Ultrasonics 107 (2020) 106170

Contents lists available at ScienceDirect

Ultrasonics

journal homepage: www.elsevier.com/locate/ultras

A feasibility study fornoninvasive measurement of shear wave speed in live zebrafish

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ARTICLE INFO

Keywords:
Ultrasound vibro-elastography
Zebrafish
Shear wave speed
In vivo zebrafish testing

ABSTRACT

Zebrafish are being increasingly used as animal models for human diseases such as cardiomyopathy and neuroblastoma. Owing to a nearly fully sequenced genome and efficient genetics/chemical genetics, zebrafish open new research opportunities for human diseases research. The purpose of this study was to develop zebrafish ultrasound vibro-elastography (ZUVE) for measuring the shear wave speed of zebrafish. An adult female zebrafish was anesthetized for three minutes for the ZUVE testing. A 0.1 s gentle harmonic vibration was generated on the tail using a sphere tip indenter with 3 mm diameter. Shear wave propagation in the zebrafish was measured using a high frequency 18 MHz ultrasound probe. Shear wave speeds were measured at 300, 400, and 500 Hz. Shear wave speeds were, respectively, 3.13 ± 1.20 (m/s) for 300 Hz, 4.28 ± 1.36 (m/s) for 400 Hz, and 5.07 ± 1.45 (m/s) for 500 Hz for zebrafish 1 in a region of interest (ROI) which covered the central body. The shear wave speed dispersions were similar for four zebrafish and shear wave speeds ranged between 2.5 (m/s) and 5 (m/s) from 300 Hz to 500 Hz. The experimental setup and testing for a zebrafish lasted less than three minutes. All tested zebrafish were alive after testing. ZUVE is safe, fast, and noninvasive, making the testing of elastic properties of zebrafish feasible.

1. Introduction

Zebrafish are currently developing animal models for human diseases such as cardiomyopathy or neuroblastoma. Owing to a nearly fully sequenced genome and genetic similarity with respect to human genomes [1], zebrafish are an ideal vertebrate model for human diseases research [2]. For example, zebrafish models have been developed for cardiomyopathies such as doxorubicin-induced cardiomyopathy or diabetes-induced cardiomyopathy [3,4], and neuroblastoma including a recently developed MYCN model [5,6]. Current methods of phenotyping these zebrafish models include echocardiography for cardiomyopathy and microscope imaging for neuroblastoma. There are no noninvasive techniques to measure elastic properties of zebrafish.

Vibro-acoustography is a technique based on the acoustic radiation force of ultrasound [7]. In vibro-acoustography, the acoustic radiation force of two focused ultrasound beams with slightly different frequencies generates a vibration at an object at a lower different frequency; the acoustic response of the object to this force is detected by a hydrophone. Vibro-acoustography has been tested on various tissues including breast, prostate, arteries, liver, and thyroid [8]. We also developed an ultrasound stimulated optical vibrometry technique to analyze arterial tube vibration [9] and elastic modulus [10]. This technique uses the acoustic radiation force of ultrasound to locally generate an arterial tube and uses a scanning laser system to detect the resulting vibration or wave propagation in the arterial tube. Sonoelastography was developed in 1990 by applying low frequency vibration to induce oscillations within soft tissues and detecting the tissue motion using a Doppler ultrasound based technique [11]. This technique is technically complex.

We developed ultrasound vibro-elastography (UVE) techniques to study various tissue diseases. UVE is a safe and noninvasive technique for generating and measuring surface wave propagation on the skin and shear wave propagation in the subcutaneous tissue. In UVE, a local 0.1-second harmonic vibration at a frequency is generated on the skin using a handheld shaker. A small tissue motion in tens of μm is enough for sensitive ultrasound detection of the generated tissue motion. The excitation of the shaker on the skin is significantly less than 1 N. Patients feel very gentle motion on their skin and experience no discomfort. An ultrasound probe is aligned about 5 mm away with the shaker indenter to measure the generated tissue motion at each pixel of the ultrasound imaging plane. Particle velocity in the axial direction of the ultrasound beam is analyzed using an autocorrelation method [12]. The resulting

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https://doi.org/10.1016/j.ultras.2020.106170
Received 24 November 2019; Received in revised form 6 April 2020; Accepted 1 May 2020
Available online 11 May 2020
0041-624X/

surface wave on the skin surface is used to study the skin, and the shear wave in the subcutaneous tissue is used to study internal tissues. Both the surface wave and shear wave measurements are obtained in a single test. The wave speed measurement depends on the local elastic property of tissue and is independent of the location of wave excitation. UVE has been used for assessing interstitial lung disease [13–15,16,17], ocular disorders such as glaucoma [18,19], and erectile dysfunction [20]. However, the zebrafish size is much smaller than human organs. The purpose of this research was to study the feasibility of zebrafish ultrasound vibro-elastography (ZUVE) for measuring shear wave speed in this vertebrate model.

2. Materials and methods

Adult female WIK zebrafish were obtained under approved Institutional Animal Care and Use Committee (IACUC) protocol A0000351100 and maintained at 28.5 °C. The zebrafish were anesthetized using 0.016% Tricaine-S (MS-222, Syndel, Ferndale, Washington 98248, USA) in fish water. A zebrafish was considered anesthetized when its respiration rate decreased as visually noticead by its gill movement. Three minutes of anesthesia was designed for the zebrafish study, because the zebrafish experimental setup and tests could be finished within three minutes. Fig. 1a shows a schematic drawing of the ZUVE technique. The coordinator × represents the lateral direction of an ultrasound image and the coordinator y the depth direction of the ultrasound image. A zebrafish is positioned in a fish holder. A 0.1 s gentle harmonic vibration is generated on the tail using a sphere tip indenter with a 3 mm diameter. Shear wave propagation in the zebrafish is measured using a high frequency 18 MHz ultrasound probe. Fig. 1b shows a zebrafish in the fish holder. A zebrafish was mounted ventral side facing upwards in the fish holder. The testing fixture was a circular plastic tube plate with a precut foam insert to hold the zebrafish in place. Fig. 1c shows a photo of the experimental setup. A thick rubber material plate is between the fish holder fixture and the testing table to reduce wave reflection.

The 0.1-second harmonic vibration signal is generated by a function generator (Model 33120A, Agilent Inc., Santa Clara, CA, USA). The signal is amplified using a power amplifier (Model PYLE PRO PCA4, PYLE PRO Service Center, Brooklyn, NY 11204, USA). The signal is then sent to a small vibrator shaker (Model: FG-142, Labworks Inc., Costa Mesa, CA 92626, USA). The shaker generates a local small vibration on the tail of fish using a spherical indenter with a plastic ball 3 mm in diameter. A layer of ultrasound transmission gel (Aquasonic®100, Parker Laboratories, Inc., Fairfield, NJ 07,004 USA) was applied on the fish. A Verasonics ultrasound system (Vantage, Verasonics, Inc., Kirkland, WA, USA) with a high frequency ultrasound probe L22-14vXLF with a central frequency of 18 MHz was used for collecting the ultrasound data. The L22-14vXLF probe has 128 elements with a pitch of 0.10 mm. A high frame rate of 2000 frames per second was achieved by using a plane-wave pulse transmission method [17]. The measurement of shear wave speed was independent of the location of excitation.

Radio-frequency data of the ultrasound echo from the tissues were obtained. By demodulation of the radio-frequency data using quadrature detection, the in-phase/quadrature data of ultrasound signals were processed. The IQ data consisted of 2D intensity information for the duration of the vibration excitation. Particle velocity in the axial direction of the ultrasound beam (V) caused by wave propagation was used for wave speed estimation. V was calculated from in-phase/quadrature data of consecutive frames using an autocorrelation method [20,21]. Wave speed measurements were performed by cross-correlating two particle velocities from two imaging pixels (denoted by $v\left(m-\frac{w}{2}, n, t\right)$ and $v\left(m+\frac{w}{2}, n, t\right)$), where m is the lateral dimension, n is the axial dimension, t is the slow time dimension, and w is the window size) that are a fixed distance apart, which is equal to window size w, to estimate the wave speed of the pixel at location (m, n) [22]. The normalized cross-correlation coefficient of each pixel in the lateral direction (CC_x) is calculated by [23],

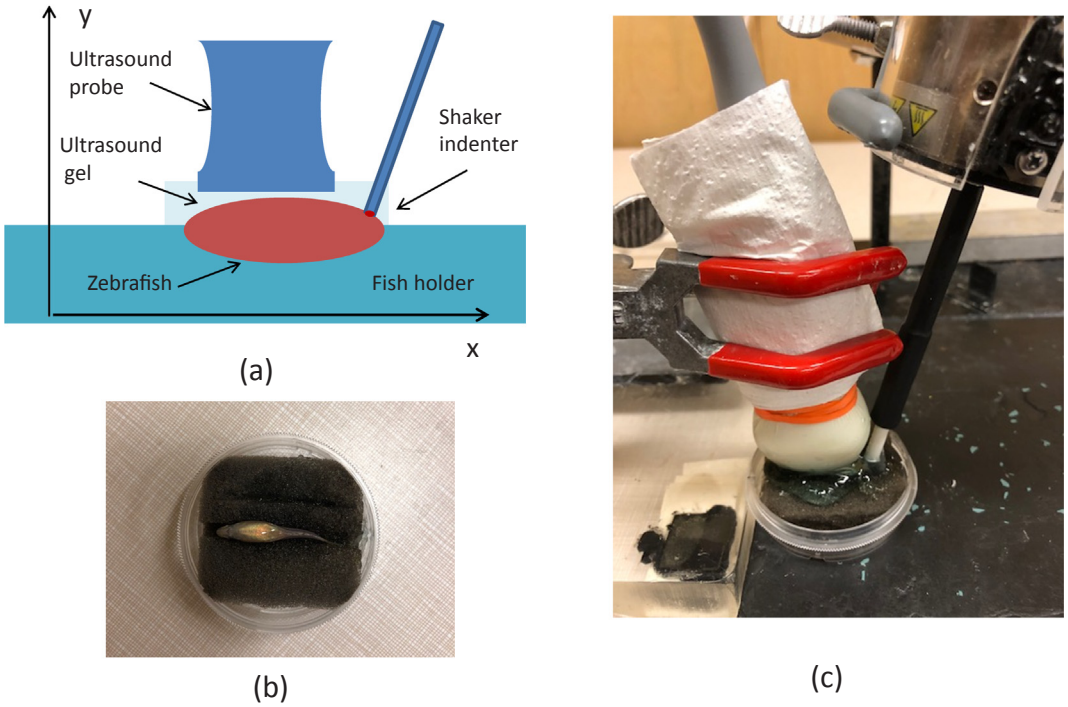

Fig. 1. (a) Schema of the ZUVE technique. the zebrafish is positioned in a fish holder. A 0.1 s gentle harmonic vibration is generated on the tail of the zebrafish using a sphere tip indenter with 3 mm diameter. Shear wave propagation in the zebrafish is measured using a high frequency 18 MHz ultrasound probe. (b) Fixture to hold the zebrafish stably using a fish shaped foam material. (c) Shows the experimental setup. A thick rubber material plate is between the fish holder and the testing table to reduce wave reflection.

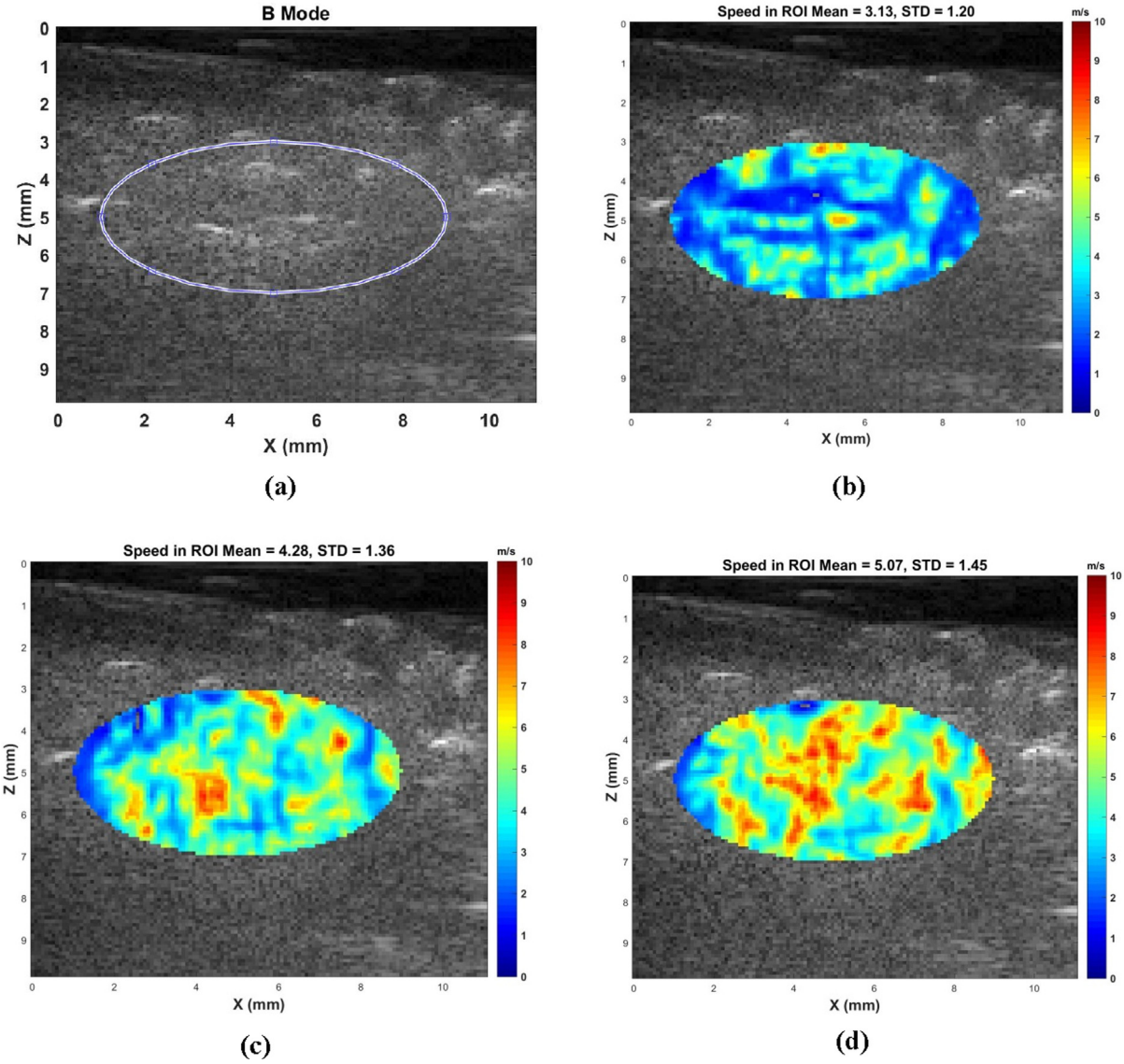

Fig. 2. (a) Representative B-mode image of a zebrafish. A region of interest (ROI) covering the central body of the zebrafish is selected to analyze wave speed. (b) The shear wave speed is 3.13 ± 1.20 (m/s) in the ROI. (c) Shear wave speed is 4.28 ± 1.36 (m/s) in the ROI. (d) Shear wave speed is 5.07 ± 1.45 (m/s) in the ROI.

$$CC_x(j) = \frac{\sum_{i=-M/2}^{M/2}\left[v\left(m-\frac{w}{2},n,i\right)-\bar{v}(m-\frac{w}{2},n)\right]\left[v\left(m+\frac{w}{2},n,i+j\right)-\bar{v}(m+\frac{w}{2},n)\right]}{\sqrt{\sum_{i=-M/2}^{M/2}\left[v\left(m-\frac{w}{2},n,i\right)-\bar{v}(m-\frac{w}{2},n)\right]^2}\sqrt{\sum_{i=-M/2}^{M/2}\left[v\left(m+\frac{w}{2},n,i+j\right)-\bar{v}(m+\frac{w}{2},n)\right]^2}} \quad (1)$$

where M is number of wave signal data points along slow time direction. A directional filter in the lateral direction is then applied upon the correlation coefficient field to extract the correlation coefficient peak. The directional filter removes the reflected waves. Temporal delay (Δt) between the two pixel velocities in the lateral direction is then given by, $\Delta t = \left[\frac{arg_j maxCC(j)}{PRF}\right]$, where PRF is the pulse repetition frequency. The PRF is 2000 in this study. Wave speed of the center pixel at location (m, n) is given by,

$$c_x(m,n) = \frac{w\hat{A}\cdot\Delta x}{\Delta t} \quad (2)$$

where $\Delta x = \frac{c}{f}$ is the spatial resolution of the imaging pixels, and f is the ultrasound frequency.

To increase the robustness of the 2D wave speed calculation while preserving the spatial resolution of the wave speed map, a 2D processing window technique was used [24,25]. In the 2D window processing technique, all pixels within the 2D window are used to estimate wave speeds along the axial and lateral directions, respectively. The 2D wave speed c at the center pixel (m,n) can be calculated by,

$$c = \frac{c_X c_Z}{\sqrt{c_X^2 + c_Z^2}} \quad (3)$$

In this study, a region of interest (ROI) was selcted as shown in Fig. 2(a) for analyzing wave speed in the ROI. The zebrafish we tested had a typical length of 15–20 mm and thickness of 6–8 mm. The ROI is 9 mm long and 4 mm thick, which covered the central body of the zebrafish.

3. Results

Fig. 2 (b), (c), and (d) show, respectively, for zebrafish 1the shear wave speed of 3.13 ± 1.20 (m/s) (mean ± standard devivation) in the ROI for 300 Hz, 4.28 ± 1.36 (m/s) in the ROI for 400 Hz, and 5.07 ± 1.45 (m/s) in the ROI for 500 Hz.

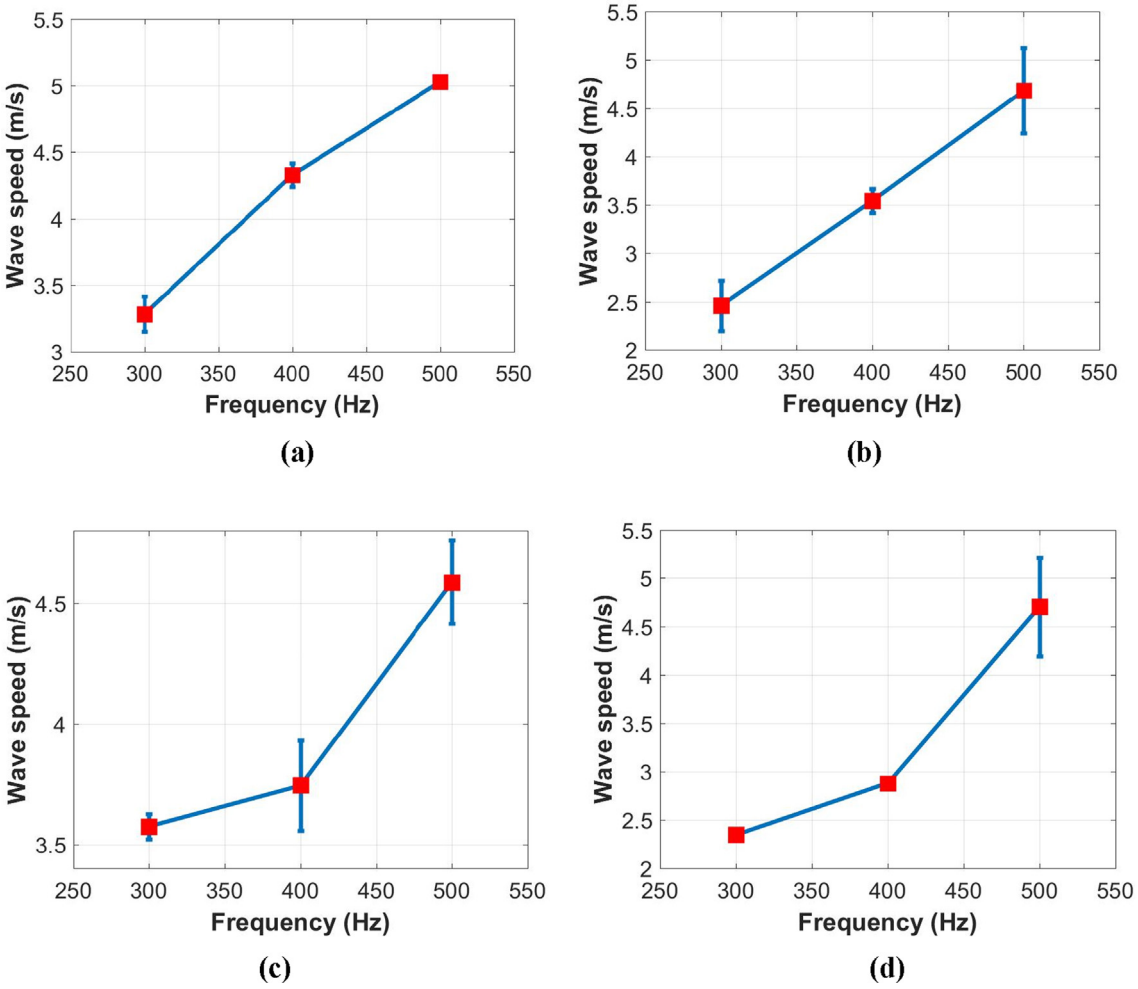

Fig. 3. (a) Wave speed dispersion from 300 to 500 Hz for zebrafish 1. Three measurements were performed at each frequency. (b) Wave speed dispersion from 300 to 500 Hz for zebrafish 2. (c) Wave speed dispersion from 300 to 500 Hz for zebrafish 3. (d) Wave speed dispersion from 300 to 500 Hz for zebrafish 4.

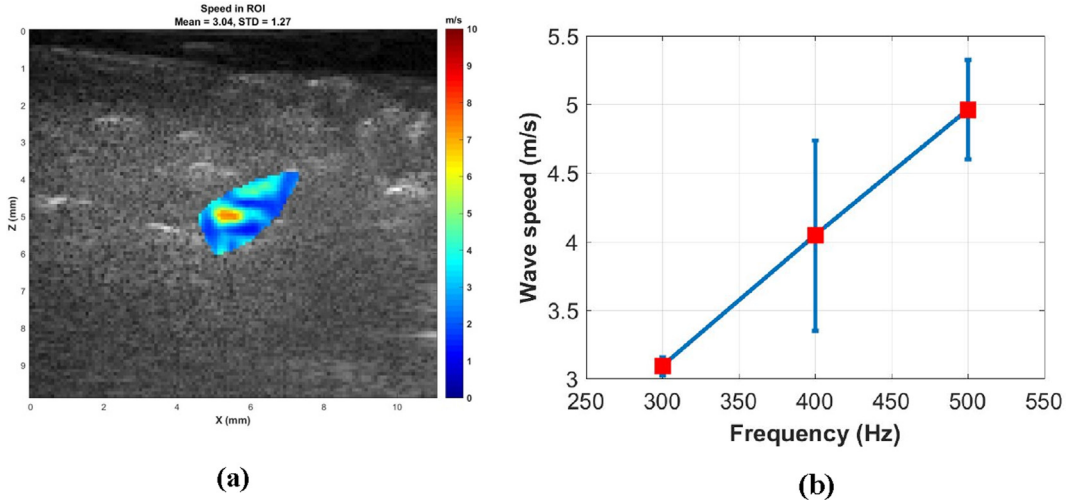

Fig. 4. (a) Shear wave speed map at 300 Hz in the ROI covering the heart ventricle of zebrafish 1. Shear wave speed is 3.04 ± 1.27 (m/s) in the ROI. (b) Wave speed dispersion from 300 to 500 Hz for the heart of zebrafish 1.

Fig. 3(a) show wave speed dispersion from 300 Hz to 500 Hz for zebrafish 1. Three measurements were performed at each frequency. Fig. 3(b) show wave speed dispersion from 300 to 500 Hz for zebrafish 2. Fig. 3(c) shows wave speed dispersion from 300 to 500 Hz for zebrafish 3. Fig. 3(d) shows wave speed dispersion from 300 to 500 Hz for zebrafish 4. The general pattern is that wave speed increases with frequency, which is consistent with many other tissues. There are some variations in terms of wave speed among different zebrafish, but the ranges of wave speed are similar. This technique is feasible for measuring the shear wave speed of the heart of a zebrafish. A ROI is selected

to cover the heart ventricle of zebrafish 1. Fig. 4(a) shows the shear wave speed map at 300 Hz. The shear wave speed is 3.04 ± 1.27 (m/s) in the ROI. Fig. 4(b) shows wave speed dispersion from 300 to 500 Hz for the heart of zebrafish 1. Three measurements were performed at each frequency.

4. Discussion

The aim of this study was to prove the feasibility of using the ultrasound vibro-elastography technique for noninvasively and safely measuring the shear wave speed of live zebrafish. Because a zebrafish is very small, a high frequency 18 MHz ultrasound probe was used. To reduce the wavelength of generated wave propagations, wave propagation in zebrafish was studied at 300, 400, and 500 Hz. In human studies, we typically measured wave speeds at 100, 150, and 200 Hz.[1]

The central part of a zebrafish is imaged in this research, therefore, a whole zebrafish is not visible in the images. Although the heart of the zebrafish is visible, it was difficult to delineate clearly the border of the heart with the 18 MHz probe. Therefore, our intention in this pilot study is not to measure stiffness of the zebrafish heart, but to prove the feasiblity of measuring stiffness of the zebrafish. To measure the stiffness of individual organs in a zebrafish, a higher frequency ultrasound probe and system are needed. We had experience studying heart function of zebrafish using a Vevo 3100 ultrasound system (FUJIFILM VisualSonics, Inc., Toronto, ON, Canada) with a MX700 ultrasound probe (29–71 MHz)[28]. In future studies, we plan to evaluate a higher frequency 30 MHz ultrasound vibro-elastography system for measuring shear wave propagation in the heart of zebrafish.

5. Conclusions

The feasibility of zebrafish ultrasound vibro-elastography (ZUVE) was demonstrated for measuring shear wave speed. An adult female zebrafish was anesthetized for three minutes for the ZUVE testing. A 0.1 s gentle harmonic vibration was generated on the tail of the zebrafish using a sphere tip indenter with 3 mm diameter. Shear wave propagation in the zebrafish was measured using a high frequency 18 MHz ultrasound probe. Shear wave speeds were measured at 300, 400, and 500 Hz. Shear wave speeds were, respectively, 3.13 ± 1.20 (m/s) in a ROI for 300 Hz, 4.28 ± 1.36 (m/s) for 400 Hz, and 5.07 ± 1.45 (m/s) for 500 Hz for zebrafish 1. The shear wave speed dispersions were similar for four zebrafish and shear wave speeds ranged between 2.5 (m/s) and 5 (m/s) from 300 to 500 Hz. The experimental setup and testing for a zebrafish lasted less than three minutes. All tested zebrafish survived after testing. It is feasible to develop this technique as a routine laboratory test for measuring the elastic properties of zebrafish for phenotyping various diseases.

Acknowledgement

We thank Mrs. Jennifer Poston for editing this manuscript.

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