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In-vivo measurement of the blood flow in the heart of Medaka embryo

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Abstract

In order to examine the generation mechanism of the vertebrates, including humans, and to explore the diverging point of the genetic abnormalities, it is effective to investigate the growth of the embryo of an artificial mutant. As the embryos of Medaka or Zebrafish hatch outside of the body and their egg membrane are transparent, it is quite easy to carry out the in-vivo observation of their organs. Moreover, the development and the structure of their major organs are quite similar to those of human. Thus, the observation of the developmental abnormalities of Medaka embryos may serve as a clue to the pathologic investigation of the genetic disease and hopefully give some insight into the future effective treatment.

Presently, the intracardiac blood flows of Medaka embryos have been observed by a high-speed camera under microscope. The obtained images are processed and the velocity fields in the heart are measured by PIV. In this study, red blood cells flowing inside the heart of Medaka embryos are used as tracer particles, but the images of red blood cells are dark in the captured image, so PIV measurement cannot be performed with the images of the red blood cell as it is. Therefore, image pre-processing was applied to capture the image of red blood cells. First, the brightness of the image is inverted so that the red blood cell can been captured as the bright fragments as is the case for the tracer particles. Second, the brightness of the darkest instance has been collected to obtain the background brightness. Finally, by subtracting the background brightness, only the image of the red blood cell is highlighted and thus the cross-correlation method can be applied. The PIV results are super-imposed onto the original image of the Medaka embryo with the outline of the heart. As a result, the movie of the shape changes of the organs together with the velocity vectors of the red blood cell is prepared. According to the movie, it is found that the beating of the heart corresponds to the pulsation of the flow velocity of the red blood cell in the heart. Consequently, the in-vivo velocity in the heart of the Medaka embryo has been measured under microscope with a high-speed camera using PIV and the pre-processed images.

1 Introduction

In order to examine the generation mechanism of the vertebrates, including humans, and to explore the diverging point of the genetic abnormalities, it is effective to investigate the growth of the embryo of an artificial mutant. As the embryos of Medaka or Zebrafish hatch outside of the body and their egg membrane are transparent, it is quite easy to carry out the in-vivo observation of their organs. Moreover, the development and the structure of their major organs are quite similar to those of human (Orman et al. 2015). Moreover, the DNA information of Medaka has been already read and it is easy to cause the genetic abnormalities to the mutant. Thus, the observation of the

developmental abnormalities of Medaka embryos may serve as a clue to the pathologic investigation of the genetic disease and hopefully give some insight into the future effective treatment.

2 Visualization

Presently, the in-vivo intracardiac blood flows of Medaka embryos have been observed by a high-speed camera under microscope as shown in Fig. 1. The microscope used in this study is Leica Microsystems MZ16 and the high-speed camera is Photron FASTCAM Mini WX100, working frame rate is 125fps with the shutter speed of 1/1000 sec is selected and the resolution is 2048 x 2048.

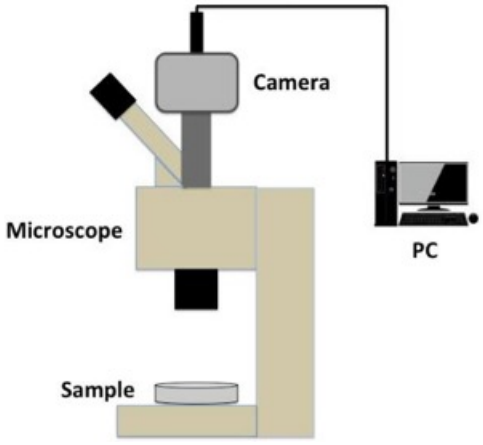


Figure 1: Experimental Setup

The obtained images are processed and the velocity vectors in the heart are measured by PIV (Sakakibara and Ninomiya 2018). In this study, red blood cells flowing inside the heart of Medaka embryos are used as tracer particles, but the images of red blood cells look dark in the captured image as shown in Fig. 2(a), so PIV measurement cannot be performed with the images of the red blood cell as it is. Therefore, image pre-processing has been applied to the image of Medaka embryo. First, the brightness of the image is inverted as shown in Fig. 2(b) so that the red blood cell can been captured as the bright fragments as is the case for the tracer particles. Second, the brightness of the darkest instance has been collected to obtain the background brightness as shown in Fig. 2(c). Finally, by subtracting the background brightness, only the image of the red blood cell is highlighted and thus the cross-correlation method can be applied.

(a) original image (b) inverted image (c) background image

Figure 2: Pre-processing to the image

3 PIV measurement

The typical result of the PIV in the vena cava is shown in Fig. 3. The color of the velocity vectors indicates the velocity magnitude from blue to red and the velocities magnitudes are shown by μm/s.

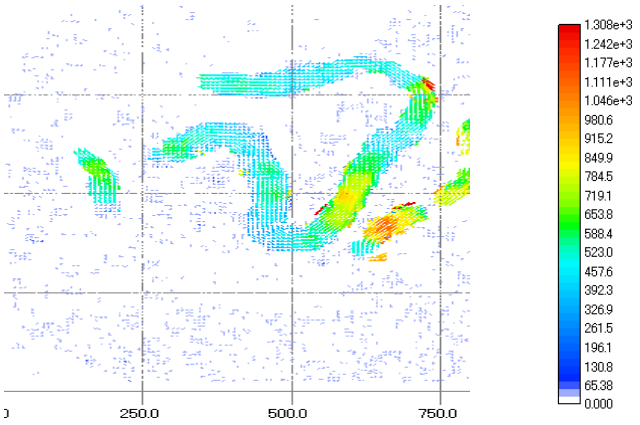
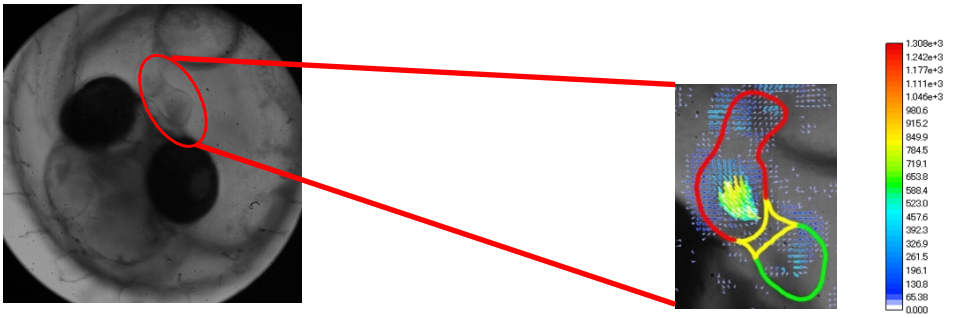


Figure 3: PIV results in the vena cava

The typical result of the PIV in the heart is shown in Figs. 4. The PIV results are superimposed onto the original image of the Medaka embryo together with the manually drawn outline of the heart. The outline of the atrium is drawn by red, the valve by yellow and the ventricle by green.



(a) Original image (b) PIV results

Figure 4: PIV results in the heart

As the embryo of Medaka is spherical and the organs inside of the embryo is located three-dimensionally, the embryo has been placed upon an agar culture medium so that a good focus can be obtained on the vena cava or heart as shown in Figs. 2. The size of the interrogation window is set as 32 x 32 and the search regions are set as 16 x 16 for the vena cava and 32 x 32 for heart, and the overlaps are 50% and 75% respectively. It can be seen that the velocity vectors in the vena cava or in the heart are clearly obtained by cross-correlation algorithm of PIV with the aid of pre-processing to the images obtained by the microscopy and the high-speed camera.

The typical size of the embryo is 1 mm and the typical width of the vena cava is about 50 μm and the typical velocity in the vena cava is about 700 μm/s. As the blood in the Medaka embryo is dilute, it may be feasible to assume the physical properties of the blood is almost same as those of normal saline solution. Thus, the Reynolds number is estimated as 0.035 and thus the flow is laminar.

Figure 5 shows the time variation of the velocities in the vena cava at different locations close to the inlet of the heart from A to E, where E is closest to the heart. The velocities at the center of the vena cava are phase-averaged by synchronizing the velocity changes by the velocity minimum in each heart beat and the 20 cycles are averaged to obtain the time variation of the velocity in the vena cava during an averaged cycle of 0.48 sec.

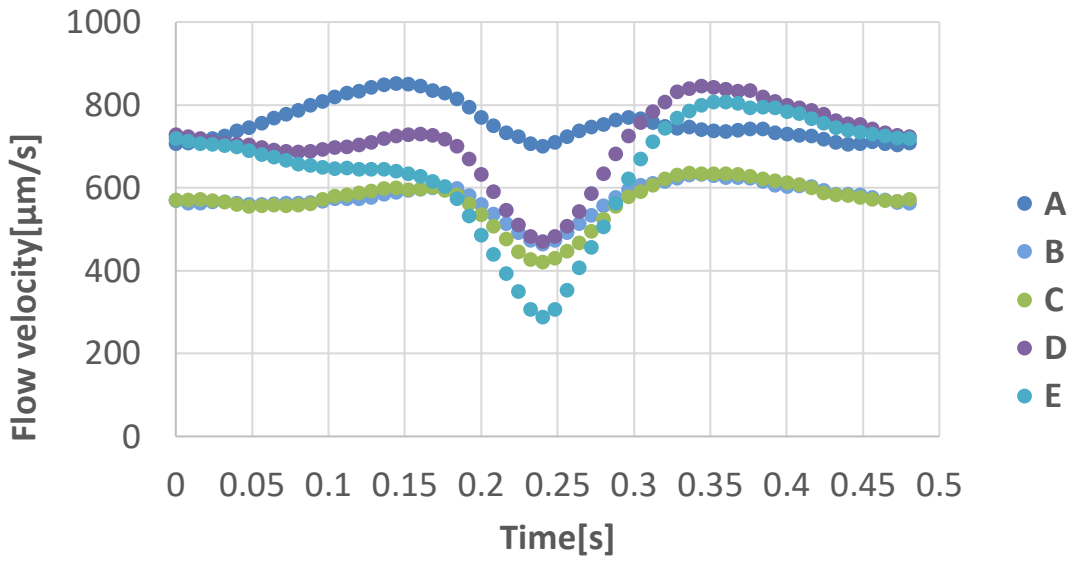
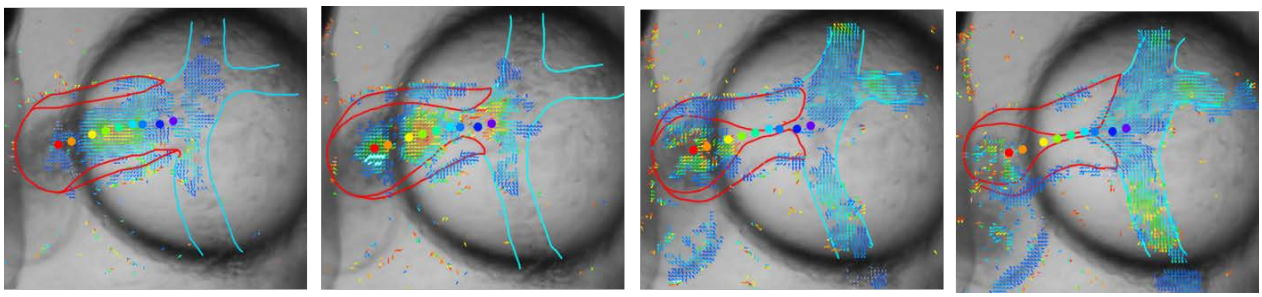


Figure 5: Time variation of the velocities in the vena cava

Due to the continuity constraints, the velocity decreases with the increase in the diameter of the vena cava. Moreover, negative pulses in the velocity are observed according to the heart beat. One interesting finding is that the pulse in the velocity does not have a time delay along the vena cava. Assuming that the blood is incompressible, it suggests that the change in the diameter of the vena cava is very small. Another interesting finding is that the magnitude of the velocity defects due to the heart beat decreases with the distance to the heart. This implies that the membrane of the vena cava has a compliance to reduce the velocity change.

Figures 6 show the PIV results in the atria and in the end point of the venae cavae. Three venae cavae merge into one at the inlet of atria. The outline of the atria is drawn by red and those of the vena cava by pale blue. The time variation of the velocity in the atria is also obtained as shown in Fig. 7. The phase average is obtained by synchronizing the sharp velocity decrease at the inlet of the atria, which is shown by purple dot in Fig. 6 and labeled by [A] in Fig. 7. One cycle of the embryo at this age was 0.60 sec. The typical age of the embryo used in this study is about 2 days after birth.



(a) 0.34s (b) 0.38s (c) 0.42s (d) 0.46s

Figure 6: PIV results in the atria of the heart

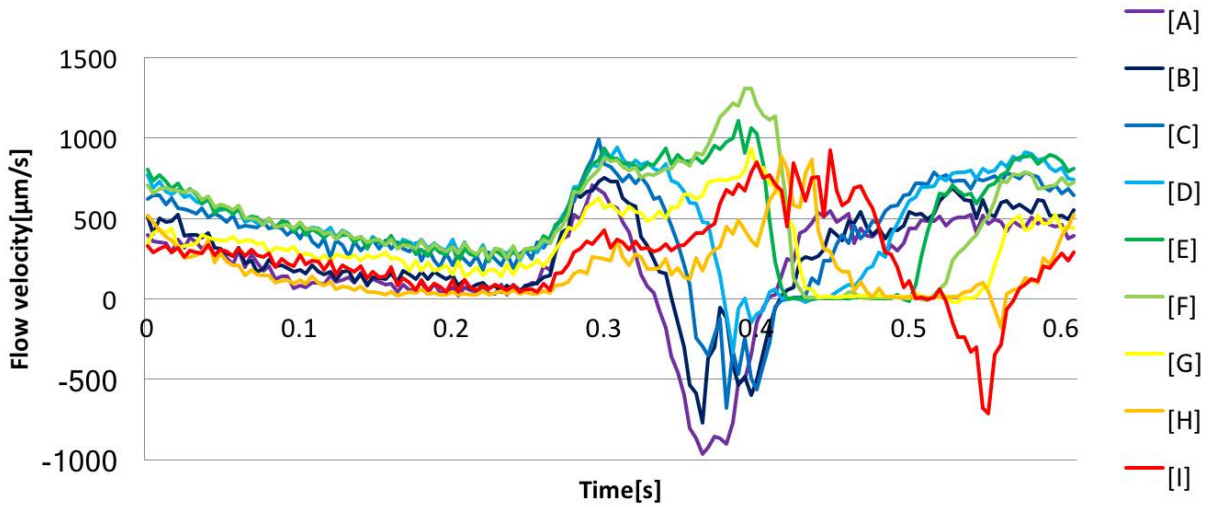


Figure 7: Time variation of the velocity in the atria.

It is found that there is no valve at the inlet of the atria of the Medaka heart and a pyloric structure at the inlet of the heart controls the flow direction. The blood flows as if a man shuts the mouth and sucks and swallows the liquid into the palate. As a result, the movie of the shape changes of the organs together with the velocity vectors of the red blood cell is prepared. According to the movie, it is found that the beating of the heart corresponds to the pulsation of the flow velocity of the red blood cell in the heart.

The measurement of the velocity in the ventricle has also been tried, but it was very difficult to see the red blood cells in the ventricle in the plane parallel to the main flow direction because the ventricle is located close to the eyeballs.

Consequently, the authors observed the blood flow in the heart of Medaka embryo under microscope with a high-speed camera and measured the in-vivo velocities of the red blood cells in the heart by PIV measurement using the pre-processed images.

4 Future study

Presently, the in-vivo velocity measurement in the heart of Medaka embryo has been carried out and the velocity change in the heart has been investigated along with the shape change of the atrium. In the future study, by comparing the velocities in the healthy embryo and those in the embryo with the heart disease that has been artificially caused by DNA manipulation, the early detection of the developmental abnormalities may be possible and it may serve as a clue to the pathologic investigation of the genetic disease and hopefully give some insight to the future effective treatment at early stage.

The present PIV measurement has been carried out in the two-dimensional plane which is parallel to the blood flow in the heart of the Medaka embryo and thus the velocity information obtained in this study has been limited to two-dimensional information. But, in order to fully investigate the flow conditions in the heart, three-dimensional velocity measurement should be carried out. The authors of this study have invented the three-dimensional velocity measurement by using Doppler phase-shifting holography which can be used with single camera observation. The three-dimensional velocity measurement in the heart of the Medaka embryo using Doppler phase-shifting holography remains for the future study.

5 Conclusion

The in-vivo velocity measurement of the blood flow in the heart of Medaka embryo has been carried out. The images of the red blood cell in the heart of the Medaka embryo have been taken by the high-speed camera and the microscope. The images have been pre-processed to highlight only the image of the red blood cell and to enable the PIV measurement. Consequently, the phase-averaged velocity changes in the vena cava and in the atria has been obtained. The results help in finding the developmental abnormalities in the early stage and may give some insight to the future effective treatment.

Acknowledgements

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