High-contrast 3D in vivo microvascular imaging using scanning 2D ultrasound and acoustic sub-aperture processing (ASAP)

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***Abstract*—Non-invasive techniques for microvascular environment assessment are invaluable for clinical diagnosis and treatment monitoring. We recently developed a super contrast processing to suppress noise background in ultrafast Power Doppler, known an acoustic sub-aperture processing (ASAP), and demonstrate using 2D contrast enhance ultrasound. However, 2D imaging is insufficient to represent the 3D complex vascular environment. We therefore extend our study to demonstrate the feasibility of our technique for volumetric imaging. A pseudo-3D imaging technique was developed and demonstrated using a research system and pre-clinical transducer. A mouse liver was scanned using 2D ultrafast ultrasound and a mechanical translation stage. Initial results not only demonstrated a substantial noise reduction in 2D vascular images using ASAP, but also a high contrast volumetric rendering of a mouse liver. Our technique is ready for clinical use to provide better evaluation of angiogenesis.**

Keywords—Volumetric imaging, plane wave ultrasound, microenvironment, Doppler, preclinical in-vivo

I. INTRODUCTION

Angiogenesis is an important biomarker to study the progression and response of various diseases. The growing of new vessels in the microenvironment and their corresponding vessel morphology, provide a valid opportunity to dinstinct normal from diseases tissues. Although histology is a routine clinical practice which can accurately diagnose cancer and other diseases through microscopy examination, it is highly invasive as biopsy is performed repeatedly to collect tissue samples. Early detection the malignant masses is not possible as the procedure may not be used unless area of abnormality can be seen. This therefore motivated the extensive use of non-invasive imaging techniques for early disease detection and treatment monitoring.

Non-invasive imaging technique that are able to visualise tumour vascular environment include computed tomography (CT), magnetic resonance imaging (MRI), and ultrasound imaging. Both CT and MRI have the advantages of full 3D volumetric imaging and reasonable spatial resolution, although the long acquisition time and relatively high cost result in a lack of accessibility. CT is also less preferential than other imaging modalities, predominantly due to the use of ionising radiation. Ultrasound, on the other hand, is portable and affordable. It has the highest temporal resolution among these modalities, high and scalable spatial resolution, and the capability for real-time imaging making ultrasound an indispensable tool for front-line clinical use.

Recent advancements of high frame rate (HFR) ultrasound further enhance the capabilities of ultrasound for blood flow quantification[1]–[5], offering visualisation of microvasculature with and without the need to inject microbubbles. Ultrafast Doppler [1], [6]–[8], combining high frame rate imaging with Doppler processing, has been demonstrated for small vessel imaging and potential for real-time microflow monitoring. However, the practicabilities of Doppler processing to separate microvasculature at deeper regions are challenged by the low SNR in non-contrast imaging, diminution of the flow signal intensity due to attenuation, and the amplification of the noise background due to depth gain-compensation.

In order to reduce the noise background of ultrafast Doppler, we recently developed acoustic sub-aperture processing (ASAP) for super-contrast vasculature imaging, and demonstrated its feasibility for improving 2D HFR-CEUS imaging [9], [10]. However, 2D imaging is insufficient to represent the volumetric structure of the vascular environment. In this study, we aim to develop a high contrast volumetric microvascular images using a scanning 2D ultrasound and ASAP. In-vivo experiment was performed on a mouse model and initial 3D results of a mouse liver vasculature is presented.

II. METHODS

A. Animal model

In vivo study was conducted on a female athymic nude mice. All procedures complied with the Animal Act 1986 and were approved by the Animal Welfare and Ethical Review Body of the Institute of Cancer Research.

B. 3D imaging

The experiment setup is depicted in **Fig. 1**. The mouse was first anesthetised by subcutaneous injection of 20 µl ketamine before placing the animal on a warming platform to regulate its temperature. Ultrasound gel was applied to couple the mouse skin with a polyethylene bag containing water. The probe was placed in the water bag so that it could move freely without affecting the position of the mouse during volumetric data acquisition.

A L22-14v linear probe connected to a Verasonics

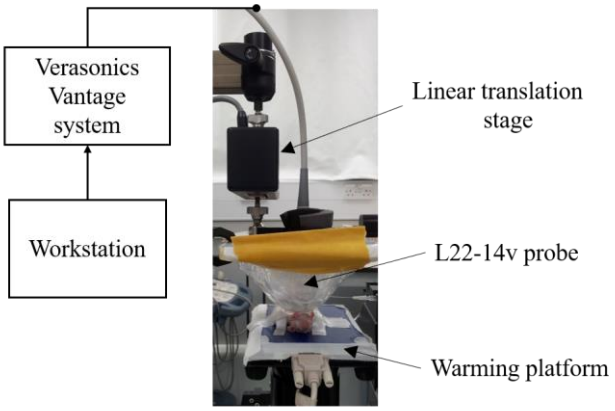

Fig. 1. Experimental setup of the 3D imaging system.

Vantage system (Verasonics Inc., Redmond, WA, USA) was used to acquire the high frame rate images with the imaging parameters shown in **Table I**. The radio-frequency (rf) data is subsequently transferred to a workstation for post-processing. During the acquisition, a stepper motor was synchronised with the ultrasound system to move the transducer along the elevation direction with a step size of 400 µm to cover the mouse liver with approximately 8 mm.

TABLE I. IMAGING PARAMETERS

Parameter	**Value**
Mode	Coherent compounded plane wave
Centre Frequency	18 MHz
Number of angle	15
Angle range	15^0
MI	0.12
Effective frame rate	500 Hz

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C. Data processing

All data was processed offline to produce ASAP and Power Doppler (PD) images with the method described in [9]. Comparing to the PD, ASAP preserved the coherency of the Doppler signal and exploited the spatial coherence of the Doppler signal between channels to significantly reduce noise.

The working principle of ASAP is similar to the PD. In-phase quadrature (IQ) data was reconstructed, clutter-filtered, before autocorrelation and ensemble average to produce vascular images. Instead of beamforming into a single image in PD, RF channel data were split into two non-overlapping sub-apertures in ASAP and reconstructed separately into a pair of images before clutter filter and cross-correlation was computed on the image pair to generate ASAP images. Since the noise is not correlated between channel, high contrast vascular image with significant noise reduction is therefore generated.

III. RESULTS AND DISCUSSION

Fig. 2 shows the B-mode, PD and ASAP images of a mouse liver. Due to the relatively strong clutter signal in comparison to the blood signal, the vasculature is not visible in the B-mode images. Such high amplitude clutter signal can be efficiently suppressed using singular value decomposition (SVD) filter and the blood signal can be emphasised using PD processing. The correlated background noise, however, is amplified with the imaging depth and further reduce the image contrast and discernibility of the small vessel at the deeper region. The ASAP technique, on the contrary, can substantially reduce the background noise in comparison to the PD to reveal weak micro-vessels signal and produce high contrast vascular images. The average signal-to-noise ratio quantified from the signal region (white boxes in Fig 2) and noise regions (green boxes) were 2.23 ±1.25 dB and 9.70 ± 2.09 dB for PD and ASAP, respectively.

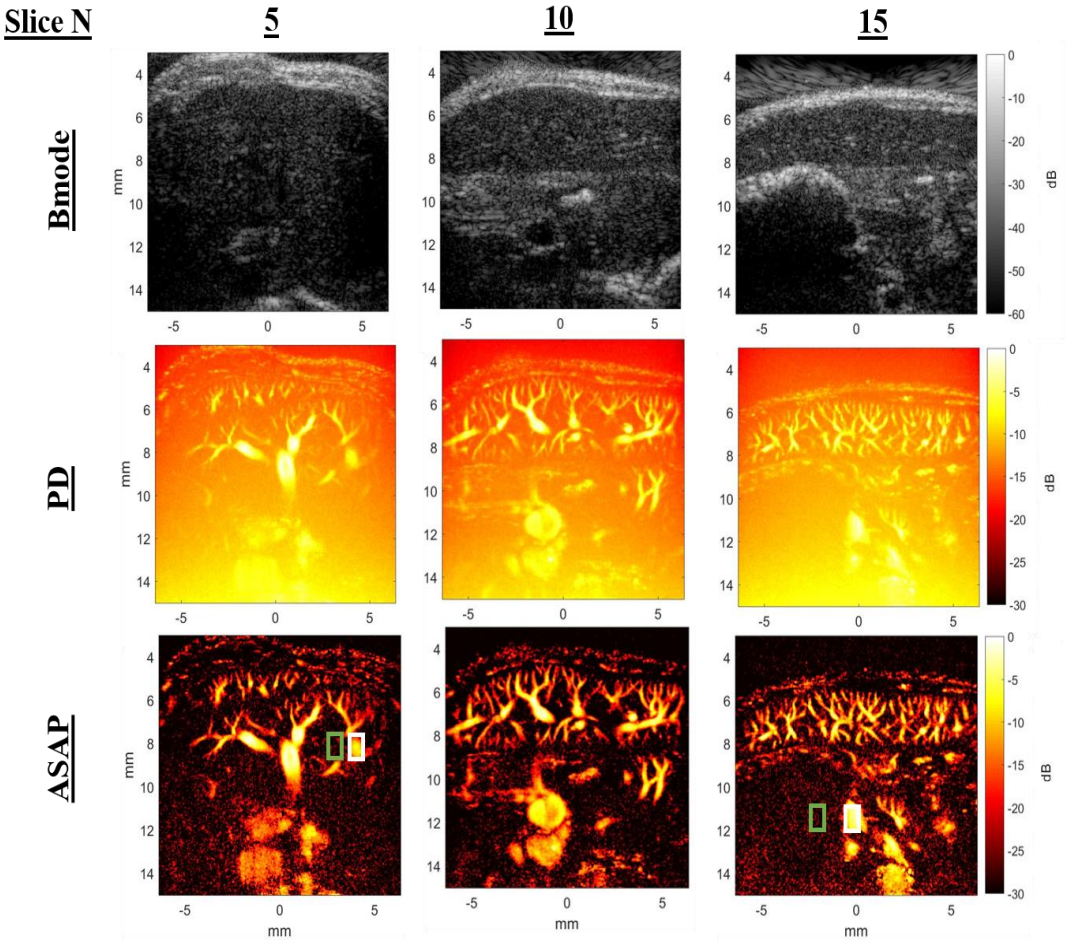

Fig 2.: 2D comparison of the B-mode, power Doppler (PD), and acoustic sub-aperture processing (ASAP) from 3 elevation positions. The white and green boxes were manually selected to quantify the signal–to-noise ratio

Fig. 3 shows the volumetric images of the vasculature image of the mouse kidney. All the 2D ASAP images were stacked accordingly and rendered in a volumetrically with constant slice thickness equivalent to the motor step-size. Qualitatively, the microvessels structure of the mouse liver are homogenous, organised and branching from larger vessel from the bottom of the imaging plane.

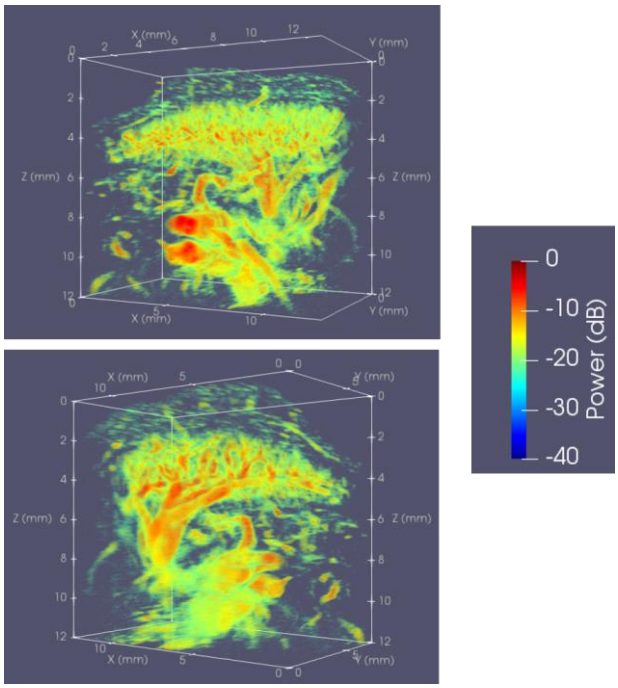

Fig. 3. 3D microvascular images of a mouse liver visualised from two different viewpoints.

IV. CONCLUSION

We presented a technique for high contrast volumetric microvascular imaging using a linear transducer and computer-controlled translational stage. High contrast mages generated using ASAP not only facilitate the visualisation of the vascular information in 2D without the need of contrast agents, but also produce high quality volumetric vasculature image. In the future, we expect to extract and quantify the vascular structural information generated using ASAP to study various diseases such as cancer.

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