Published in final edited form as:

IEEE Trans Ultrason Ferroelectr Freq Control. 2013 April ; 60(4): 718–726. doi:10.1109/TUFFC.2013.2620.

Ultrasound Bio-Microscopic Image Segmentation for Evaluation of Zebrafish Cardiac Function

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Abstract

Zebrafish can fully regenerate their myocardium after ventricular resection without evidence of scars. This extraordinary regenerative ability provides an excellent model system to study the activation of the regenerative potential for human heart tissue. In addition to the morphology, it is vital to understand the cardiac function of zebrafish. To characterize adult zebrafish cardiac function, an ultrasound biomicroscope (UBM) was customized for real-time imaging of the zebrafish heart (about 1 mm in diameter) at a resolution of around 37 μm . Moreover, we developed an image segmentation algorithm to track the cardiac boundary and measure the dynamic size of the zebrafish heart for further quantification of zebrafish cardiac function. The effectiveness and accuracy of the proposed segmentation algorithm were verified on a tissue-mimicking phantom and *in vivo* zebrafish echocardiography. The quantitative evaluation demonstrated that the accuracy of the proposed algorithm is comparable to the manual delineation by experts.

I. Introduction

Coronary heart disease is among the leading causes of disability and mortality worldwide. A human heart injured by myocardial infarction (MI) results in decreased cardiac performance and, eventually, the development of heart failure [1]. In contrast to mammals, zebrafish hearts have a remarkable regenerative ability [2], [3]. Zebrafish fully regenerate myocardium after 20% ventricular resection and provides an excellent model system to study the mechanisms of heart regeneration [2], [3]. 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 particularly efficacious [4].

Although heart regeneration in zebrafish has been characterized using molecular and genetic tools [5], the physiological properties and functions of the regenerative heart have not been fully investigated because of the lack of suitable tools. Although various imaging modalities have been used in zebrafish study [6]–[8], most of them were invasive or not fast enough to capture dynamic information of the zebrafish heart [9]. Recently, ultrasound biomicroscopy (UBM) imaging has been shown to be feasible for obtaining *in vivo* structural, physiological, and functional information of the zebrafish heart noninvasively [9], [10]. The real-time, high-resolution, and noninvasive features of UBM imaging may facilitate longitudinal study of individual zebrafish hearts to precisely determine their regenerative properties.

Unlike human beings, zebrafish have two-chambered hearts and no pulmonary vasculature. However, cardiac parameters such as the ejection fraction (EF) and the heart rate are still important parameters for evaluating cardiac function, and they can be obtained by segmenting the zebrafish echocardiographic images acquired by the UBM. Hence, image segmentation is an important task in the study of zebrafish cardiac function.

Obtaining physiological and functional information of the human heart using echocardiography has been extensively studied [11, ch. 11]. Also, many image segmentation methods have been proposed for quantitative analysis of human echocardiography. Generally, these methods tried to improve the image quality and integrate more features and priors. For example, besides the B-mode intensity, parameters derived from the RF signal [12] and motion analysis [13] also proved to be helpful for segmentation. The shape prior was used to tackle the difficulties caused by occlusion, missing signals, and other noises [14], [15], and the Kalman filter was also used to track the myocardium border in the cardiac image sequence. The reader is referred to [16] for a comprehensive review.

To the best of our knowledge, there has been no study on quantification of zebrafish cardiac function from zebrafish echocardiography. As a key technique, identification of the heart boundary by automatic segmentation is very challenging because of the ambiguous feature definition. Fig. 1 shows a UBM image of the zebrafish heart. The myocardium and cardiac chambers are poorly presented because the zebrafish heart consists of trabecular structure without well-defined endocardium. Segmenting such an image is difficult even for manual tracing. Moreover, the appearance of the heart is not uniform. Various tissues around the heart make the background inhomogeneous. Furthermore, other misleading features also cause difficulties (e.g., the stomach labeled by *D* in Fig. 1 makes a sharp edge). Directly applying existing segmentation techniques will fail because of these challenges.

In this paper, our goal is to segment the UBM cardiac images of zebrafish and measure the regional size of the zebrafish heart. We propose an automatic and robust algorithm, in which the image is decomposed into two layers according to the information from temporal correlation analysis. It is demonstrated that the proposed *decorrelation layer* is very useful

for segmenting tissue with high echogenicity and irregular motion, like the zebrafish heart. To tackle region inhomogeneity and attenuation, we take advantage of the local region statistics to locate the active contour [17]. Shape priors and the Kalman filter are also integrated to improve the robustness of our method.

To evaluate the performance of our method, experiments on a tissue-mimicking phantom and *in vivo* zebrafish echocardiography were performed. With the phantom data, the feasibility of using the decorrelation layer was illustrated. With the *in vivo* echocardiographic data, the merits of our algorithm were demonstrated, and then the accuracy and variability of the proposed method were evaluated by comparing the automatic results against the manual delineation by three experts.

II. Materials and Methods

A. Tissue-Mimicking Phantom Preparation

A phantom mimicking a zebrafish heart was constructed to evaluate the proposed method. Briefly, it was made from agarose, deionized water, propylene glycol, bovine milk, antiseptic, glass beads, etc., following the procedure by Madsen *et al.* [18]. The diagram of the phantom experiment is shown in Fig. 2. A hole with a radius of around 1 mm was drilled in the phantom center to mimic the zebrafish heart chamber. A tube was connected to the phantom hole from a syringe. By pressing the syringe plunger, a tissue-mimicking solution could flow into the phantom hole to simulate the blood flow. The phantom was submerged in a water bath for the coupling of ultrasound transmission. An ultrasound transducer fixed on an imaging stand was placed in the water bath. While acquiring UBM images, the position of the hole was adjusted so that it was at the focal point of the ultrasound beam.

B. In Vivo Data Acquisition

The zebrafish experiments were performed with a protocol approved by the Institutional Animal Care and Use Committee (IAC UC) at the University of Southern California. Zebrafish were obtained from Aquatica Tropicals (Aquatica Tropicals, Plant City, FL) and maintained in a recirculating aquarium system at a temperature of 28°C. The fish were at one year of age. During *in vivo* experiments, the fish were anesthetized by being placed in the 0.08% tricaine solution (MS-222, ethyl 3-aminobenzoate methanesulfonate salt, Sigma-Aldrich, St. Louis, MO) for 30 s. Afterward, the fish were maintained in the 0.04% tricaine solution throughout the experiments.

Zebrafish echocardiography was acquired by the UBM with a similar setup to the tissue-mimicking phantom, as illustrated in Fig. 2. The fish was imaged upside down with the chest and abdomen facing the transducer. The focus of the transducer was positioned at the center of the zebrafish heart (9 mm from the transducer surface). The central frequency of the transducer was 45 MHz with a -6-dB bandwidth of 27 MHz. For each 2-D image, 108 RF lines were acquired with a resolution of 37.5 μm and a sampling rate of 200 MHz. The RF data was processed by the Hilbert transform and logarithmic transformation to obtain the B-mode image¹. The temporal frame rate was 33 frames per second (fps).

C. Feature for Segmentation

We propose to use the active contour in the level set formulation [19] as the basic framework for image segmentation. The B-mode image in Fig. 1 shows that the edges for the heart boundary are obscure and the image is full of intensity variations. Thus,

¹In signal processing, the Hilbert transform is used to obtain the amplitude of an RF signal, and the logarithmic transformation is used to adjust the dynamic range of the image.

segmenting the whole sequence based on edge information is difficult. Although the method using region statistics is more reliable, region-based methods usually have the assumption that the feature used for segmentation should be homogeneous on each side of the boundary [20]. To address these issues, we would construct a parametric image called a *decorrelation layer*, which is desirable for region-based segmentation.

In practice, segmenting the zebrafish heart from a static UBM image is difficult even for manual tracing. Besides image intensity, another cue that can be used for segmentation is the motion pattern of the beating heart. However, reliable motion estimation is unavailable inside the heart region because of the large and irregular motion of the zebrafish heart, which raises the feature-motion decorrelation problem [21]. Instead of relying on motion estimation, here we propose to make use of the decorrelation phenomenon.

Speckle tracking is usually used in motion analysis of ultrasound images [21], [22]. In speckle tracking, speckle patterns are assumed to be stable except for a shift before and after tissue motion. Thus, the displacement of tissue corresponding to the signal shift can be estimated by maximizing the correlation coefficient between images, which is defined as

$$\rho(T;X)=\frac{\text{cov}[I_1^{W(X)}, I_2^{W(X+T)}]}{\sqrt{\text{var}[I_1^{W(X)}]}\sqrt{\text{var}[I_2^{W(X+T)}]}}, \quad (1)$$

where I_1 and I_2 represent two successive frames, $I_*^{W(X)}$ means the subimage enclosed by a window W centered at X , T is the displacement to be estimated, and cov and var denote covariance and variance, respectively. In speckle tracking, the optimal T that gives the maximum correlation coefficient (MCC) is searched for each location X over the image.

Ideally, MCC will be equal to 1 if speckle patterns remain unchanged before and after tissue deformation. Thus $1 - \text{MCC}$ measures the decorrelation effect between frames. Here, we decompose the original image I into two layers:

$$I = \underbrace{\text{MCC} \cdot I}_{\text{correlation layer}} + \underbrace{(1 - \text{MCC}) \cdot I}_{\text{decorrelation layer}}. \quad (2)$$

The *correlation layer* shows the magnitude of stable signals and the *decorrelation layer* shows the magnitude of fast varying signals.

In zebrafish echocardiography, the heart has high intensity in the decorrelation layer because the signal in this region is generated from mixtures of trabecular myocardium and blood. In the background with high echogenicity, the MCC is close to 1 because the rigid translation is dominant. In the background with low echogenicity, the B-mode intensity is low. Thus, the intensity in the decorrelation layer is high for the heart and low for other parts, which satisfies the region-homogeneity assumption for region-based segmentation.

D. Local Region-Based Segmentation

Region-based methods are widely used in ultrasound image segmentation because they are robust to noise and insensitive to initialization. In region-based deformable models, the contour is driven by the force from region competition and evolves to minimize the energy function [19]

$$E_{\text{region}}(C) = \int_{\text{inside}(C)} (I - u_{\text{in}})^2 dX + \int_{\text{outside}(C)} (I - u_{\text{out}})^2 dX + \lambda \cdot \text{length}(C), \quad (3)$$

where I is the image, C is the contour, λ is a constant, and u_{in} and u_{out} are mean values of the image intensity inside and outside the contour, respectively. The limitation of the original region-based methods is the assumption that the intensity inside the same region should be uniform, which may not be true in ultrasound images where attenuation, shadows, and other artifacts cause intensity variation inside the region. To solve this problem, we make use of the local region-based energy [23]. The idea is that the intensity means are computed based on local statistics instead of global statistics:

$$u_{in}(X) = \frac{\int_{inside(C)} B(Y;X)I(Y)dY}{\int_{inside(C)} B(Y;X)dY}, \quad u_{out}(X) = \frac{\int_{outside(C)} B(Y;X)I(Y)dY}{\int_{outside(C)} B(Y;X)dY}. \quad (4)$$

Y is the integration variable, X is the coordinate for the current position, and $B(Y;X)$ is a kernel centered at X , which masks a local region. An average mask or a Gaussian mask is often used. For the whole image, $u_{in}(X)$ and $u_{out}(X)$ can be computed by 2-D convolution, which can be implemented efficiently by fast Fourier transform.

E. Shape Tracking and Curve Evolution

We use a level set framework with shape priors. It is similar to the method in [15]. The major difference is that we make use of the local region statistics instead of global statistics, and incorporate a Kalman filter into the framework. Image I in the following equations is the decorrelation layer computed before segmentation.

In image segmentation, the shape prior usually means a representation of the desired object boundary which is statistically learned from a collection of segmented images [14]. The shape prior is usually used in medical image segmentation, where the image quality is poor because of the difficulties such as missing edges and high noise level. Due to the lack of annotated data and the large shape variation of zebrafish hearts among individuals, we simply use a template contour derived by manual tracing on the first frame of each sequence as the shape prior in our method. To match the heart boundary in different frames, the template contour can be warped by the rigid coordinate transformation:

$$X \rightarrow \mu RX + T, \text{ where } R = \begin{bmatrix} \cos\theta & \sin\theta \\ -\sin\theta & \cos\theta \end{bmatrix}, \quad (5)$$

where X is the coordinate, μ is the scale parameter, θ is the rotation angle, and T is the translation vector.

The pose parameters are estimated by minimizing the local region-based energy:

$$E_{shape}(\mu, \theta, T) = \int_{inside(C_{\mu,\theta,T}^*)} (I - u_{in})^2 dX + \int_{outside(C_{\mu,\theta,T}^*)} (I - u_{out})^2 dX, \quad (6)$$

where $C_{\mu,\theta,T}^*$ means the prior shape after the rigid transformation. The optimal parameters μ^* , θ^* , and T^* are computed by using gradient descent to minimize (6). To make use of the temporal continuity of the heart motion, we fuse the measurements of pose parameters from the image data with a motion model describing the temporal correlation of the pose parameters in a classic Kalman filtering framework. Here, we simply use a constant acceleration model [17]:

$$\ddot{\Theta}(t) = \ddot{\Theta}(t-1), \quad (7)$$

where $\Theta(t)$ is the vector composed of pose parameters at time t and $\ddot{\Theta}$ means the second derivative of Θ to t .

To detect the shape variation that can't be captured by rigid transformation, we further evolve the curve based on the local region contrast and use the registered template contour as the initial contour and the shape constraint. The energy for the curve evolution is:

$$E_{\text{curve}}(C) = E_{\text{region}}(C) + \lambda \int_0^1 d^2(C(s), C_{\mu^*, \theta^*, T^*}^*) |C'(s)| ds, \quad (8)$$

where $C(s)$ is the parametric curve ($s \in [0, 1]$); $d(C(s), C_{\mu^*, \theta^*, T^*}^*)$ is the distance from a point on C to the contour $C_{\mu^*, \theta^*, T^*}^*$. The second term in (8) measures the similarity between the considered contour and the registered prior contour. The energy functional is minimized by the Euler–Lagrange equation, in which the contour C is evolving based on the partial derivative of the functional. Shape priors and curve evolution are implemented using the level set scheme in [14].

III. Results

A. Tissue-Mimicking Phantom Validation

The experiment procedure for imaging the tissue-mimicking phantom is described here. First, the tissue-mimicking solution without glass beads was injected into the hole, and B-mode images were acquired as shown in Fig. 3(a). Because of the low echogenicity of the solution, the hole boundary was sufficiently apparent to be used as the reference boundary to verify the automatic segmentation result. Computed from the B-mode image, the size of the hole is 4.308 mm². Next, the tissue-mimicking solution with glass beads was injected into the hole to imitate random blood flows inside the heart chambers. The solution had similar echogenicity with the phantom. Therefore, the hole boundary was indistinct on the B-mode image, as shown in Fig. 3(b). Then, two frames were acquired as the input of our algorithm. Fig. 3(c) shows the computed decorrelation layer. The hole is obvious, with higher intensity. Fig. 3(d) displays the segmentation result superposed on the reference image. The contour was obtained by running the local region-based active contour on the decorrelation layer. It almost matched the reference boundary. The hole area estimated from the automatic segmentation was 4.304 mm². The testing was repeated 50 times and the error of area estimation was 2.1% $\pm$ 1.8% compared with the ground truth.

B. In Vivo Zebrafish Experiments

First, we demonstrate the merits of our segmentation method with the image shown in Fig. 1. To illustrate the necessity of using the decorrelation layer for segmentation, we selected data from three different regions, as shown in Fig. 4(a), and plotted their distribution in different feature spaces. Fig. 4(b) shows the data distribution in the 2-D space of the B-mode intensity and the MCC value. The data from the heart (Region 1) is nearly Gaussian distributed with a single mean, whereas the data from the background (Region 2 and Region 3) has multiple modes, which violates the homogeneity assumption of region-based methods. Correspondingly, Fig. 4(c) shows the intensity distribution on the decorrelation layer. The data from the heart region and the background roughly belong to two separate Gaussian distributions, which provides a proper feature for region-based segmentation.

Next, we give a qualitative evaluation of our method on the image shown in Fig. 1. Figs. 5(a) and 5(b) show the decorrelation layer and the segmentation obtained by our method, respectively. The algorithm-generated contour coincided well with the manual delineation. For comparison, we also tested two related methods. The first alternative for comparison

differed from our method only in that it used global statistics to segment the image. The result in Fig. 5(c) shows that the bottom boundary of the heart was mislocated in global-region based segmentation. This was because the image intensity inside the heart was not uniform because of signal attenuation, which violated the region-homogeneity assumption. The second method for comparison applied the same segmentation scheme with our method but on the vector image, which consisted of the B-mode intensity and the MCC value. The external force for the contour evolution was the weighted sum of local region-based forces derived from two layers of the vector image. It is a commonly used strategy to integrate multiple features [13], [20]. The result in Fig. 5(d) shows that the contour was misled by the high contrast between the bright and dark regions inside the background.

Quantitative indices could be derived from the segmentation results to better understand the heart regeneration mechanism. Fig. 6 plots the change of the estimated heart area along the sequence of 150 frames. Periodicity is obvious, which shows heart cycles. The estimation had relatively large variation around end-systole because the epicardium was shaded by other tissue when the heart contracted to minimum. The mean value of end-diastole area (EDA) was $2.98 \pm 0.10 \text{ mm}^2$ and the mean value of end-systole area (ESA) was $2.10 \pm 0.12 \text{ mm}^2$, which gave an EF around 0.30. Because only 2-D images were available, the EF was approximated by $(\text{EDA} - \text{ESA})/\text{EDA}$. The heart rate estimated from the curve was about 125 beats per minute.

Finally, we give a quantitative evaluation of the proposed algorithm on 4 UBM sequences. For each sequence, 8 frames were selected for evaluation, and manual outlines were given by 3 different experts for each frame. Fig. 7 displays the results on a pair of ED and ES frames for each sequence, where the manual outlines were averaged to be a single outline. Three metrics are used to measure the difference between two contours C_1 and C_2 : the mean absolute distance d_M , the Hausdorff distance d_H , and the relative non-overlapping area d_A ,

$$d_M(C_1, C_2) = \frac{\int_0^1 d(C_1(s), C_2) |C_1'(s)| ds}{2|C_1|} + \frac{\int_0^1 d(C_2(s), C_1) |C_2'(s)| ds}{2|C_2|}, \quad (9)$$

$$d_H(C_1, C_2) = \max \left\{ \sup_s d(C_1(s), C_2), \sup_s d(C_2(s), C_1) \right\}, \quad (10)$$

$$d_A(C_1, C_2) = 1 - \frac{|\Omega_{C_1} \cap \Omega_{C_2}|}{|\Omega_{C_1} \cup \Omega_{C_2}|}. \quad (11)$$

Here, $d(p, C)$ is the minimum distance from point p to contour C , $|C|$ represents the contour length, Ω_C denotes the region inside C , and $|\Omega_C|$ means the area of the region. The results are summarized in Table I, where we can see that the distance from the algorithm-generated contour to the manual contours was comparable to the intra-observer variability among the manual contours.

We also evaluated the algorithm in terms of the estimated heart area. The comparison between the areas given by the algorithm and the manual outline is shown in Fig. 8(a) using the Bland–Atman plots. It shows that the heart area was a little underestimated by the algorithm compared with the manual results. The underestimation was caused by the shrinkage effect of the smoothness regularization on the active contour, especially when the object boundary was not sharp. The correlation between the automatic results and the manual results is presented in Fig. 8(b). The manual results were averaged over three

experts. The Pearson's correlation coefficient was 0.99, which shows that the automatic algorithm would give an accurate estimate of the dynamic change of the heart size.

IV. Discussion

In this paper, we developed an image segmentation algorithm to precisely acquire the dynamic size of the zebrafish heart from UBM images. Compared with manual segmentation, the proposed method achieved comparable accuracy. The algorithm was implemented with Matlab (The MathWorks Inc., Natick, MA) and tested on a Windows desktop (Microsoft Corp., Redmond, WA) with an Intel i5 CPU (Intel Corp., Santa Clara, CA) and 4 GB of RAM. The time required to process each frame was about 1.5 s, whereas that for manual segmentation was more than 1 min. We demonstrated that the decorrelation layer provided a desirable feature for segmenting tissue with high echogenicity and irregular motion. One point worth mentioning is that segmentation solely based on the MCC is not feasible. The MCC in low-echogenicity regions is usually as small as that of the heart region because of the existence of noise. In some previous works that used the MCC for segmentation, the MCC was usually integrated with other parameters to form a vector image. Then, the deformable model was evolved according to the weighted sum of external forces derived from different layers of the vector image [13]. The problem with applying this approach to the problem considered here is that the multivariate distribution of the data in the background usually has multiple means, as shown in Fig. 4(b), which may cause an erroneous result in region-based segmentation. Also, determination of the weights between different features is a difficult problem. To the best of our knowledge, the method presented in this paper was the first effort toward the quantification of zebrafish cardiac parameters using ultrasound images. It provides an automatic tool for us to process large-scale data sets composed of thousands of zebrafish cardiac images acquired at different stages of the heart regeneration process. At the current stage, the parameters were derived from 2-D images. In the future, we would like to extend our method to obtain volumetric parameters from 3-D volumes reconstructed from 2-D slices.

Acknowledgments

The authors thank Mr. F. K. Tsang for experimental validation, and Mr. C. W. Cheung for manual delineation.

The authors gratefully acknowledge financial support from Hong Kong RGC/GRF (PolyU5301/09E), the Proof of Concept Fund (PCF 04. 09/10) from the Hong Kong University of Science and Technology, the Innovation and Technology Fund (ITS 180/11) from the Hong Kong Innovation and Technology Commission, and the National Institutes of Health (1R01HL079976).

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Fig. 1.

An exemplar B-mode ultrasound bio-microscope image of a zebrafish. The heart is roughly inside the ellipse marked A. B is the gill. C is the bony structure of the fish's mouth, which occludes part of the heart in the image. D is the stomach. E is the liver. F is the intestine.

Fig. 2.
The diagrams of the experiment to acquire ultrasound bio-microscope images of (a) the tissue-mimicking phantom of the zebrafish heart and (b) the *in vivo* zebrafish heart.

Fig. 3.

The phantom experiment. (a) The reference image with low-echogenicity solution. The hole boundary is clear and sharp. (b) The ultrasound image with tissue-mimicking solution. The hole is indistinct. (c) The decorrelation layer. (d) The segmentation result superimposed on the reference image.

Fig. 4.

Data distribution in the feature space. (a) Region selection. Region 1 is inside the heart. Regions 2 and 3 are background regions with different echogenicity. (b) Data distribution in the feature space (maximum correlation coefficient versus B-mode intensity). Data from Regions 2 and 3 have different modes. (c) Histogram of the decorrelation layer. Red bins with the solid fitting curve denote data from Region 1. Blue bins with the dashed fitting curve denote data from Region 2 and 3. The distribution is roughly the mixtures of two Gaussians.

Fig. 5.

Comparison of alternative methods. (a) The computed decorrelation layer. (b)–(d) show results from three related methods. The yellow solid curve is the result of automatic segmentation. The cyan dashed curve is the manual delineation. Contours are plotted on top of the original B-mode image. Results are obtained from: (b) the proposed method, (c) global region-based segmentation on the decorrelation layer, and (d) local region-based segmentation on the vector image, in which the external force is the sum of region-based forces from B-mode intensity and maximum correlation coefficient.

Fig. 6.
The change of the heart area over time, estimated by our algorithm.

Fig. 7.

In vivo zebrafish heart segmentation results on four ultrasound bio-microscope image sequences. Each column shows an end-diastole image and an end-systole image selected from a sequence. The yellow solid curve is the result of the proposed algorithm. The cyan dashed curve is the averaged outline given by three experts.

(a)

(b)

Fig. 8.

Comparison between the heart areas estimated by the algorithm and by the manual delineation. (a) Bland–Altman plot (algorithm versus manual). (b) Regression plot (algorithm vs. manual).

TABLE I
Quantitative Comparison Between Contours Generated by the Algorithm and the Manual Outline.

	Sequence 1			Sequence 2			Sequence 3			Sequence 4		
	d_M	d_H	d_A	d_M	d_H	d_A	d_M	d_H	d_A	d_M	d_H	d_A
Inter-	0.040	0.136	0.111	0.051	0.180	0.104	0.047	0.139	0.114	0.044	0.142	0.098
Intra-	0.041	0.133	0.114	0.056	0.195	0.113	0.048	0.144	0.121	0.053	0.163	0.124

The difference is measured by the mean absolute distance d_M (in millimeters), the Hausdorff distance d_H (in millimeters), and the relative nonoverlapping area d_A . Inter- is the mean difference between the algorithm contour and the manual outlines; Intra- is the mean difference between the manual outlines given by different experts. For each sequence, 8 frames were selected for comparison.