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A Study of the Adult Zebrafish Ventricular Function by Retrospective Doppler-Gated Ultrahigh-Frame-Rate Echocardiography

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Abstract

The zebrafish (*Danio rerio*) has become a preferred animal model for studying various human diseases, particularly those related to cardiovascular regeneration; therefore, a noninvasive imaging modality is needed for observing the cardiac function of zebrafish. Because of its high resolution, high-frequency ultrasound B-mode imaging has recently been used successfully to observe the heart of adult zebrafish. However, ultrahigh-frame-rate echocardiography combining B-mode imaging and color flow imaging is still needed to observe the detailed transient motions of the zebrafish ventricle. This study develops an 80-MHz ultrahigh-frame-rate echocardiography system for this purpose, based on retrospective Doppler-gated technology. B-mode and color flow images of the cardiovascular system of the zebrafish were reconstructed by two-dimensional autocorrelation at maximum frame rates of up to 40 000 and 400 fps, respectively. The timings of end diastole (E_D) and end systole (E_S) of ventricle can be determined by using this high-resolution image system. Two ventricular function parameters—fractional shortening (FS) and fractional area change (FAC)—were measured for evaluating the ventricular function by using E_D and E_S with their corresponding ventricular dimensions. The experimental results indicated that the measured FS values were $42 \pm 4\%$ (mean $\pm$ standard deviation) and $60 \pm 13\%$ for the long axis and short axis of the ventricle, respectively, and that FAC was $77 \pm 9\%$. This is the first report of these ventricular function parameters for a normal adult zebrafish. The results showed that retrospective high-frequency echocardiography is a useful tool for studying the cardiac function of normal adult zebrafish.

I. Introduction

The adult zebrafish has recently become an important small-animal model for studying human pathologies in many preclinical applications. An adult zebrafish can fully regenerate its heart even after 20% of its ventricle has been removed [1], [2], which has prompted many scientists to study zebrafish cardiovascular regeneration for applications in tissue regeneration [3], [4] and gene therapy [5]. Two ventricular function parameters—fractional shortening (FS) and fractional area change (FAC)—have often been measured to assess the cardiac function of the zebrafish embryo using optical methods [6]. However, it is difficult to apply this modality to adult zebrafish because of the opaqueness of the fish body. The zebrafish cardiac morphology has mainly been observed using histological specimens, but some noninvasive imaging modalities have been proposed to obtain images of the heart structure of adult zebrafish, such as micro-computed tomography [7] and magnetic resonance imaging [8]. However, the temporal resolutions of these modalities are insufficient for monitoring the cardiac motions. Fluorescence microscopy has also been used to image the zebrafish heart, however, only for the transparent adult zebrafish (genetically modified zebrafish) [9]. The drawbacks of this approach include that it is not easy to reproduce genetically modified transparent zebrafish and the low optical penetration, making it difficult to analyze cardiac function.

High-frequency ultrasound can provide high sensitivity in characterizing biological tissues [10]–[13], and it has been proposed as a useful tool for small-animal imaging because it is noninvasive and has real-time capability [14]–[16]. For instance, the zebrafish liver tissues were characterized by 50-MHz ultrasound for tumor study [17] and the blood flow velocities of heart were also measured by 7.5-MHz ultrasound for estimating the cardiac functions of zebrafish [18]. However, the imaging resolutions of these systems were inadequate for monitoring zebrafish cardiac function. A high-frequency ultrasound array imaging system with a frequency bandwidth from 30 to 70 MHz is available for small-animal imaging applications (Vevo 2100, Visualsonics, Toronto, ON, Canada) [19], however, the image resolution can be improved by using higher frequency ultrasound [20]. By using sector scanning of a 75-MHz single-element transducer, a B-mode image resolution of 25 μm and a maximum frame rate of 200 fps can be achieved for real-time zebrafish heart imaging [20], [21]. Chirp-coded tissue harmonic imaging with a frame rate of 68 fps has also been used to characterize the zebrafish heart [22]. Even though a high resolution and high frame rate were achieved, no cardiac function and color flow images of the zebrafish heart were presented because it is difficult to measure the chamber variations and blood flow synchronously in those approaches. In addition, a sector scan may introduce errors in the 2-D autocorrelation used to estimate the active contraction phase and the passive relaxation phase of heart, particularly for deeper regions. In other words, high-frame-rate color flow images are still needed for evaluating the cardiac function of zebrafish.

Although the operating frequency and frame rate in commercially available ultrasound array systems are inadequate for imaging the zebrafish heart, the use of a higher frequency single-element transducer with mechanical scanning is still a possible option. A common method used to obtain high-frame-rate ultrasound images based on electrocardiogram (ECG)-gated data acquisition has been proposed for cardiac and vascular applications. Based on the

acquisitions of echo signals from the heart at a high pulse repetition frequency (PRF) synchronized by ECG timing, a reconstructed echocardiograph with a high temporal resolution of 8000 fps can be obtained for observing the cardiac cycle in detail. Similar ECG-gated technology has been also used to reconstruct high-frame-rate B-mode and color flow images for measuring the heart chamber motions and cardiovascular blood flow in mice [23], [24]. Even though this ECG-gated technology can theoretically be applied for zebrafish heart imaging, one potentially serious problem is the invasiveness of using needle electrodes for the ECG measurements, increasing the risks of infection and death for the zebrafish. Therefore, the ability to noninvasively detect physiological signals from the zebrafish heart is needed as a substitute for the ECG. The relationship between the diastole blood flow velocity and R-wave of the ECG [25] prompted us to consider using the Doppler flow velocity as a trigger for reconstructing high-frame-rate cardiac images. For instance, the continuous-wave Doppler flow has been used as a trigger to obtain high-frame-rate images of the mouse embryo using an annular array transducer with mechanical scanning [26]. Doppler-gated technology was also used for 3-D imaging of the fetal heart [27]. These studies prompted us to use retrospective Doppler-gated high-frame-rate and high-frequency echocardiography to study the ventricular function of adult zebrafish *in vivo*.

The present study developed a method of ultrahigh-frame-rate (UHFR) echocardiography with a high spatial resolution based on incorporating high-frequency pulsed-wave (PW) Doppler-gated technology for assessing the cardiac function of zebrafish. A 35-MHz PW Doppler flowmeter was built for measuring the Doppler spectrogram from a zebrafish artery, and the timing of the peak flow velocity was detected by analog circuits as a trigger signal to synchronize a high-frequency ultrasound mechanical scanning system incorporating a transducer with a frequency bandwidth up to 100 MHz. UHFR retrospective B-mode images (RBIs) and retrospective color flow images (RCFIs) were obtained by the echocardiography system. Experiments were performed with adult AB-line zebrafish. The ventricular function of zebrafish was determined quantitatively by measuring the ventricular function parameters FS and FAC. The emphasis was on obtaining information about the blood flow and the corresponding variations in ventricular volume.

II. Materials and Methods

A. Retrospective Doppler-Gated Echocardiography System

Fig. 1 shows a block diagram of the UHFR retrospective Doppler-gated echocardiography system. As mentioned in the previous section, the pulsatile flow velocity from the zebrafish artery was measured as a substitute for the ECG trigger for the reconstruction process in retrospective Doppler-gated echocardiography. The high-frequency PW Doppler flowmeter designed in this study included a PRF timing controller, bipolar-pulse generator, preamplifiers, demodulation circuits, sample/hold circuits, wall filters, PRF filters, and an audio amplifier [28], [29]. A 35-MHz high-frequency needle transducer (NIH Ultrasonic Transducer Resource Center, University of Southern California, Los Angeles, CA) with a focal depth of 1.3 mm was used for measurements. The characteristics of the Doppler flowmeter are summarized in Table I. The Doppler shift frequencies were measured by a frequency detector to create the heartbeat timing triggers to synchronize the motor for

mechanical scanning. The main component of the frequency detector was a tone decoder (LM567, National Semiconductor Corp., Santa Clara, CA). The detector outputs an impulse signal when it receives a Doppler signal whose frequency is higher than a preset threshold frequency. The threshold frequency of detector was adjusted by the external resistances and capacitances of the voltage-controlled oscillator. The threshold of Doppler frequency was set to be 3 kHz in the present study. When a function generator (AFG 3102, Tektronix, Beaverton, OR) received the Doppler triggers from the frequency detector, accurate synchronous triggers were generated for the other devices.

The echocardiography system was equipped with an 80-MHz single-element ultrasound imaging transducer (NIH Ultrasonic Transducer Resource Center). The axial and lateral resolutions of this imaging system were approximately 21 and 56 μm , respectively. The transducer was driven by a customized high-frequency bipolar-pulse generator [28], [29], and connected via an expander (Matec Instrument Co. Inc., Northborough, MA) to eliminate electrical noise from the pulse generator. The center frequency of the bipolar excitation pulses was 80 MHz, and their peak-to-peak output voltage was 85 V. The echocardiography frame rates were adjusted from 1000 to 40 000 fps, depending on the PRF of the pulse generator. An electronic limiter (Matec Instrument Co. Inc.) was connected in front of a low-noise amplifier (AU-1313, MITEQ Inc., Hauppauge, NY) to avoid device damage. Echo signals were filtered by a band-pass filter and then digitized by an 8-bit analog-to-digital convertor (ADC; PXI 5152, National Instruments Corp., Austin, TX) at a sampling rate of 250 MHz. The imaging transducer was mounted on a one-axis piezoceramic motor (HR8, Nanomotion, Yokneam, Israel) for mechanical scanning. The imaging transducer could also be moved in the other two orthogonal directions using two axial microstep motors (CM1-C-17L30A, Cool Muscle, Tokyo, Japan). The 3-D motor stage was controlled by a motor controller (DMC-1842, Galil Motion Control, Rocklin, CA). A software program was developed for motor control and echo signal acquisition using Lab-VIEW software (National Instruments Corp.).

B. Reconstruction of Retrospective Duplex Images

The reconstruction procedure of retrospective Doppler-gated UHFR echocardiography is shown in Fig. 2. The procedure of timing control for the Doppler-gated technology was as follows:

- Step 1: The Doppler shift frequency from a zebrafish artery was measured using the PW Doppler flowmeter.
- Step 2: The audio signal of the Doppler shift frequency was fed to the frequency detector. When the frequency of the Doppler audio signal was higher than a certain threshold, an impulse clock was generated as a trigger (TRIG 1 in Fig. 2) to the function generation.
- Step 3: When the function generation received a trigger, two kinds of clock signals were produced via internal functions to synchronously control both the pulse generator (TRIG 2) and Doppler flowmeter (switch TRIG). One synchronous broad rectangular pulse was generated to switch the Doppler flowmeter on and off. This design avoided interference between the imaging transducer and Doppler needle

transducer, by ensuring that the Doppler flowmeter was turned off when the imaging transducer was working. Another synchronous clock with a higher PRF was produced to trigger the pulse generator as well as the ADC to record the ultrasound echo signals.

- Step 4: The imaging transducer was set at an initial location ($K = 1$). When the pulse generator and ADC were triggered by the function generator, the transmitted and received ultrasound signals were respectively produced and digitized at a high PRF of 40 kHz. The total acquisition time was controlled by software according to how much of the cardiac cycle was being monitored. The transducer was then moved $4 \mu\text{m}$ to the next location ($K = 2, 3, \dots, k$) by using a three-axial-motor stage. Subsequently, the blood flow velocity was measured by Doppler flowmeter to provide the synchronous signal (TRIG 2) to trigger the ADC and pulse generator. Then, the RF signals were recorded from when TRIG 2 was fed to the ADC and pulse generator. This procedure ensured that the onset time of each scanning location was consistent with the cardiac cycle.

The method used to reconstruct retrospective duplex images is illustrated in Fig. 3. The retrospective images were reconstructed by echo signals acquired from different locations. Because the zebrafish heart is smaller than $2 \times 2 \times 2 \text{ mm}$, the heart was scanned from 500 different locations (i.e., $K = 500$). At each location, a total of N A-lines were used to reconstruct the retrospective duplex images, with N depending on the acquisition duration multiplied by the PRF. First, the A-line signals coming from different locations ($K = 1, 2, \dots$) and timings ($1/\text{PRF}$) ($F = 1, 2, \dots$) were combinatorially stored in an individual 3-D matrix. The interval between successive 2-D matrixes was $1/\text{PRF}$, which corresponds to the frame rate of the echocardiography system. After the 2-D matrix was formed, the RBI and RCFI were reconstructed. For RBI reconstruction, the signal processing of A-lines includes demodulation, logarithmic compression, and gray-level transformation to implement UHFR echocardiography. In addition, the echo signals were used to obtain estimates of the blood flow by a 2-D autocorrelation color flow function given by (1), see above [30], where V is the estimated blood flow velocity, c is speed of sound in blood, f_s is the sampling frequency, f_{dem} is the ratio of the center frequency to the sampling frequency, I and Q are the in-phase and quadrature sampled signals, respectively, M is the window depth in samples, N is the total number of A-line signals, and indexes m and n correspond to the fast time (depth direction) and the slow time (pulse number) directions, respectively, of I and Q data [31]. In this study, the sampling rate was set to 250 MHz, the PRF was 40 kHz, and the pulse-generator transmission frequency was 80 MHz. For RCFI reconstruction, the slow-time window was 100 lines from continuous frames and the fast-time window was 20 points to calculate the 2-D autocorrelation functions. Therefore, the maximum frame rate (F') for the RCFI was reduced to $\text{PRF}/100 \text{ fps}$.

$$V = \frac{c}{2} \frac{\frac{\text{PRF}}{2\pi} \tan^{-1} \left\{ \frac{\sum_{m=0}^{M-1} \sum_{n=0}^{N-1} [Q(m,n)I(m,n+1) - I(m,n)Q(m,n+1)]}{\sum_{m=0}^{M-1} \sum_{n=0}^{N-1} [Q(m,n)Q(m,n+1) + I(m,n)I(m,n+1)]} \right\}}{\frac{f_s}{2\pi} \left\{ 2\pi f_{\text{dem}} + \tan^{-1} \left\{ \frac{\sum_{m=0}^{M-2} \sum_{n=0}^{N-1} [Q(m,n)I(m+1,n) - I(m,n)Q(m+1,n)]}{\sum_{m=0}^{M-2} \sum_{n=0}^{N-1} [Q(m,n)Q(m+1,n) + I(m,n)I(m+1,n)]} \right\} \right\}} \quad (1)$$

C. Anesthesia of Adult Zebrafish

Experiments were carried out using healthy AB-line adult zebrafish (GENDANIO Ocean Technology, New Taipei City, Taiwan) aged 4 to 5 mo. The zebrafish were reared in separate tanks at a density of 24 fish per 10 L of unchlorinated water, with the temperature maintained within the range 27.5°C to 28.5°C. Each zebrafish was anesthetized with ethyl 3-aminobenzoate methanesulfonate salt (MS-222, Sigma-Aldrich, St. Louis, MO). The MS-222 was solved in water at pH 7.2 to a concentration of 0.16% as a stock. The stock was kept at 4°C. The fish were anesthetized by immersion in 4.2% MS-222 solution. The zebrafish were anesthetized to stage-4 anesthesia, defined as the zebrafish turning abdominal side up for more than 5 s but with the operculum still moving [32]. After the zebrafish was anesthetized and fixed in the sponge, UHFR echocardiography was used to assess its ventricular function. The animal experimental protocol was approved by the Institutional Animal Care and Use Committee of Fu-Jen Catholic University.

D. Estimation of Zebrafish Cardiac Function

The ventricular function of the zebrafish heart was estimated off-line from retrospective duplex images. To simplify the assessment of ventricular size changes during heartbeats, the zebrafish ventricle was assumed to be a prolate spheroid and separated ventricle with a short axis (SAx) and long axis (LAx). The zebrafish heart structure including ventricle, bulbus arteriosus (BA), and atrium is illustrated in Fig. 4. The scanning direction and SAx and LAx at the systole and diastole phases are also plotted in Fig. 4. The zebrafish ventricular area was calculated as [33]

$$\text{Area} = \frac{\pi \times \text{LAx} \times \text{SAx}}{4}. \quad (2)$$

The detailed motions of SAx and LAx were measured using high-temporal-resolution M-mode structure images (i.e., with a high imaging frame rate) obtained from two axial directions (note that the main reason for needing high-frame-rate B-mode images is for assessing LAx). The diameter changes of the ventricle in M-mode images were subsequently measured using a semiautomatic program implemented in Matlab (The MathWorks Inc., Natick, MA). To observe the diameter changes in both axial directions, sequential M-mode images were analyzed to identify the diameters at E_D and E_S . E_D and E_S were found by measuring the largest and narrowest diameters during the cardiac cycle, respectively [34]. After the diameters of E_D and E_S were found, the FS ventricular function parameter is given by [6]

$$\text{FS} = \frac{D_D - D_S}{D_D} \times 100\%, \quad (3)$$

where D_D and D_S are the diameters at E_D and E_S , respectively. The FS values represent the percentage diameter variations from E_D to E_S along LAx and SAx. The other (2-D) ventricular function parameter, FAC, is given by [6]

$$FAC = \frac{EDA - ESA}{EDA} \times 100\%, \quad (4)$$

where EDA and ESA are the ventricular areas at E_D and E_S , respectively. Both FS and FAC can be used to determine the cardiac contractile performance of adult zebrafish. E_D and E_S refer to the “time” of the cardiac phase. D_D and D_S refer to the “diameter” at the phases of E_D and E_S , respectively. It took about 16 min to collect the RF data for image reconstruction and about 20 min for computing the FS and FAC.

III. Results

A typical RBI and a typical RCFI of the zebrafish heart acquired in 80-MHz UHFR echocardiography are shown in Figs. 5(a) and 5(b), respectively. The RBI shows the major cardiac anatomy of the zebrafish heart, including the ventricle, BA, and atrium. The atrioventricular valve (located at the interior portion of the atrium, and connecting the ventricle and atrium) and the bulboventricular valve (located at the anterior portion of the ventricle, and connecting the BA and the ventricle) are evident in the UHFR RBI (video 1, , which has a frame rate of 1000 fps). After the UHFR RBI was obtained, the detailed transient motions of SAX and LAX can be measured for the two axial directions at a high spatial resolution. UHFR echocardiography also provides high-temporal-resolution RCFIs, as shown in Fig. 5(b). The flow into and out of the ventricle are clearly evident in the UHFR RCFI (video 2, , which has a frame rate of 400 fps). The colors indicate both flow directions and velocity. Because the PRF of transmission pulses was 40 kHz, a maximum peak flow velocity of 192 mm/s can be measured with this system. The tissue motion of the zebrafish heart was removed by a clutter filter [35] with a cut-off velocity of 0.6 mm/s.

The LAX and SAX values for the ventricle were further determined quantitatively in RBIs, as shown in Fig. 6(a). The zebrafish ventricular M-mode images were subsequently captured in both the SAX and LAX directions, as shown in Fig. 6(b) and (c). The zebrafish ventricular endocardial boundaries were traced along the temporal axis in both the inferior-superior [Fig. 6(b)] and anterior-posterior [Fig. 6(c)] directions. It is evident that the diameter of the endocardium increased for both directions during the diastolic phase, but decreased during the systolic phase. The quantitative variations of the diameter during cardiac cycles for LAX and SAX are plotted in Fig. 6(d). Fig. 7 shows the average results of LAX and SAX over a cardiac cycle from eight zebrafish ($N = 8$). The lines and error bars present the mean value and standard deviation (SD), respectively. Based on the interval between cardiac cycles, the zebrafish heart rate was found to be 97 ± 14 beats/min (mean $\pm$ SD). Fig. 7 also shows that the change in the diameter between systole and diastole was less for LAX than for SAX.

The measured two axial diameters were used to calculate the changes in the zebrafish ventricular area by using (2). Fig. 8 shows that the area of the ventricle increased during the diastolic phase. The largest and narrowest areas during the cardiac cycle were 0.54 ± 0.03 and 0.13 ± 0.04 mm², respectively. The variations of ventricular blood flow were measured from RCFIs (gated near the center of the ventricle), as shown in Fig. 8. Because the measurements of blood flow and area are simultaneous, the relationship between the area changes and blood flow during the cardiac cycle can be found. The experimental results

indicated that the end of A-flow and end of outflow occurred at 0.23 ± 0.04 and 0.53 ± 0.08 s, respectively, and corresponded to ventricular areas of 0.45 ± 0.05 and 0.23 ± 0.03 mm².

Fig. 9 shows the measured D_D and D_S values; they were 0.8 ± 0.08 and 0.32 ± 0.11 mm, respectively, for SAx, and 0.85 ± 0.08 and 0.50 ± 0.05 mm for LAx. The measured results of D_D and D_S were used to estimate the FS values of the zebrafish ventricle using (3); they were $60 \pm 13\%$ and $42 \pm 4\%$ for SAx and LAx, respectively. Also, the measured FAC for the normal adult zebrafish obtained using (4) was $77 \pm 9\%$. The zebrafish ventricular function parameters are summarized in Table II.

IV. Discussion

The present study is the first to propose the use of high-frequency ultrasound color flow imaging for studying the cardiac function of adult zebrafish. UHFR imaging is needed to provide detailed information about the blood flow from the heart. Even though ultrasound biomicroscopy at a frame rate of 200 fps has been applied to zebrafish heart imaging [20], [21], the technique employed in the present study yielded B-mode images with greatly improved quality and resolution. Furthermore, the use of retrospective Doppler-gated technology provided B-mode and color flow images at frame rates of up to 1000 and 400 fps, respectively. The frame rate depends on the PRF of data acquisition, and so it can be varied according to the experimental requirements. The flow velocity estimation in 2-D autocorrelation algorithm (1) depends not only on the PRF but also on the operating frequency of the transducer (f_{dem}), with the maximum velocity measured being reduced because of the higher operating frequency used in this system. Therefore, a high PRF provides the advantages of 1) increasing the range of velocity estimations; and 2) reducing the likelihood of aliasing in Doppler measurements. The technique used in this study could measure a maximum flow velocity of 192 mm/s. A conventional mechanical-scanning color flow imaging system [36] requires a clutter filter to remove tissue motion artifacts while keeping blood flow information. The cut-off velocity of the clutter filter depends on both the tissue motion and moving speed of the mechanical scanning [37]. However, the effect of transducer motion can be ignored in our retrospective imaging method because the backscatter signals were acquired with a high PRF at a fixed location. Therefore, a cut-off velocity of 0.6 mm/s was set to remove the tissue motion. A wide velocity estimation range from 0.6 to 192 mm/s can be achieved by using RCFIs, as shown in Fig. 5(b) (video 2,). In other words, the RCFI exhibits a high sensitivity for lower flow velocities while maintaining an excellent capability for large flow velocities. In addition, the color flow information can be displayed for a larger region of interest in RCFIs than for existing commercial systems. The SAx for zebrafish heart can be measured by traditional M-mode imaging with the transducer fixed outside the thoracic cavity, whereas a high-temporal-resolution RBI is needed to measure the variation of LAx in the ventricle. Both SAx and LAx can be obtained synchronously from UHFR RBIs at a PRF of 40 kHz. The innovation of present study is that we used the arterial blood flow velocity to replace the ECG signal for synchronizing the M-mode and heart motion because the ECG (R wave) and peak flow velocity of artery exhibit the same interval of heart beat. The variations of SAx can be measured by using M-mode image without the synchronizer; however, the synchronizer is needed for measuring the lateral variations of heart because the LAx was obtained from B-

mode image under a very high frame rate. Because the RCFI can provide very high temporal resolution (400 fps) for imaging blood flow in the ventricle of the zebrafish, the instant variations of flow can be measured as a function of cardiac cycle, as shown in Fig. 8. We compared our results (Fig. 10) to the same measurements which were obtained from an array system (Vevo2100, VisualSonics). Because the commercial system cannot provide the raw data of color Doppler image, the blood flow velocity from zebrafish heart was obtained by counting the color variations of color Doppler image. The detailed variations of instant blood flow velocities, including E-flow (early diastolic flow), A-flow (late diastolic flow), and outflow [20], [21], were also measured by using RCFI in present study. However, some instant information about blood flow was missed as using a commercial system because the frame rate is not fast enough, as shown in Fig. 10.

Because the adult zebrafish is not transparent, the optical modality cannot be used for imaging the heart of adult zebrafish. Optical technique can be only used to measure the FS and FAC parameters for assessing the ventricular function of zebrafish embryo and larva (embryo and larva are transparent). That is the main reason why the acoustic modality is needed for evaluating the cardiac functions of adult zebrafish. The measured results of E_D , E_S , FS, and FAC parameters from adult zebrafish were compared with those of embryo and larva from previous research [6], [33] to understand the cardiac functions of zebrafish during development. The ventricular functions of zebrafish embryo [72 hours post fertilization (hpf)] were studied by using an optical microscope [6]. The 72 hpf means the zebrafish development through the end of embryogenesis (the protruding-mouth stage), which occurs at 72 hpf at the standard temperature [38]. The timings of E_D and E_S for the embryo ventricle were found by measuring the peaks of blood flow into and out of the ventricle, respectively. However, the peak of blood flow velocity within ventricle is not synchronous with the area change of ventricle for adult zebrafish. The peak of A-flow was found to lead the peak of ventricular area for adult zebrafish, as shown in Fig. 8. In other words, use of blood flow velocity to measure the E_D and E_S is incorrect for adult zebrafish. Therefore, the determinations of E_D and E_S for ventricle were measured depending on the largest and narrowest motions of endocardium. The FAC parameter was obtained based on the difference in the ventricular areas between E_D and E_S . The measured FAC values for the zebrafish ventricle were approximately 51%, 42%, and 77% for the embryo (72 hpf) [6], larva (2 to 3 d) [33], and adult (4 to 5 mo), respectively. The measured results of FS are approximately 60% and 42% for SAX and LAX, respectively, for adult zebrafish and about 57% and 39% for the zebrafish embryo (72 hpf) [6]. The measured FAC indicated that the cardiac contractile performances are higher for the adult zebrafish than for the embryo heart, which may reflect differences in ventricular development between the embryo and adult zebrafish. It is well known that the heart rate, ventricular blood pressure, and myocardium increase during zebrafish development [39]. The higher FAC and FS were obtained from adult zebrafish because its ventricle exhibits a higher systolic strength. In other words, the ventricle of adult zebrafish needs to compress more volume to pump blood for circulation. Similar result was obtained in a previous mice study [34] at different ages. Furthermore, the FS and FAC parameters are used in imaging-based research of mammals and afford the opportunity to translate results across species [6].

Because the ECG (R wave) and peak flow velocity of artery exhibit the same interval of heart beats, both ECG and Doppler-gated technologies can provide good accuracy and repeatability for image reconstruction of heart. Besides its noninvasive nature, Doppler-gated technology is more suitable for zebrafish heart imaging. In ECG measurement, the electrodes must be inserted into the ventral midline about 2 mm posterior to the gill. In other words, the scanning space of transducer between two ECG electrodes is limited for single-element or array transducers. However, this problem doesn't exist in Doppler-gated technology. In addition, the Doppler-gated technology can be also used for 3-D image reconstruction of zebrafish heart by using an array or single-element transducer system. The main reason for measuring the FS and FAC in present study is that these two parameters are often used for estimating the cardiac functions of zebrafish heart during development. It may provide more information about cardiac functions if the volume size of zebrafish heart can be measured by the present system. However, there are still some technical problems for estimating the volume of heart. For instance, the processing speed is not fast enough for calculating the mass data under a very high frame rate. In addition, it is possible to obtain the FS, FAC, and volume of heart by using a commercial array system if the frame rate of imaging and the central frequency of probe can be increased.

V. Conclusions

Retrospective Doppler-gated technology was proposed in this study for improving the frame rate of high-frequency echocardiography for studying zebrafish ventricular function. The transient ventricular motions of normal AB-line adult zebrafish were measured using UHFR RBIs on both the long and short axes. The transient information of blood flow within ventricle at different cardiac phases were also measured using high-temporal-resolution RCFIs. These results were used to calculate the ventricular function parameters FS and FAC of adult zebrafish—this is the first paper reporting those ventricular parameters for normal adult zebrafish. The higher cardiac parameters of the adult zebrafish demonstrated that the developed ventricle can contract more to maintain the blood circulation in the whole body; however, the contractibility of the embryo ventricle is smaller because of its low systolic strength. The results obtained in the present study have demonstrated the feasibility of using retrospective Doppler-gated UHFR echocardiography for studying the cardiac function of adult zebrafish. Future studies should assess the potential of this method as a useful tool for preclinical studies in medicine.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Fig. 1.

Block diagram of retrospective Doppler-gated ultrahigh-frame-rate (UHFR) high-frequency echocardiography.

Fig. 2.
Timing control of retrospective Doppler-gated echocardiography.

Fig. 3. Reconstruction procedure of retrospective Doppler-gated ultrahigh-frame-rate (UHFR) echocardiography. RBI = retrospective B-mode image; RCFI = retrospective color flow image.

Fig. 4.

Illustration of cardiac function measurements in zebrafish, showing the mechanical scanning position for adult zebrafish (a) and the ventricular shape variations from the systolic phase to the diastolic phase (b). LAX = long axis; SAX = short axis.

Fig. 5.

In vivo zebrafish retrospective duplex images: (a) retrospective B-mode image (RBI) and (b) retrospective color flow image (RCFI).

Fig. 6.

Estimation of the zebrafish ventricular motion. (a) Typical retrospective B-mode images. The ventricle boundary, LAX, and SAX are plotted in M-mode images along the (b) interior-superior direction (SAX) and (c) anterior-posterior direction (LAX). The variations of the endocardium boundary are plotted in dotted lines. (d) Variations of the two axial diameters of the zebrafish ventricular endocardium as functions of the cardiac cycle.

Fig. 7.
Variations of ventricular axial diameters during a cardiac cycle. Data are mean and standard deviation (SD) values.

Fig. 8. Zebrafish ventricular area changes and corresponding ventricular blood flow variations during a cardiac cycle. Data are mean and standard deviation (SD) values.

Fig. 9.

SAX and LAX measurements from systole and diastole. D_S , diameter at systole; D_D , diameter at diastole.

Fig. 10. Measurements of ventricular blood flow velocity by using retrospective color flow image (RCFI) and commercial system (Vevo2100, VisualSonics).

TABLE I

Characteristics of the Pulsed-Wave Doppler Flowmeter.

Central operating frequency	35 MHz
Pulse repetition frequency	40 kHz
Transmitted pulse duration	85.7 ns
Output voltage	± 50 V
Wall filter	100 Hz

TABLE IICardiac Parameters of the Zebrafish Ventricle ($N = 8$).

Parameter	Value (mean $\pm$ SD)
Heart rate (beats per minute)	97 ± 14
Fractional shortening of short axis (%)	60 ± 13
Fractional shortening of long axis (%)	42 ± 4
Fractional area change (%)	77 ± 9