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Speckle variance optical coherence tomography of blood flow in the beating mouse embryonic heart

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Abstract

Efficient separation of blood and cardiac wall in the beating embryonic heart is essential and critical for experiment-based computational modelling and analysis of early-stage cardiac biomechanics. Although speckle variance optical coherence tomography (SV-OCT) relying on calculation of intensity variance over consecutively acquired frames is a powerful approach for segmentation of fluid flow from static tissue, application of this method in the beating embryonic heart remains challenging because moving structures generate SV signal indistinguishable from the blood. Here, we demonstrate a modified four-dimensional SV-OCT approach that effectively separates the blood flow from the dynamic heart wall in the beating mouse embryonic heart. The method takes advantage of the periodic motion of the cardiac wall and is based on calculation of the SV signal over the frames corresponding to the same phase of the heartbeat cycle. Through comparison with Doppler OCT imaging, we validate this speckle-based approach and show advantages in its insensitiveness to the flow direction and velocity as well as reduced influence from the heart wall movement. This approach has a potential in variety of applications relying on visualization and segmentation of blood flow in periodically moving structures, such as mechanical simulation studies and finite element modelling.

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Four-dimensional speckle variance OCT imaging shows the blood flow inside the beating heart of an E8.5 mouse embryo.

Graphical Abstract

Robust and automatic separation of the cardiac wall tissue from blood flow is essential for experiment-based computational modeling of early-stage cardiac mechanics. However, blood flow imaging in a beating embryonic heart, a highly dynamic tissue environment was previously not achieved. Here we demonstrate a four-dimensional speckle variance optical coherence tomography method to provide blood contrast from the cardiac wall tissue in the live mouse embryo, indicating high-quality structural separation to reveal the dynamic heart morphology.

Keywords

optical coherence tomography; speckle; mouse embryo; live imaging; heart; blood flow; four-dimensional imaging

1. Introduction

Mechanical simulation analysis of the cardiac dynamics in the embryonic heart can provide valuable information that might not be available from experimental studies [1].

Computational modelling with finite element method has recently provided important insights into biomechanics of the early cardiac development, including the looping [2–4] and the hemodynamics [5–7] of the heart. Such computational analysis essentially requires experimental data on the morphological and structural details of the embryonic heart to simulate the dynamics and assess the mechanical parameters [2, 5]. Thus, three-dimensional (3D) high-resolution imaging and efficient separation of the heart wall motion from the blood flow are critical for developing accurate numerical models to describe and predict the mechanical behaviour of the embryonic heart.

Optical coherence tomography (OCT), a noninvasive 3D imaging modality, has been established as a powerful live imaging tool for cardiovascular embryology [8–10]. The micro-scale spatial resolution and millimetre-level field of view of OCT enabled a number of significant applications contributing to an improved understanding of the early cardiovascular development [11–14]. Recent progress in OCT imaging of embryonic heart provided the direct volumetric structural and functional imaging of the beating mouse embryonic heart [15], the dynamic and quantitative analysis of genetically induced cardiac mutations in early development [16, 17], as well as the morphological and hemodynamic characterization of the embryonic heart phenotypes from chemical treatments [18] and physical manipulations [6, 19]. The continuous technical advancement of OCT has enhanced its unique role in the research of congenital heart defects [20].

Speckle variance OCT (SV-OCT) relies on the higher variation of speckles from blood flow in comparison to surrounding static tissue to form contrast for functional blood perfusion

imaging [21]. SV-OCT has been applied for embryonic microvascular imaging in the chick [22] and mouse [23] models. Recently, based on the speckles from OCT imaging, Kulkarni et al. developed a robust and scalable algorithm for an improved reconstruction of the yolk sac vasculature in the mouse embryo, providing superior contrast and imaging quality than the traditional SV methods [24]. In spite of the efforts in continuously refining SV algorithms [25–27], SV-OCT of blood flow in the beating embryonic heart, a highly dynamic tissue environment, remains challenging and not achieved. This significantly limits the efficient and automatic structural separation between heart wall and blood, thus preventing experiment-based simulation studies of the biomechanics in an early heart.

To address this demand, here we demonstrate a four-dimensional (4D) dynamic SV-OCT approach for blood flow imaging in the beating heart of live mouse embryo. The method relies on the periodic cardiac motion where for every phase of the heartbeat the heart wall returns back to the very similar spatial location while the blood cells do not. Therefore, a relatively much higher speckle variation is created from the blood flow compared to the dynamic cardiac wall tissues, providing the contrast for imaging. A number of OCT image processing techniques were previously developed based on the periodic feature of the embryonic heart wall movement, including the 4D cardiodynamic synchronization [28–31], noise reduction [32], and image mosaicing [33]. These suggest that with a sufficient temporal resolution, it is possible to assume that the cyclic activity of the embryonic heart produces a high-enough identity of the 3D spatial location of heart wall at the same phase of each heartbeat, allowing for the separation from the blood flow based on the variance of OCT speckles.

In this paper, we first describe in detail the method and then present the 4D SV-OCT imaging of the cardiac blood flow in the mouse embryo at embryonic day 8.5 (E8.5). Then, we validate SV-OCT imaging results with the Doppler OCT imaging from the same dataset. Finally, based on a comparative analysis with Doppler OCT, we show the proposed SV-OCT approach has advantages of insensitivity to blood flow direction and velocity as well as smaller influences from the cardiac wall motion. These features of the presented speckle-based method are important for separation of the dynamic heart wall in the live embryo, which could improve further computational modelling and analysis of early-stage embryonic heart.

2. Materials and Methods

2.1 Mouse embryo

Mouse embryos at E8.5 were used in this study. The vaginal plugs from the females in the timed mating cage were monitored and the day of seeing a plug is counted as E0.5. Mouse embryos at E8.5 were dissected live in the dissection medium that contained 89% DMEM/F12, 1% penicillin-streptomycin and 10% fetal bovine serum. Details of live mouse embryo culture can be found in our previously established protocols [34]. Upon dissection, the embryos within the intact yolk sac were placed in a humidified incubator (37°C and 5% CO₂) for recovery before imaging experiments. The decidua of the embryo was carefully shaped during dissection to orient the embryo in the culture dish with the cardiac region facing up towards the imaging beam.

All the animal manipulation procedures in this work were approved by the Institutional Animal Care and Use Committee at Baylor College of Medicine. Experiments were conducted based on the approved guidelines.

2.2 OCT system

A spectral-domain OCT system with a stabilized phase from low coherence interferometry was employed for the live imaging of mouse embryos. This home-built OCT system was previously described in detail [35]. With the laser source that has a ~808 nm central wavelength and a ~110 nm bandwidth, the OCT system has a measured axial resolution of ~5 μm in tissue (assuming refractive index of 1.4). A fiber-based Michelson interferometer is used for the light interference and a line-field CMOS camera is utilized for the spectrometer to resolve the interference fringes. The A-line rate of the system can reach up to ~68 kHz with the full 4096 pixels from the camera. The available imaging depth of the system covers the entire heart region of the E8.5 mouse embryo. A transverse resolution of ~4 μm ensures the details of cardiac structure to be well visualized. The OCT system can reach a sensitivity of ~97 dB (optical path length difference of 50 μm and exposure time of 18 μs) with a depthwise sensitivity drop of ~4 dB per millimetre. During imaging experiments, the sample arm of the OCT system was placed inside a humidified incubator where the temperature was maintained at 37°C with 5% CO₂. A B-scan rate of 100 Hz was set for dynamic imaging of the beating mouse embryonic heart.

2.3 SV-OCT method

Our proposed SV-OCT algorithm is based on the assumption that the cyclic behaviour of embryonic heart results in an identical location of the heart wall from the same phase of each heartbeat, which has been used as the basis for a number of previous algorithms for analysis in the embryonic heart [28–33]. The blood cells that do not return to the same spatial positions over cardiac cycles generate a higher variation of OCT speckles with respect to time, thus creating an imaging contrast. The specific details of implementing this principle in data acquisition and post-processing are described below and illustrated in Figure 1.

A 3D scanning scheme with densely sampled B-scan locations is conducted to acquire the dataset for the 4D SV-OCT imaging. Since the heart is beating during data acquisition, the recorded B-scans are not only spatially resolved, but also temporally resolved and cover multiple heartbeat cycles. The B-scan spatial sampling interval is designed to fulfil the requirement that the distance from one B-scan to its closest B-scan that has the same phase of cardiac cycle is within 2 μm (half of the transverse resolution of OCT system), which allows at least two B-scans to be considered as from the same spatial location and from the same phase of different heartbeats. Such B-scans are utilized later for SV calculation. For the results in this paper, the spatial distance of such B-scans is ~1 μm , enabling four frames for calculating the variance of speckles. Since a healthy mouse embryo at E8.5 generally has a heart rate of around 2 Hz, this particular requirement in 3D data acquisition needs a pre-recording estimation of the heart rate, which can be obtained with a real-time 2D imaging of the embryonic heart over time.

First, the acquired structural OCT dataset is split into individual heartbeat cycles and is synchronized to the same phase of the heartbeat using the established approach reported before [16, 36, 37]. This approach brings the B-scans for each phase of heartbeat into spatial 3D volume, where adjacent B-scans have the distance of $\sim 1 \mu\text{m}$. For example, if the scan is set to cover $600 \mu\text{m}$ and 30,000 frames are acquired at 100 frames per sec (600 A-lines per B-scan), with the heartrate of $\sim 2 \text{ Hz}$, ~ 600 heart beats are acquired over one volume, which would allow to assign them to ~ 600 spatial positions, $\sim 1 \mu\text{m}$ apart.

A nonlinear counter-harmonic filter (with an order of 5) is then applied to the images to reduce the Gaussian noise while preserve the edges of structures and increase SV contrast. At every time point of the 4D data, the SV signal of each B-scan of the 3D is calculated as [21]

$$SV_{jk} = \frac{1}{N} \sum_{i=1}^N \left[I_i(j, k) - \frac{1}{N} \sum_{i=1}^N I_i(j, k) \right]^2, \quad (1)$$

where j and k are the depthwise and transverse indices of pixels, I is the OCT intensity, and N in this study is 4, representing the number of adjacent frames used to for variation calculation. After obtaining all SV B-scans, a 3D median filter with the window size of three pixels in all spatial dimensions is applied to the time-resolved 3D SV data to reduce noise and smooth the image. Binary mask is then created based on the SV images with a threshold of 0.17 for the normalized SV signals of 0–1. The binary values are inversed and applied to the structural OCT data to have the heart wall and static structures separated out. Finally, the blood flow from the SV data and the cardiac wall with static tissues obtained through masking are fed into two separate channels in Imaris software (Bitplane) and overlapped for visualization purpose. The described data processing for SV signals is achieved using Matlab (MathWorks).

2.4 Doppler OCT

Doppler OCT imaging of blood flow in the embryonic heart and vasculature was employed for validation and comparative analysis of the dynamic SV-OCT approach. Doppler OCT was conducted based on windowed Kasai autocorrelation function [38]. The details of the Doppler OCT imaging of the cardiac blood flow in the mouse embryo were previously reported in [39] by our group and the same method was applied in this study. Doppler processing was performed from the same dataset where SV-OCT imaging was obtained. Thus, the Doppler result acts as a good reference to verify whether the contrast provided by the SV-OCT algorithm is from the blood flow and to evaluate the advantages of SV-OCT over the existing approach.

3. Results

The 4D SV-OCT imaging of the mouse embryonic heart at E8.5 is shown in Figure 2 and Media 1. As illustrated in Figure 2(A), the 3D imaging field of view covers the sinus venosus, primitive atrium and primitive ventricle of the heart, as well as the dorsal aorta.

Figure 2(B)–(D) show the structural OCT images from three selected time points. In Figure 2(E)–(G), the SV-OCT imaging of the blood flow and the structurally separated heart wall with static tissues are overlapped, which clearly shows the volumetric reconstruction of circulation and permanent structures. The amplitude of SV signal is presented with normalized values from 0 to 1. The bright signal inside the lumen of the heart and vasculature indicates a good contrast for the blood flow imaging. Also, the separation between the blood and the cardiac wall tissues can be clearly seen, suggesting the capability of this SV-OCT approach to produce high-quality time-resolved 3D morphological data for computational modelling and analysis of the biomechanics in early embryonic heart.

Verification of the SV-OCT imaging of blood flow is shown in Figure 3, where both Doppler and SV-OCT results from the same dataset are presented. The field of view covers the yolk sac blood vessel that feeds blood into the sinus venosus of the heart. Comparing Figure 3(B) and 3(C), it is clear that based on the Doppler data, the contrast provided by the SV-OCT method is indeed from the blood, which demonstrates that the presented SV-OCT approach is effective for blood flow imaging.

As a major approach for blood flow imaging in the mouse embryo, Doppler OCT is generally not capable to generate contrast when flow direction is perpendicular to the imaging beam. This is revealed in Figure 3(B) for the atrium part of the heart. For generating morphological data to serve the modelling purpose, this can potentially result in the issue that the blood and the heart wall are not well separated at the particular flow direction. We compared the SV-OCT method with Doppler OCT to determine whether the presented speckle-based approach has the advantage in this aspect. Figure 4 shows the selected images of SV-OCT and Doppler OCT from a 4D imaging of the yolk sac vasculature in the E8.5 mouse embryo. Since blood flow from parts of the vasculature appears perpendicular to the imaging beam, it can be seen from Figure 4(E) and 4(F) that certain region of the vasculature does not show sufficient contrast for blood flow in comparison with the SV-OCT imaging in Figure 4(B) and 4(C). This suggests that the SV-OCT method is insensitive to the blood flow direction relative to the imaging beam. Also, as shown in Figure 4(D), when the blood flow velocity gets close to zero at the particular phase of heartbeat cycle, Doppler OCT is unable to pick up the region of blood. However, because the SV-OCT approach relies on the inter-cycle variation of the speckles, even though the blood flow is transiently stopped, the imaging contrast from the blood region can still be obtained with high quality, as shown in Figure 4(A). A 4D visualization of the comparison is presented in Media 2. These results indicate that the SV-OCT method is capable of blood flow imaging from more comprehensive temporal and spatial positions over the 4D embryonic cardiodynamics, suggesting it could be a robust approach for blood separation from the heart wall and static tissue structures.

A further comparative analysis of the SV-OCT with Doppler OCT focuses on the influence from the cardiac wall motion, as shown in Figure 5. From Figure 5(B) and 5(C), it can be seen that both SV and Doppler signals provide good contrast for the blood inside the primitive atrium and ventricle. Specifically, the SV signals well fill in the lumen of the atrium and ventricle, demonstrating a high-quality separation of the blood from the heart wall tissues. At this time point, the embryonic heart is under relaxation and the squared

myocardium regions in Figure 5(B) and 5(C) move up towards the imaging beam. This movement creates a positive Doppler frequency, shown as the red-colour signals from the heart wall in Figure 5(B). In comparison, since the SV-OCT method employs speckle calculation from the same phase of cardiac cycle, the heart wall is relatively immobile, producing less or no effects on the blood flow imaging, as shown in Figure 5(C). Such an advantage makes the presented SV-OCT approach more suitable for automatic separation between the blood and the cardiac wall. In addition, as pointed by the arrows in Figure 5(B) and 5(C), the transverse blood flow that could not be picked up by Doppler OCT can be imaged by SV-OCT with a good contrast, demonstrating again the insensitiveness of the SV-OCT method to the blood flow direction, which will be beneficial to a more robust separation of the cardiovascular system in the embryo.

4. Discussions and Conclusion

Although inter-volume analysis of OCT speckles for 4D blood flow imaging was previously developed [15, 40], such imaging has only been reported with static tissue environment [15, 40]. To the best of our knowledge, this study is the first demonstration for 4D speckle-based OCT blood flow imaging in dynamic tissue environment, the beating embryonic heart. Relying on periodic cardiac tissue movement, the SV-OCT method shows superior imaging contrast from blood flow, as verified by Doppler OCT imaging. In comparison with Doppler OCT, the SV approach indicates no effect from blood flow direction, consistent contrast with zero velocity of flow, and little influence from the heart wall motion, suggesting that the high-quality separation between cardiac wall and blood is possible for all phases of the heartbeat cycle and more comprehensive spatial locations of the embryonic heart. This is of significant value for automatic processing of morphological data to serve computational modelling and analysis for the biomechanical study of cardiogenesis.

As one of the limitations of the presented SV-OCT method, the approach relies on the heart wall returning to the identical spatial locations at the same phase of different heartbeats. From the technical aspect, this assumption requires a high enough temporal sampling rate to have the cardiac activity well resolved in time, which ensures the same movement of the heart wall tissue at the specific time points are repeatedly captured over cardiac cycles. In this study, we have a temporal resolution of 10 ms, demonstrating as sufficient to fulfil this requirement. An improved time resolving ability will help to further reduce the noise signal generated from the heart motion. From the biological aspect, such an assumption excludes the imaging and analysis of the arrhythmia models where the embryonic heart rate varies over cycles. In these cases, optical pacing with pulsed infrared laser [41] might potentially be useful to control and obtain a uniform heart rate from diseased embryos.

Since the SV is calculated based on sections acquired over different heart beats and slightly different spatial locations, the edges of the tissue structures also generate SV signal (can be seen in Figure 2 and Media 1). While this creates an artefact for blood flow segmentation, this might potentially be utilized for segmentation of surfaces for periodically moving structures, which would require further optimisation and exploration.

Previously we have utilized the OCT data acquisition strategy of sequential time lapses acquired at spatial locations with a small step to obtain 4D cardiodynamics through synchronization [16]. In contrast to the previous scanning protocol, here we utilized slightly different strategy, when densely sampled B-scans are continuously acquired within a 3D volume. While these two approaches are overall similar since there is the same total number of frames over the volume is acquired, there are advantages for the new method. It provides a flexibility in splitting the total data set into individual heartbeats assigning them to different positions. Besides, since the dataset is split into heartbeats precisely, no extra frames (which would be discarded after synchronization) are assigned to spatial locations. Therefore, it provides for more efficient utilization of acquired data.

For the goal of separation between embryonic heart wall and blood flow, the SV-OCT method demonstrates advantages over the Doppler OCT approach. However, velocity quantification from the presented speckle-based approach is challenging. Although the OCT speckle (or intensity) decorrelation was employed for flow velocity measurement [42–47], presented 4D SV-OCT approach utilizes frames with the time interval of one heartbeat cycle (~0.5 seconds), where the decorrelation of OCT speckle (or intensity) is complete, thus not allowing for velocity assessment. In order to have quantitative hemodynamic information, the Doppler OCT obtained from the same dataset can be conveniently employed to reveal velocity information of the blood flow. This can also provide a potential experimental verification of the biomechanical predictions from the computational model built based on morphological data.

Numerical analysis, such as the computational fluid dynamics, combined with 4D OCT imaging has recently been employed for study of the biomechanics in chick embryonic heart [5–7]. In comparison with the chick, the mouse is a well-established mammalian model with a number of genetic tools available for generating models of congenital heart disease [48]. The presented SV-OCT method allows for automatic separation of the dynamic heart wall from the blood, providing the opportunity for computational modelling and mechanical evaluation of the mouse embryonic heart at early development, which, as our future work, could contribute to an improved understanding of mammalian normal cardiogenesis and congenital heart defects.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1.

Illustration of 4D SV-OCT approach for blood flow imaging in the embryonic heart. Hearts of different colors represent different frames in the final reconstruction of SV in the 4D volume.

Figure 2.

4D SV-OCT imaging of the blood flow in the E8.5 mouse embryonic heart (Media 1). (A) Illustration and annotation of the cardiovascular structure of an E8.5 mouse embryo. (B–D) Selected 3D structural OCT images of the beating mouse embryonic heart at three time points of the 4D data show the cardiodynamics. (E–G) SV-OCT imaging of blood flow overlapped with the heart wall and static tissue imaging from masking shows good separation of the blood from the cardiac wall tissues. The “hot” scale for the SV-OCT data represents the normalized SV signals. (E–G) are from the same time points as (B–D).

Figure 3.

Verification of SV-OCT imaging of blood flow with Doppler OCT imaging. (A) Structural OCT, (B) Doppler OCT and (C) SV-OCT imaging of the same cardiovascular region where the blood from the yolk sac vasculature is fed into the sinus venosus of the E8.5 mouse embryonic heart. The comparison between (B) and (C) show the SV-OCT method is indeed picking up the blood flow and separating out the heart wall and static structures with a good contrast.

Figure 4.

Comparative analysis of SV-OCT with Doppler OCT for the sensitiveness of blood flow direction and velocity (Media 2). (A–C) SV-OCT and (D–F) Doppler OCT of the same region of yolk sac vasculature from the E8.5 mouse embryo show that the SV-OCT method is insensitive to velocity of blood flow and has sufficient contrast for the flow direction that is perpendicular to the imaging beam, indicating good separation of blood from the heart wall and static tissues at all the phases of heartbeat cycle.

Figure 5. Comparative analysis of SV-OCT with Doppler OCT for the sensitiveness of heart wall motion and blood flow direction. (A) Structural OCT, (B) Doppler OCT, and (C) SV-OCT imaging of the E8.5 mouse embryonic heart and yolk sac vessels show the SV-OCT imaging has reduced influence from the cardiac wall movement (yellow square) and is able to pick up the transverse flow that is perpendicular to the OCT beam (yellow arrows).