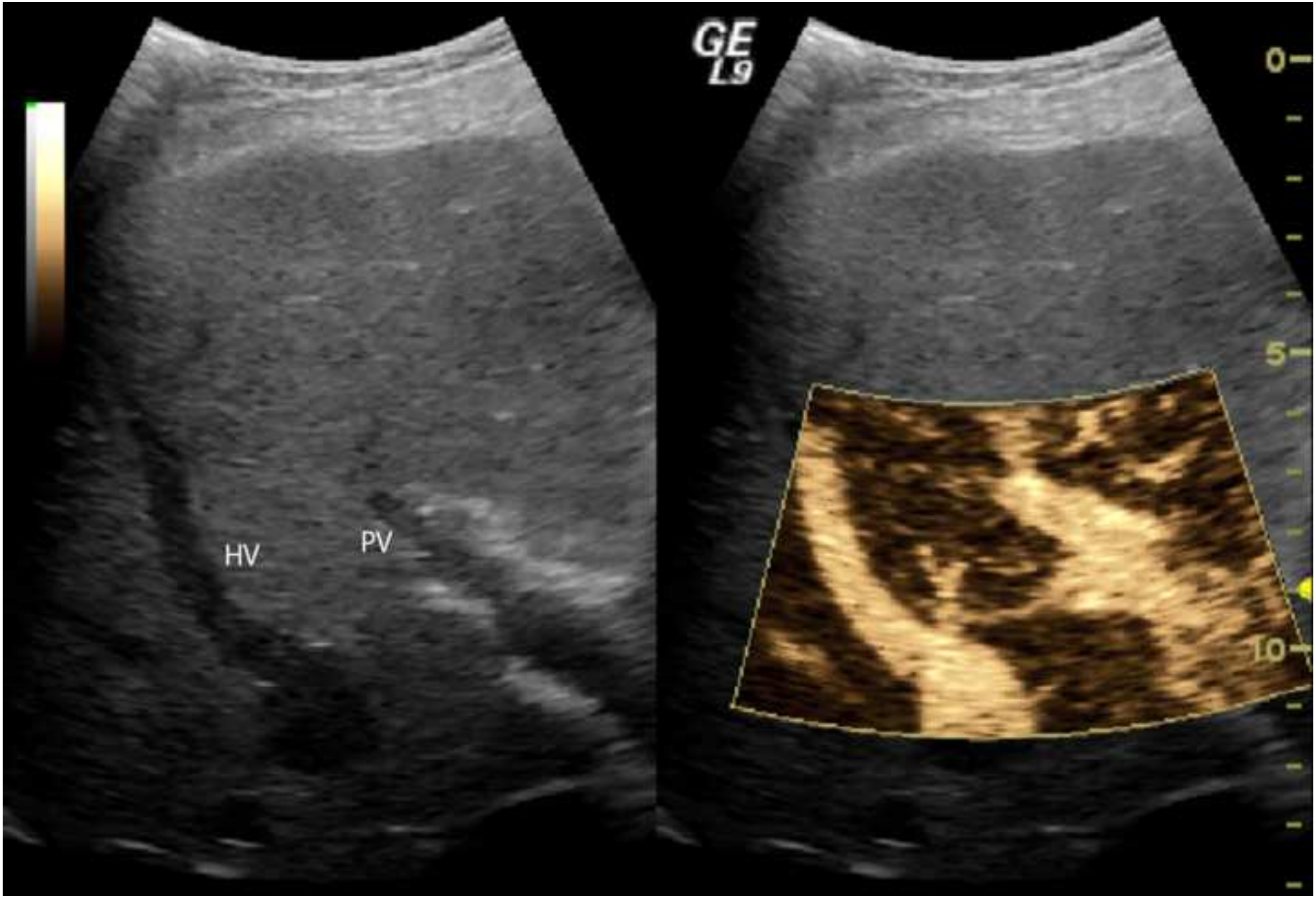


Fig 4

Click here to access/download;Figure;Fig 4 final.psd



Table

Click here to access/download;Table;SHAPE rev1 JoVE Table 1.xlsx

Table 1: Subharmonic response (and correlation) of commercial UCAs to a pressure increase of approximate

UCA	Manufacturer	Subharmonic signal reduction (dB)	Linear regression (r^2)
Definity	Lantheus Medical Imaging, N Billerica, MA, USA	11.0 ± 0.3	0.98
Levovist	Schering AG, Berlin, Germany	9.6 ± 0.2	0.98
Lumason aka SonoVue	Bracco, Milan, Italy	1.0 ± 1.3	0.20
Optison	GE Healthcare, Princeton, NJ, USA	10.1 ± 0.2	0.97
Sonazoid	GE Healthcare, Oslo, Norway	13.3 ± 0.2	0.99

ely 185 mmHg.

Table of Materials

Click here to access/download;Table of Materials;SHAPE rev1 JoVE_Materials Table.xlsx

Name of Material/ Equipment	Company	Catalog Number	Comments/Description
2 mL syringe	Becton Dickinson	309637	Used for reconstituting Sonazoid
10 mL saline-filled syringe	Becton Dickinson	306545	Used for flushing line to verify IV access
500 mL saline bag	Baxter Healthcare Corp	2131323	Used for co-infusion with Sonazoid
C1-6-D curvi-linear proble	GE Healthcare	H40472LT	Used for liver imaging
Chemoprotect Spike	Codan USA	C355	Chemospike used for reconstituting Sonazoid
Discofix C Blue	B. Braun Medical Inc	16494C	3-way stopcock
Intrafix Safeset 180 cm	B. Braun Medical Inc	4063000	Infusion tubing
Logiq E10 ultrasound scanner	GE Healthcare	H4928US	Used for conventional ultrasound imaging as well as for SHI and SHAPE
Luer lock 10 mL syringe	Becton Dickinson	300912	For infusion of Sonazoid
Medfusion 3500 syringe pump	Smiths Medical	3500-500	Used for infusing Sonazoid at 0.18 mL/kg/hour
Perfusor-leitung tubing 150 mm	B. Braun Medical Inc	8722960	Extension line enabling syringe connection to patient's IV access
SHI/SHAPE software	GE Healthcare	H4920CI	Contrast-specific imaging software
Sigma Spectrum infusion system	Baxter Healthcare Corp	35700BAX	Pump used for co-infusing saline at 120 mL/hour

Sonazoid	GE Healthcare		Gas-filled microbubble based ultrasound contrast agent
sterile water, 2 mL	B. Braun Medical Inc		Used for reconstituting Sonazoid
ultrasound gel	Cardinal Health	USG-250BT	Used for contact between probe and patient
Venflon IV cannula 22GA	Becton Dickinson	393202	Cannula needle for obtaining IV access

Response to the Reviewers:

We would like to thank the reviewers for such detailed and insightful comments. We have addressed each comment below in blue and have included the location of the changes made in the marked manuscript wherever relevant.

Editorial comments:

Changes to be made by the Author(s):

1. Please take this opportunity to thoroughly proofread the manuscript to ensure that there are no spelling or grammar issues.

We have done as requested.

2. Unfortunately, there are sections of the manuscript that show overlap with previously published work. Please revise the following lines: 57-60, 65-67,

Given the nature of the journal, this is to be expected. However, the text has been rewritten as: "Hence, significant energy components will be produced in the received echoes ranging from subharmonic and harmonic to ultraharmonic frequencies.[1,2] These nonlinear signal components can be extracted from tissue and linear bubble echoes (e.g., using pulse inversion) and used to create contrast -specific imaging modalities such as subharmonic imaging (SHI),…" lines 57-62 as well as "We have advocated the use of UCAs not as vascular tracers, but as sensors for noninvasive pressure estimation in the circulatory system by monitoring subharmonic contrast bubble amplitude variations." lines 67-70.

3. Please sort the materials table alphabetically.

Amended as requested. We also reorganized Table 1 to be in alphabetical order.

4. JoVE policy states that the video narrative is objective and not biased towards a particular product featured in the video. The goal of this policy is to focus on the science rather than to present a technique as an advertisement for a specific item. To this end, we ask that you please reduce the number of instances of "Sonazoid" within your text. The term may be introduced but please use it infrequently and when directly relevant. Otherwise, please refer to the term using generic language.

5. JoVE cannot publish manuscripts containing commercial language. This includes trademark symbols (™), registered symbols (®), and company names before an instrument or reagent. Please remove all commercial language from your manuscript and use generic terms instead. All commercial products should be sufficiently referenced in the Table of Materials.

We have reduced the mention of commercial products including Sonazoid whenever possible; lines 75, 141, 142, 146, 161, 174, 215, 273, 310. We have retained company names when specifically discussing which scanners can perform the SHAPE algorithm (as not all of the scanners are in the Materials Table); lines 328-331.

6. Please tone down the marketing language regarding the SHAPE protocol.

We have done as requested.

7. Are there any inclusion/exclusion criteria?

There are no specific inclusion criteria, but we do exclude subjects with unstable cardiopulmonary conditions or who are generally medically unstable as well as subjects with known allergies to any component of the UCA used for the study. This is now listed in bullets 2.1 and 2.2; lines 122-126.

8. Please discuss limitations of the protocol in more details in the Discussion section.
The most critical limitations, such as the power optimization algorithm and the benefit of pulse-shaping, are addressed, but we have expanded the Discussion section with the following text "Moreover, the SHAPE algorithm as currently implemented has a failure rate of approximately 10%, where the subharmonic signal-to-noise ratio is too low for reliable pressure estimates to be calculated.[14] No differences in age, body-mass-index, depth of imaging or liver status have been identified between subjects with successful and with failed SHAPE studies." lines 313-317.

9. Please spell out journal titles in the references.
Amended as requested.

Reviewers' comments:

Reviewer #1:

Manuscript Summary:
Authors provide the background and technical details on using subharmonic aided pressure estimation (SHAPE) in the noninvasive measurement of portal hypertension.

Major Concerns:
1. This reviewer could not find description on how to convert/render dB values to mmHg, which would be an important practical item. It is assumed authors have such information at hand based on their using HVPG as a reference standard.
The regression line between HVPG and SHAPE has been included in the text along with a warning that this is scanner and UCA dependent. Specifically, the text now reads: "Based on our current studies, the optimal operating point for identifying CSPH is -0.11 dB and the linear regression equation is HVPG = 0.81 x SHAPE + 9.43.[14] It is important to note that this cutoff and equation iare both scanner and UCA dependent." lines 232-234

2. Authors refer to their 2013 paper in Radiology (line 257-259), stating that "showed significantly higher SHAPE gradients between the portal and hepatic veins in subjects with CSPH (i.e., a HVPG ≥ 10 mmHg) than in those with lower HVPGs (1.37 ± 0.59 dB vs. -1.68 ±259 0.27 dB, p < 0.001)" - while this is a technical paper, it would be useful to see sensitivity/specificity parameters at mild and clinically significant portal hypertension. The key reason for this is that detection of PH in the range of 5-10 mmHg is indeed where noninvasive methods could shine, providing an easy alternative for early detection in less sick patients.
The text has been rewritten to state: "Similarly, the sensitivity and specificity for diagnosing all subjects with portal hypertension (i.e., HVPG ≥ 5 mmHg) were 71% and 80%, respectively." lines 278-279.

3. As discussed in lines 250-252 and illustrated in Figure 4, fistula formation between the portal vein and hepatic vein can result in erroneous SHAPE readings, and free HV pressure can reach remarkably high

values due to direct portal inflow. Authors describe this phenomenon as fistula between the "hepatic veins", which is misleading although in a sense portal vein is also a hepatic vein.
Agreed and the text now reads “a fistula between the portal and hepatic vein” line 261.

Minor Concerns:
None

Reviewer #2:
Manuscript Summary:
This is an invited Methods Article regarding Contrast-Enhanced Subharmonic Aided Pressure Estimation (SHAPE) Using Ultrasound Imaging with a Focus on Identifying Portal Hypertension.

Major Concerns:
It is a very interesting application, which may be promising.

Minor Concerns:
Line 140-150
These are just a methodology for the preparation of Sonazoid, which could be obtained by drug package insert.
I think these sentences could be deleted.
We do agree with the reviewer that Section 3 of the protocol is specific to Sonazoid. However, this is explicitly mentioned and the need to modify this procedure if using another UCA is also stated unequivalently. Hence, the protocol was not modified.

Line 170-173
Select an abdominal preset on the ultrasound scanner and use a curvi-linear array (typically with a 1-6 or 2-8 MHz bandwidth) to acquire grayscale images of both the portal and a hepatic vein in the same imaging plane and at similar depths (Figure 1). This is generally best achieved via a subcostal approach.
The text quoted above is bullet point 4.2, but we are unsure about the issue the reviewer is highlighting here as there are no comments or questions. Hence, the protocol was not modified.

Line 228-232
REPRESENTATIVE RESULTS:
As with all ultrasound imaging examinations, the first consideration for SHAPE is to obtain the best possible baseline grayscale images of the target region. In the case of liver imaging for diagnosing portal hypertension the key is to visualize both the portal vein and a hepatic vein at the same depth in order to minimize the impact of attenuation (Figure 1).

The contrast results may be influenced if there is a intrahepatic portalvenous shunt between PV and HV. The operators need to check whether this kind of vascular abnormality prior to the examination. Please mention it.
Agreed and this is now explicitly mentioned “… the first consideration for liver SHAPE is to obtain the best possible baseline grayscale images of the target region and to ensure (using Doppler imaging) that there are no intrahepatic portal venous shunts or other vascular abnormalities present.” lines 237-240.