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# Cardiac parameters analysis for zebrafish heart regeneration based on high frequency ultrasound imaging

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*Abstract*— **Zebrafish can fully regenerate their myocardium after up to 20% ventricular resection within 2 months without evidence of scar tissues. The extraordinary regenerative abilities provide a significant model system to study the activation of the regenerative potential of human heart tissue. In order to characterize cardiac functions of zebrafish during heart regeneration, we used high-frame-rate high-frequency ultrasound system with the capabilities of 75 MHz B-mode imaging to monitor real-time cardiac parameters. Longitudinal *in vivo* experiments was carried out to capture echocardiographic images from individual fishes. A pilot study on 6 fish over 30 days post amputation (dpa) was performed using this technique. A total of over 400 videos were captured. To process the large video data, an automatic image segmentation algorithm was developed. By using information obtained from temporal decorrelation of the B-mode image sequence, the epicardium is determined in a region-based level set framework combined with shape priors and Kalman filtering. Afterwards, the size of the heart was estimated frame by frame using the outlined epicardium to obtain its dynamic variation. Subsequently, the maximum and minimum of the heart size was used to calculate ejection fraction (EF). The time course of the mean EF (n=6) with a "V" shape indicates the strong ability of zebrafish to recovery cardiac functions along its morphological regeneration.**

*Keywords; high frequency ultrasound, zebrafish heart regeneration, automatic segmentation, ejection fraction*

## I. INTRODUCTION

Understanding the functions and mechanisms of heart regeneration in a vertebrate model system is a highly relevant public health concern. Because injured human hearts caused by myocardial infarction (MI) results in decreased cardiac performance and eventually the development of heart failure. Activation of the regenerative potential of human heart tissue may become a novel therapeutic approach that can supplement or replace traditional pharmacotherapy and mechanical interventions for MI, which can be invasive or not particular efficacious [1]. In contrast to mammals, zebrafish fully regenerate myocardium after 20% ventricular resection [2], [3]. These remarkable regenerative abilities provide an excellent model system to study the functions and mechanisms of heart regeneration.

Although zebrafish heart regeneration has been characterized using molecular, genetic and immune-histochemical tools , the physiological properties and functions of the regenerative heart have not been determined. Recently, high-frequency ultrasonic imaging system has been used in the zebrafish heart study, and the results significantly improved delineation of detailed cardiac structures and accurate estimation of cardiac dimensions [4].

In this paper, a pilot study on 6 fish over 30 days post amputation (dpa) was performed using this technique. During the experiments, longitudinal experiments were carried out to capture images from individual fishes *in vivo* non-invasively, a series of images were acquired on parallel imaging planes separated by 50μm. Such a procedure was repeated on individual fish before the amputation and throughout of 30 dpa. A total of over 400 videos were captured. In order to process the large video data, an automatic image segmentation algorithm was developed. Decorrelation coefficient was used to separate the heart region from the fish body by evaluating changing rate of the signal patterns frame by frame. Moreover, local region statistics was integrated into a level set framework, in which the active contour will be driven by both the local region contrast and the geodesic distance from the shape prior. Shape priors and Kalman filtering were also applied to improve the robustness of the auto segmentation method. Comparison between manual segmentation and automatic segmentation shows very similar contour. Afterwards, the size of the heart was estimated frame by frame using the outlined epicardium to obtain its dynamic variation. Subsequently, the maximum and minimum of the heart size was used to calculate ejection fraction (EF). The time course of the mean EF (n=6) with a "V" shape indicates the strong ability of zebrafish to recovery cardiac functions along its morphological regeneration.

## II. MATERIALS AND METHODES

### A. Ultrasound Image Acquisition

To perform zebrafish imaging experiments, one year old Ekk wild type fish was obtained and used for heart amputation according to the described procedure [2]. Briefly, the fish was anaesthetized in tricaine and placed ventral side up on a moist sponge. A small incision at the chest was made using

iridectomy scissors. The heart ventricle was exposed by gentle pressure and 20% of the ventricle at the apex was removed with the scissors.

Longitudinal imaging experiments were carried out to capture echocardiographic data from individual fishes. First, the fish was anesthetized with 0.08% tricaine (MS-222, Ethyl 3-aminobenzoate methanesulfonate salt (Sigma-Aldrich, St. Loius, MO) for 30 seconds, followed by gently removing the scales at the ventral side between the gills. Afterwards, the fish was maintained in 0.04% tricaine solution during the experiments. Afterwards a series of images were acquired on parallel imaging planes separated by 50μm. For each parallel imaging plane, we recorded a short period of real-time ultrasound images including at least 10 cycles of heart beats. High-frame-rate high-frequency ultrasonic images were acquired and analyzed. A total of over 400 videos were captured from 6 fish over 30 dpa using high frequency ultrasound imaging system.

### B. Automatic Image Segmentation and Analysis

The method used to extract heart area mainly consists of two parts, decorrelation layer calculation and local region-based segmentation, as shown in the flow chart (Fig. 1).

*1) Decorrelation Layer:* Fig. 2 shows a typical B-Mode image of the zebrafish using high frequency ultrasound. Outside the heart region are organs and body structures with different echogenicity. Considering the speckled feature of the B-Mode image, decorrelation coefficient was used to separate the heart region from other body structures.

$$\rho(T) = 1 - \frac{\int_W I_{t1}(X) I_{t2}(X+T) dX}{\sqrt{\int_W |I_{t1}(X)|^2 dX} \sqrt{\int_W |I_{t2}(X+T)|^2 dX}} \quad (1)$$

$I_{t1}$ and $I_{t2}$ represent intensity from two sequential frames. X is the spatial coordinate, T represents the translation vector, and W denotes the computation window in two frames.
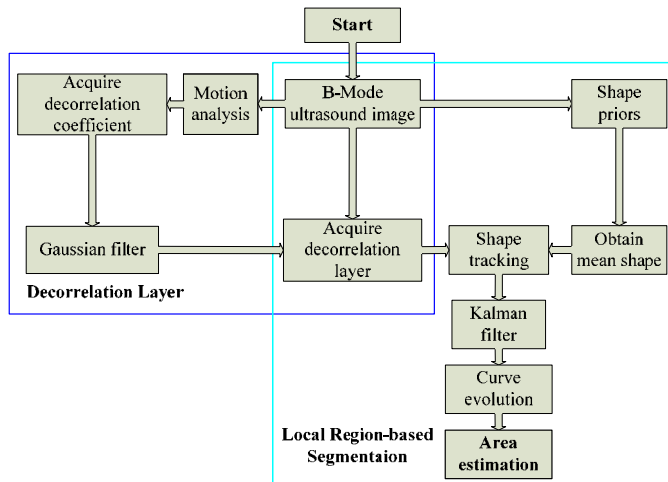


Figure 1.   The flow chart of automatic segmentation algorithms

The minimum decorrelation coefficient (MDC) is the minimum value of the above function, and the corresponding T is an estimate of the translation between computation windows in two sequential ultrasound images. If tissue only undergoes translation, these two images in computation windows are totally correlated with each other and the MDC should be equal to 0. But when large deformation is occurred in tissue, the signal change results in feature-motion decorrelation. Then the MDC value will be bigger than 0. Thus MDC is a measure of the change rate of the signal patterns from frame to frame. Based on MDC values, decorrelation layer (Fig. 3) was obtained using MDC*I after Gussian smoothing. The relative high intensity in the center indicates the heart region.

*2) Local region-based segmentation:*

*a) Local region-based statistics:* Intensity variations in decorrelation layer may result in region inhomogeneity. Local region-based statistics [5] was employed to solve this problem:

$$u_{in}(X) = \frac{\int_{inside(C)} B(Y;X) I(Y) dY}{\int_{inside(C)} B(Y;X) dY} \quad (2)$$

$$u_{out}(X) = \frac{\int_{outside(C)} B(Y;X) I(Y) dY}{\int_{outside(C)} B(Y;X) dY}$$

The idea is that the intensity means are computed based on local statistics. Y is the integration variable and X is the coordinate for the current position. B(Y; X) is a kernel centered at position X, which masks a local region. I means the decorrelation layer. C is counter initialized with shape prior, and determined by iteration.

The prior shape is derived from a set of training contours drawn manually by the expert on several initial frames of the image sequence. First training contours are aligned to remove the dependence on the pose parameters, and then the mean shape is obtained and used as the prior shape.

*b) Shape tracking and curve evolution:* Based on local region-based statistics, segmentation with shape priors and level set framework was implemented similar to the method developed in [6], [7]. Shape tracking was realized by matching the prior shape to the object in the image. First, pose parameters (scale parameter μ, rotation angle θ and translation
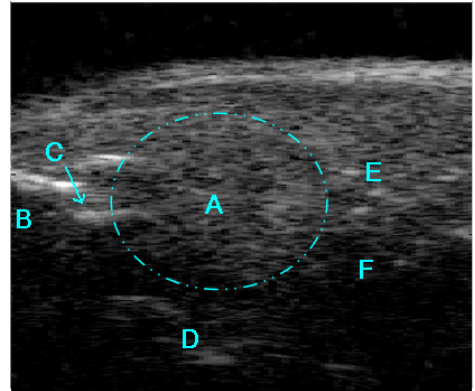


Figure 2.   B-Mode ultrasound image of the zebrafish heart. A is the heart. The heart is roughly inside the circle. B is the gill. C is the bony structure of the fish's mouth. D is the stomach, E is the liver and F is the intestine.
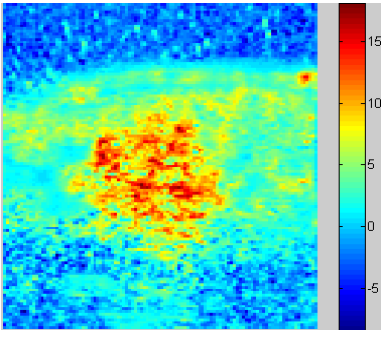
Figure 3. The decorrelation layer.

vector T) need to be estimated. Here we make the prior shape registration by minimizing the following local region-based energy:

$$E_{shape}(\mu,\theta,T) = \int_{inside(C^*_{\mu,\theta,T})}(I-u_{in})^2 dX$$
$$+ \int_{outside(C^*_{\mu,\theta,T})}(I-u_{out})^2 dX$$

where $C^*_{\mu,\theta,T}$ means the prior shape after the rigid transformation. The optimal values of $\mu$, $\theta$ and T are obtained by using gradient descent to minimize the above energy. I means the decorrelation layer.

To make use of the temporal continuity of the heart motion, we integrated the measurements of the pose parameters and a second-order autoregressive model [8] in a classic Kalman filtering framework.

To detect the shape variation that can't be captured by rigid transformation, we further evolved the curve based on the local region contrast again and constrained the evolution by the prior shape. The energy for the curve evolution is:

$$E_{curve}(C) = E_{region}(C) + \lambda \oint_C d^2(C,C^*_{\mu,\theta,T})|C'(s)|ds$$

$d(C, C^*_{\mu,\theta,T})$ means the distance from a point on C to the registered prior shape $C^*_{\mu,\theta,T}$. The second energy term measures the similarity between the interested contour and the registered prior shape. Finally, the size of the heart region was obtained from outlined epicardium extracted using this technique.

## C. Statistical Analysis

A total of over 400 videos obtained by high-frequency ultrasonic imaging were processed using the above method programmed in MATLAB (Math Works Inc., Natick, MA). The heart size was estimated frame by frame within a video clip. The dynamic result was then plotted as a function of time. The peaks and valleys correspond to the cardiac cycles at end-diastoles and end-systoles. Arrhythmia and fibrillation were noted during some experiments. Therefore, cardiac parameters obtained from abnormal states were not used. In order to minimize the estimation errors at different imaging planes, the ones passing through the long axis with large heart size were selected to compute the eject fraction (EF) using equation (5).

$$EF = \frac{1}{MN}\sum_{i=1}^{M}\sum_{j=1}^{N}\frac{S_d(i,j)-S_s(i,j)}{S_d(i,j)} \tag{5}$$

M means the number of plane, N means the oscillation number in one image plane. $S_d$ and $S_s$ indicate the heart size at end-diastoles and end-systoles separately.

Such a process was applied to estimate the ejection fraction on each fish at every dpa. The mean and standard deviation of ejection fraction among the total fish were calculated at different dpa, and expressed as means ± SD.

## III. RESULT AND DISCUSSION

Fig.4 shows the segmentation of one frame of zebrafish echocardiographic video clip outlining the epicardium. Yellow solid curve represents the result by automatic segmentation method. Red solid curve is the result by manual segmentation. It can be noted that the segmentation result obtained by both methods nearly coincide with each other, indicating that automatic algorithm provided a reliable means to estimate the variation of heart size during the regeneration process.

Using the automatic segmentation method, the epicardium was outlined frame by frame, and the heart size estimation was performed frame by frame as well. Fig. 5 shows a cyclic dynamic variation of heart size due to heart beat during a 4.5s period of echocardiography video acquired at each parallel imaging plane. The peak heart size at end-diastole is 2.445mm$^2$, and minimum heart size is 1.605mm$^2$ at end-systole with an estimated cardiac output of 0.84mm$^2$. The heart ejection fraction value is about 0.34. The heart rate is estimated 165 beats/min.

Various echocardiographic videos were acquired at different parallel planes separated by 50µm at the same dpa. Independent processing was performed on each videos acquired at different planes. The results from two videos containing relatively similar heart size from the same fish at the same dpa were calculated and compared. Fig. 6(a) and 6(b) plots the heart size as a function of time. It can be noted that the plane position influenced the waveforms. However, the ejection fractions are similar, 0.408 and 0.362, respectively. The heart rates are close too, 150 beats/min and 142 beats/min, respectively.
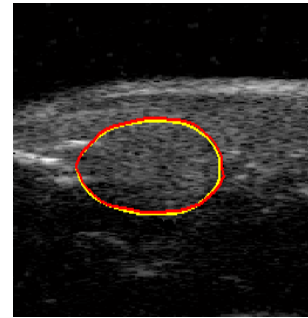


Figure 4. result of segmentation B-Mode ultrasound image of the zebrafish.

Despite the difference of the position of imaging plane, the heart rate and ejection fractions remain relatively the same, indicating that these parameters serve as a reliable indicator for assessing cardiac function during zebrafish heart regeneration.

Repeating the above automatic analysis, ejection fraction was estimated on each fish at every dpa. Finally, the mean ejection fraction with standard deviation from all fish (n=6) at individual dpa was plotted in Fig. 7. Day 0 means before amputation. The time course of ejection fraction shows a clear "V" shape. It suggested that after removing ~20% cardiac muscles by ventricular amputation, the heart lost contractile force indicated by a sharp drop of ejection fraction. After that, heart starts to regenerate and the blood clot was gradually replaced by muscles. With the increase of muscles, the heart gradually regains its force and function by slowly increasing its ejection fraction.

## IV.  Conclusion

In this paper, high frequency ultrasound imaging was used to longitudinally study zebrafish heart functional regeneration in real time and non-invasively. To process large amount of video data, an automatic image processing algorithm was developed to obtain cardiac parameters, such as ejection fraction. Compared with manual segmentation, the automatic method demonstrates good effectiveness and reliability. Based on this method, a pilot study demonstrates strong evidence that zebrafish heart possesses ability of functional recovery.

### ACKNOWLEDGEMENT
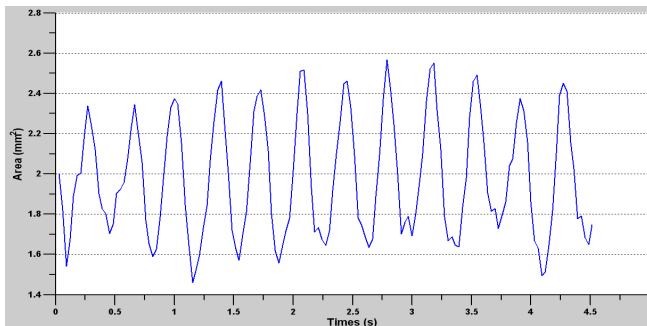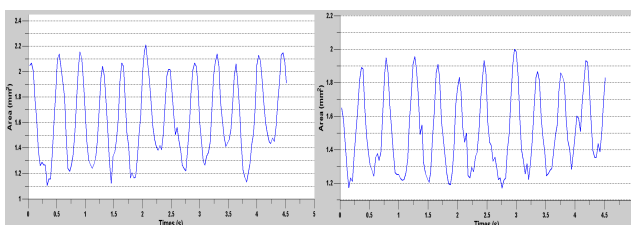
Figure 5.   Cyclic variation of heart size due to heart beating.



(a)                              (b)

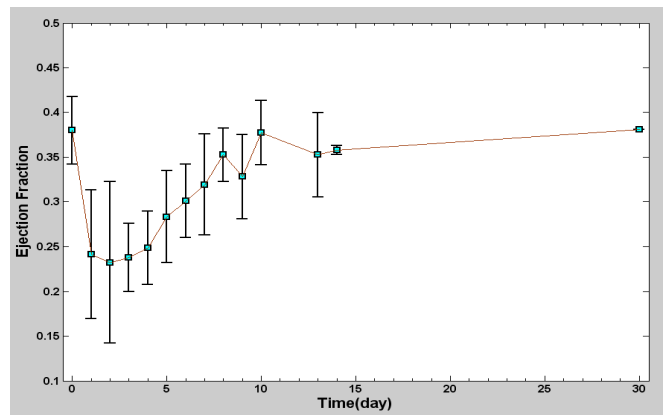Figure 6.   Cyclic variation of heart size due to heart beating from two different planes.



Figure 7.   Statistical analyses of ejection fraction from 6 zebrafish

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