

CARDIAC TISSUE AND ERYTHROCYTE SEPARATION IN BRIGHT-FIELD MICROSCOPY IMAGES OF THE EMBRYONIC ZEBRAFISH HEART FOR MOTION ESTIMATION

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ABSTRACT

Bright-field (BF) microscopy enables imaging the beating embryonic zebrafish heart at high frame rates, thereby revealing motion of both tissues that form the heart and red blood cells (RBCs). However, single-channel BF images lack the specificity seen in multi-color fluorescence microscopy since all structures in the field of view contribute similarly to image contrast. We discuss an algorithm that overcomes this limitation by separating a BF sequence of the beating heart into two distinct image sequences: one showing only the heart and surrounding tissues and the other showing only the transient structures such as RBCs. These sequences can be analyzed separately to characterize heart wall and RBCs motion using common optical flow techniques (e.g. Lucas-Kanade method). We validate our technique on a synthetically generated image sequence and show its potential for facilitating quantitative characterization of heart function during cardiac morphogenesis by examining experimental BF images of the beating embryonic zebrafish heart.

Index Terms— bright-field microscopy, segmentation, cardiac imaging, morphogenesis, optical flow

1. INTRODUCTION

Heart development in the vertebrate embryo is a highly dynamic process since the heart is beating long before it has reached its final shape. Quantifying flow and tissue deformation over the course of the cardiac development is central to reaching a better understanding of the interplay between genetic and epigenetic factors that contribute to morphogenesis [1]. The motions of heart-wall and erythrocytes (or RBCs) in the embryonic heart are fast (several millimeters per second) and need high frame-rates (typically, hundreds to thousands of frames per second) to be imaged without motion artifacts like aliasing and blurring [2].

Two widely used live imaging methods are fluorescence microscopy and bright-field microscopy. In fluorescence microscopy, the different structures of the specimen are labeled with different fluorophores that exhibit fluorescence when illuminated with light of specific wavelength. Multi-color

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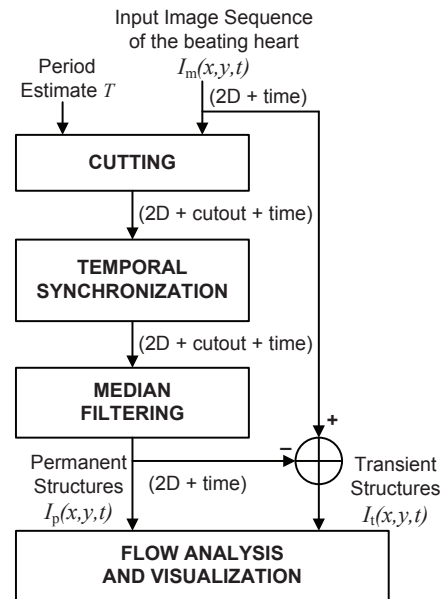


Fig. 1. Top-level block diagram illustrating the various stages of the separation algorithm.

images of the specimen can be created by assembling different fluorescence images, thereby providing a high degree of specificity. However, the equipment is costly and sample preparation can be complex. Also, the achievable frame rates with current fluorescence microscopy techniques are not sufficient for thorough flow analysis.

In contrast, bright-field (BF) microscopy can yield high frame-rates and requires only a basic setup with minimal sample preparation. However, BF images lack specificity since all structures in the field of view contribute similarly to the image contrast.

For the flow analysis to be effective on BF microscopy images, the individual contributions to the image contrast from the heart tissue and the RBCs should be separated. Cardiac segmentation techniques for magnetic resonance imaging (MRI) [3] and ultrasound imaging [4] have been widely studied. These macroscopic modalities permit analyzing the heart-wall motion. However, they cannot resolve the individual cells in the blood stream like BF microscopy. Techniques for BF image segmentation have been studied [5, 6], but they have had varied success with handling overlapping features.

Also, in the absence of specific labeling or a priori input from a specialist, generic segmentation methods would yield poor results.

In this work, we take advantage of the periodicity of the beating heart-wall, specifically the similarity across images taken at equivalent time points in the cardiac cycle to develop a novel and effective image separation algorithm. The algorithm separates the bright-field microscopy image sequence of the beating embryonic heart into two image sequences. One sequence shows only the heart and surrounding tissues without the RBCs, while the other sequence shows only the transient structures of the blood, mainly the RBCs. The two sequences can then be analyzed to characterize motion of the blood and heart-wall separately using common optical flow techniques such as the Lucas-Kanade method [7].

This paper is organized as follows. The imaging model and the separation algorithm are presented in Section 2. A validation experiment on synthetic heartbeat data and an application on experimental data acquired on a beating embryonic zebrafish heart are discussed in Section 3. We conclude in Section 4 with a summary of the separation algorithm's main features and discuss possible extensions and applications.

2. THE SEPARATION ALGORITHM

The input BF image sequence is composed of several periods (cardiac cycles) of the beating heart. It shows the flow of RBCs, the beating heart wall and other static structures in the background. The motion of the beating heart wall is periodic, while the flow of RBCs in the blood is not. This fact can be used to separate the two in the BF image sequence using the procedure described below.

We consider that the measured images $I_m(x, y, t)$ can be modeled as

$$I_m(x, y, t) = I_t(x, y, t) + I_p(x, y, t) \quad (1)$$

with $I_t(x, y, t)$ the intensity contribution from the transient structures (such as RBCs) and $I_p(x, y, t)$ the contribution from the permanent structures (such as the heart-wall). Furthermore, we assume that the permanent structures contribute to the image contrast in a periodic fashion, that is,

$$I_p(x, y, t) \approx I_p(x, y, t + T) \quad (2)$$

where T is the period of the heart beat (or cardiac cycle).

Also, the transient structures are confined to a domain \mathcal{D}_t that is

$$I_t(x, y, t) = 0 \quad \text{for } x, y \notin \mathcal{D}_t \quad (3)$$

where the shape of the domain changes with time in a periodic manner and with the same period T as above. The latter condition corresponds to the fact that RBCs are confined to the interior of the heart (whose shape changes periodically, thereby defining the permissible domain \mathcal{D}_t for transient structures) yet the exact location of RBCs varies from heartbeat to heartbeat.

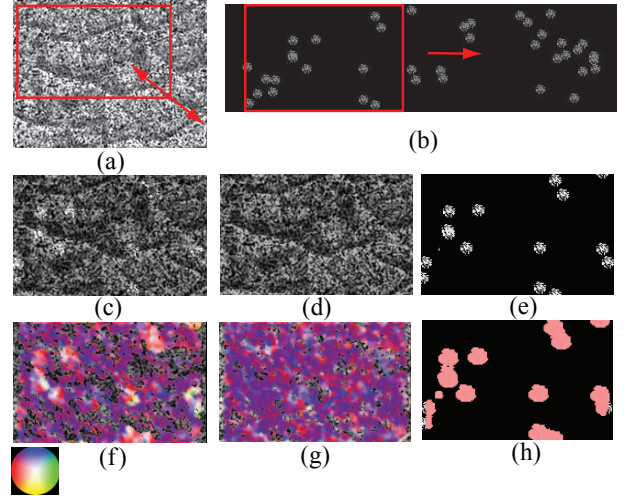


Fig. 2. Separation algorithm permits flow analysis of multiple overlapping structures without cross talk. (a) Synthetic heart-wall generation. (b) Synthetic RBC generation. (c) Frame from the synthetic heartbeat sequence. (d) Frame from the separated heart-wall sequence. (e) Frame from the separated RBC sequence. (f) Flow visualization on synthetic heartbeat sequence shows cross contamination in regions of overlap. (g) Flow visualization on separated heart-wall sequence shows features correctly tracked as moving diagonally upwards. (h) Flow visualization on separated RBC sequence shows RBCs moving from right to left. Dynamic color index [8] for flow in (f,g,h) is shown. The hue indicates the flow direction with saturation corresponding to higher velocities (2 pixels/frame)

Our algorithm aims at estimating the image contribution from transient features as $\tilde{I}_t(x, y, t)$ and periodic features as $\tilde{I}_p(x, y, t)$ given the measured $I_m(x, y, t)$. We measure I_m over N periods, that is $0 \leq t < NT$. Based on an estimate of the period T , we cut the measured sequence into R shorter sequences $I_c(x, y, r, t)$ such that

$$I_c(x, y, r, t) = I_m(x, y, \sigma r T + t), \quad 0 \leq t < 2T \quad (4)$$

with $\sigma > 1$ the step size factor of the beginning position of the cut sequences, and $r = 1, \dots, R$ the cut index, where R is such that $(\sigma R + 2)T < NT$.

Next, the cut sequences are temporally synchronized to the first cut sequence, that is, we find the shifts s_r such that

$$s_r = \arg \min_s \int \int \int_0^T |I_c(x, y, 1, t) - I_c(x, y, r, t + s)| dt dx dy, \quad 0 \leq s < T. \quad (5)$$

By construction, each cut sequence $I_c(x, y, r, t)$ has length $2T$ along the t dimension to ensure that it will match the sequence $I_c(x, y, 1, t)$ over the entire support $0 \leq t < T$ when $\sigma > 1$ is arbitrary and only a coarse (over-) estimate of T is available.

The synchronization (5) is carried out using an algorithm previously described [9]. Specifically, the minimization criterion is expressed in terms of low resolution subset of the individual images' spatial wavelet transform coefficients [10].

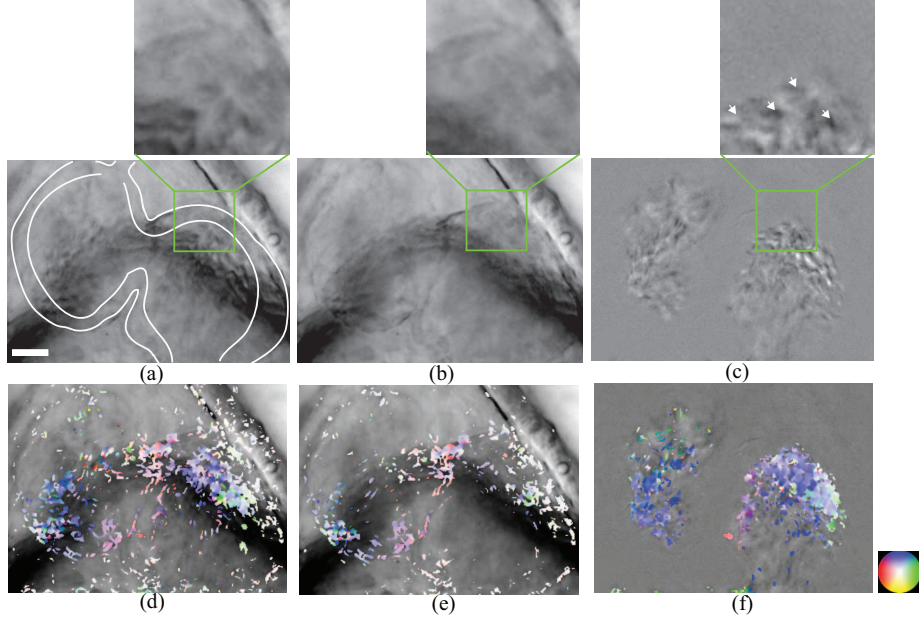


Fig. 3. (a) Frame from a BF microscopy image sequence of the beating embryonic zebrafish heart. The tissues making up the heart-wall have been marked. Scale bar is $25\mu\text{m}$ (b) Frame from the separated heart-wall sequence. (c) Frame from the separated RBC sequence. Some of the RBCs have been marked in the inset. (d) Flow visualization on BF microscopy image sequence. (e) Flow visualization on separated heart-wall sequence. (f) Flow visualization on separated RBC sequence. Dynamic color index [8] for flow in (d,e,f) is shown with saturated hues indicating flows with magnitude $\geq 254\mu\text{m/s}$. BF images courtesy of Julien Vermot and David Wu, Caltech.

Only the overall presence of RBCs (confined to a domain whose shape-change is periodic) is captured in the low resolution coefficients. Hence, proper registration is achieved, despite the fact that their intensity contribution to the overall signal is not periodic when examined at high resolution.

Finally, we estimate the contribution to I_m by permanent structures as

$$\tilde{I}_p(x, y, t) = \text{Median}\{I_c(x, y, r, t + s_r), 1 \leq r \leq R\}, \quad (6)$$

$$0 \leq t < T$$

and the contribution by transient structures, in the first measured period as

$$\tilde{I}_t(x, y, t) = I_m(x, y, t) - \tilde{I}_p(x, y, t), \quad 0 \leq t < T. \quad (7)$$

In practice, the heartbeat is not perfectly periodic and we use a non-uniform elastic registration procedure [9] to carry out the alignment. We set $\sigma = 1.5$ to avoid having repeated entries in I_c along the r dimension. In the aligned sequence, permanent structures such as heart tissues are present at the same position in every sequence, while transient structures such as red blood cells are not. This criterion is used to separate the heart-wall and the RBC image sequences using Eq. (6) and (7). No prior knowledge of the shape or motion of the RBCs is needed for the separation. The final stage of the algorithm involves optical flow analysis and visualization of the separated sequences. Fig. 1 illustrates the various stages of the separation algorithm.

3. EXPERIMENTS AND RESULTS

The separation algorithm was implemented in MATLAB. The flow analysis stage was accomplished using the FlowJ plugin for ImageJ [11]. In particular, the Lucas-Kanade algorithm [7] was used and the parameters were set according to [8]. We first validated our separation procedure on a synthetically generated data set. The procedure was then applied to BF microscopy data of beating zebrafish heart.

The synthetic data was generated as follows. A cropping window was placed on a textured background image (Fig. 2(a)) and moved diagonally down (respectively diagonally up) at a rate of $\sqrt{2}$ pixel/frame for the first (respectively the second) half of the chosen heart period. To generate frames of the synthetic RBC sequence, circular speckled particles were randomly placed on a large empty canvas and a cropping window was moved from left to right at a uniform rate of 1 pixel/frame (Fig. 2(b)). The two sequences were superimposed (added) frame-by-frame to obtain the synthetic heartbeat sequence. This sequence was processed by the separation algorithm. The algorithm was effective in separating the synthetic heart-wall (SNR = 28.55 dB) and RBCs (SNR = 21.36 dB). The signal-to-noise ratio was computed as

$$\text{SNR} = 10 \log \frac{\sum_{(x,y,t) \in \mathbb{Z}^3} I_k^2(x, y, t)}{\sum_{(x,y,t) \in \mathbb{Z}^3} (I_k(x, y, t) - \tilde{I}_k(x, y, t))^2}, \quad (8)$$

with $k = p$ and t , respectively. By comparing Fig. 2(g),(h) with 2(f) we conclude that precise and cross-contamination free flow analysis is possible on the separated sequences, despite both structures having motion components along the same direction. Further characterization of our algorithm will include quantitative evaluation of the algorithm (based on synthetic data) as a function of the number of periods R and the RBC density.

BF images comprising of several periods of a 48 hours post fertilization (hpf) beating embryonic zebrafish heart were acquired sequentially at a fixed focal plane with a Basler A602f CMOS camera running in area-of-interest mode (resolution of 305×253 pixels) for fast acquisition (216 frames per second). No external gating signals were used during the acquisition. The camera was mounted on a home built microscope equipped with an Olympus UPlanAPO water immersion objective ($60\times/1.2$) coupled with a tube lens of focal length 300 mm.

Fig. 3(b),(c) show the result of the separation algorithm on BF microscopy images. Fig. 3(e),(f) show the results of flow visualization in dynamic color (DC) format [8] on the separated image sequences. Unlike in Fig. 3(d), most of the flow in Fig. 3(e) is observed in the regions where there is movement of heart-tissues and in Fig. 3(f) flow is observed only in the regions showing movement of RBCs. Again, the algorithm permits separate analysis of RBC and heart-wall motion. The optical flow visualization for the separated sequences is easier to analyze than that for the input BF image sequence (Fig. 3(d)).

4. CONCLUSION

The algorithm presented provides a technique for separation of heart-wall and RBC images from a bright-field microscopy image sequence of the beating embryonic zebrafish heart. This technique can help in overcoming the lack of specificity in BF microscopy images. The image sequences of the separated heart-wall and RBCs generated by this technique can be subjected to flow analysis individually, thereby enabling better characterization of blood flow and heart-wall motion. This will aid the study of interactions between tissue development and blood flow and help reach a better understanding of cardiac morphogenesis. The technique is currently limited by the frame rate that was used to capture the BF images. We plan to overcome this limitation by using a camera that permits imaging at higher frame rates.

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