Development of ultrasound echocardiography technique for imaging of the cardiovascular system of small organisms in vivo

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Abstract. A technique based on a high-frequency ultrasound scanner was developed for imaging and characterization of the cardiovascular system of small organisms in vivo. An optical microscope combined with the ultrasonic unit was used in the experimental setup for simultaneous recording ultrasonic signals and video data. It was shown that combination of optical and ultrasonic data is effective to visualize dynamic processes in a living object. In addition to imaging of the cardiovascular system, video data was processed to estimate the period and phase of the cardiac cycle and to generate a trigger signal for the ultrasonic unit. The proposed approach and developed experimental setup were applied to imaging of the Danio rerio larva. In a result of the processing of the synchronous ultrasonic and optical data, the blood flow in the heart of the larva and the movement of surrounding organs were observed.

1. Introduction

Nowadays fish embryos are a popular model object for research in the fields of developmental biology, genetics, ecology, and medicine [1, 2]. Optical microscopy is widely used to observe the structure of a fish embryo, its growth, cell division, and blood flow [3–5].

Recently, it has been shown that ultrasound methods can be effectively used to study the adult Danio rerio in vivo [6–10]. Visualization of deeply located organs and their movement, monitoring of the cardiovascular system and measurement of blood flow velocity are possible using these ultrasonic techniques with minimal impact on the living organism. The phased array fast ultrasonic devices were employed in these works [7–10]. The spatial resolution of the setups was sufficient to study the cardiovascular system of an adult Danio rerio. However, higher resolution is required to image and monitor the cardiovascular system of Danio rerio at the stages of embryonic and larval development.

In this work, it is proposed to use an acoustic microscope with a high-frequency single-element transducer to achieve sufficient resolution. Since this device is based on the mechanical scanning of the transducer, an additional optical unit was employed to video record of the heart motion and
generate a trigger signal for the ultrasonic scanner. The combination of the ultrasonic and optical modalities allows ultrasonic imaging the organs and their motions through post processing of the ultrasonic and video data.

2. Experimental setup

In this work, a scanning acoustic microscope and an optical microscope were combined in one experimental setup to record ultrasonic and optical images of an organism in vivo (figure 1). The Danio rerio larva (2) was immersed in water and placed on a transparent plate. The acoustic objective, which consisted of the ultrasonic transducer (4) and the lens (3), generated probing pulsed waves and received echoes reflected by the object. The pulse generator (5), receiver (6) and controller (8) were used to produce the ultrasonic waveform and transfer it to the computer (14). To acquire an ultrasonic image, the acoustic objective was mechanically translated in the lateral directions by the scanner (7). The frequency of the transducer was in the range of 50–100 MHz, providing a spatial resolution of approximately 20–30 µm. In the two dimensional imaging mode the received ultrasonic signal is recorded as a function of the lateral positions of the mechanically translated transducer \((x, y)\) and the time of flight of ultrasonic pulses \(t\). To detect movement of the organs, the data \(s(t, T, x)\) are stored at different positions \(x\) versus the “slow” time \(T\).

![Figure 1. Scheme of the experimental setup.](image)

The optical unit of the setup consisted of the 4X objective (9), mirror (10), lens (11) and video camera (13). These optic components were arranged in accordance with a scheme of inverted microscope for recording images of the specimen (2) through the glass substrate. The white light-emitting diode (LED) (1) was used to illuminate the object. Another red LED (12) was built into the optical microscope to synchronize optical and acoustical data. At the command of the controller (8), the diode generated a short flash every time the ultrasonic objective was moved to a new \(x\) position. These flashes were recorded by the camera (13) along with video of the larva, marking the moments when the acquisition of ultrasonic frames \(s(t, T)\) began.
3. Data acquisition procedure
The data acquisition technique proposed in this work is illustrated by the diagram presented in figure 2. Suppose that the period of the heartbeat is equal $\Delta T_h$ as it is shown in figure 2(a). The intensity of the video signal $I$ measured in the region of the larva’s heart changes in the time $T$ with the same period (figure 2(b)). The strong spikes $F$ produced by the LED flashes are also presented in the video signal. Each spike indicates the start of the $s(t, T)$ frame recording executed by the ultrasonic unit. Synchronously with the trigger signal of the diode, the controller (8) (figure 1) generates a sequences of $N$ pulses with a period of $\Delta T$ which trigger the ultrasonic pulse-receiver (figure 2(c)). After recording $N$ waveforms, the controller sends a signal to the motion driver to move to the next position $x$ (figure 2(d)), and the procedure of the recording a new frame is repeated. Obviously, a period of these pulsed $\Delta T_d$ should be grater than $\Delta T\cdot N$.

To synchronize the ultrasonic $s(t, T)$ scans with the heartbeat, the moments when the intensity $I(T)$ crosses a certain threshold are determined. The interval $\Delta T_R$ between the spike $F$ and the first zero crossing point located after $F$ is used to define the position of the data window $W$ (figure 2(c)). The duration of this window $\Delta T\cdot N_1$ is less than $\Delta T\cdot N$ but it is large enough to cover several periods of the heartbeat $\Delta T_h$. The ultrasonic data within these windows $[\Delta T_R, \Delta T_R + \Delta T\cdot N_1]$ are used for further processing and analysis.

![Figure 2. Signal diagram.](image)

4. Algorithm of processing of video and ultrasonic data
To determine the heartbeat of the larva the video was recorded over entire period of time required to acquire the ultrasonic data. The ultrasonic data were measured at $K = 60$ scanner positions separated by a spatial step of 4 $\mu$m. Figure 3, shows one frame from the entire video recorded at of 30 frames per second. In this image, a window in the region of the heart was selected, and the total intensity of all pixels in the selected window was calculated as a function of the video frame number $I(n)$. The
calculated function \( I(n) \) is presented in figure 3(b) and (c) at different scales. The intensity \( I(n) \) combines the quasi periodic heart signal and \( K \) pulses from the LED used for the data synchronization. Using comparison of \( I(n) \) with a threshold, which is higher than the peak values of the heart component of the signal, the local maximal values of these pulses and their positions \( N_{\text{max}}(k), 1 \leq k \leq K \), were found. To suppress these short spikes in the signal \( I(n) \) and obtain the heartbeat component, a median filter was applied to the waveform; then a band pass filter was used to increase the signal to noise ratio and subtract the mean value.

Using Fourier transform of the filtered signal it was found that the period of the heartbeat fundamental harmonic was equal to \( \Delta T_h = 1.15 \) s. To determine the delays \( \Delta T_R(k) \), zero crossing points \( P(k) \) of the filtered intensity \( I(n) \) were found. Only the transitions from positive to negative values were taken into account, and only the points \( P \) following the synchronization pulses were used to calculate the delays \( \Delta T_R(k) = P(k) - N_{\text{max}}(k) \) (figure 3(c)). The raw ultrasonic data stored in the PC memory consisted of \( K \) frames \( s(t, T, x_i) \), traditionally called as M-scans. By application of the windows \( [\Delta T_R(k), \Delta T_R(k) + \Delta T\cdot N_1] \) over the “slow” time \( T \), the discrepancies between heartbeat and the start moments of the raw scans were compensated. The length of the window was equal to \( N_1 = 1200 \), therefore the duration of the corrected scans was 2.4 s, which was more than twice the heartbeat period \( \Delta T_h \).

![Image](image_url)

**Figure 3.** Video frame (a) and optical signals \( I(n) \) at different scales (b) and (c).

An overview of the developed algorithm for processing video and ultrasonic data is presented in figure 4 in the form of a flowchart.
5. Experimental results and discussion

The corrected M-scans $s(t, T)$ measured at $x_{39} = 156 \, \mu\text{m}$ and $x_{40} = 160 \, \mu\text{m}$ in the middle of the heart are shown in figure 5(a) and 5(b), respectively. Several distinctive ultrasonic echoes can be detected and recognized in the images. The reflection of the ultrasonic waves at the interface between the immersion liquid and the skin of larva produces the responses $F$. The internal muscle organs of the heart give the echoes $H$, whereas the ultrasound scattering on the blood elements generates the signals $B$. The surface signal $R$ is almost stable, only tiny displacements are observed. On the contrary, the signal $H$ shows quasiperiodic movement with an amplitude of about 30–40 \, \mu\text{m}. The phases of movement coincide in these images; this is an evidence of the correctness of the developed data synchronization technique.

The heartbeat period $\Delta T_h$, measured based on the video data, spreads over two peaks in the $H$ and $B$ signals. Moreover it should be noted that the behavior of the signal $H$ in the vicinity of the adjacent peaks is not identical. Thus the ultrasonic monitoring of the heart could provide detailed information in addition to the optical observations. The pattern of the blood responses $B$ also changes from period to period. This phenomenon can be explained by the fact that spatial distribution of the blood elements is not constant, and so is the ultrasonic echo. The positions of the $B$ echoes vary with time $T$ due to the movement of the blood. The blood flow velocity can be estimated by the slope of their traces: $v = \Delta t \cdot C_w / 2\Delta T_h$, where $\Delta t$ is the delay of the echo acquired over the time interval $\Delta T_h$, and $C_w \approx 1.5 \, \text{mm/\mu s}$ is the sound velocity. The typical velocities measured in the area $B_1$ and $B_2$ are 0.05 and 0.86 mm/s, respectively.

![Figure 4. Video and ultrasonic data processing flowchart.](image)

![Figure 5. Ultrasonic data of the heart.](image)
6. Conclusion
The high-frequency ultrasonic echocardiography technique has been developed for the in vivo study of the cardiovascular system in small animals. The technique is based on a combination of a single-element ultrasonic scanner and an optical microscope to obtain simultaneous ultrasonic and optical images of a living organism. It was shown that processing of the video data allows the formation trigger signal for synchronization of the ultrasonic data to the heartbeats. The experimental validation of the developed method was carried out on Danio rerio larvae. The ultrasonic images of the heart organs were obtained at different positions for a time exceeding the period of the blood cycle. The movement of the heart walls and blood flow are distinctively observed in the synchronized ultrasonic scans. The velocity of the blood elements was estimated at various moments of the heart cycle by measuring delays of the ultrasonic waves scattered on the moving cells.

Acknowledgments
This work was supported by the Ministry of Science and Higher Education of the Russian Federation under the State contract No. 0069-2019-0009.

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