Proceedings of the Second Joint EMBS/BMES Conference
Houston, TX, USA • October 23-26, 2002

# *In-Vivo* Applications of Microscale Digital Particle Image Velocimetry

G. Acevedo-Bolton[1], J.R. Hove[1], R. Koester[2], A. Forouhar[1], S.E. Fraser[2], M. Gharib[1]
[1]Option of Bioengineering, California Institute of Technology, Pasadena, CA, USA
[2]Division of Biology, California Institute of Technology, Pasadena, CA, USA

*Abstract*-**Digital Particle Image Velocimetry (DPIV) is an extremely powerful technique for quantitative flow measurements. In order to perform hemodynamic measurements in embryonic hearts, DPIV analysis was performed on both Zebrafish (*Danio rerio*) and Quail (*Coturnix coturnix japonica*) embryos. A protocol for performing DPIV at the microscale with microscopy and high-speed imaging is presented. Results show the presence of vortices and jets at low Reynolds numbers implying a high shear on vessel walls, and suggest that hemodynamics may play an important role in future heart development studies.**
*Keywords* - **DPIV, hemodynamics, cardiogenesis, microscopy**

## I. INTRODUCTION

The embryonic stage of cardiogenesis is believed to be the critical for proper heart development[1],[2]. Errors in folding or growth result can result in major heart defects later in life. Although genetics play a large role in cardiogenesis, it alone cannot fully explain how the heart develops. It is widely believed that hemodynamic forces such as shear stress and pressure play also have an important role along with genetic programming in cardiogenesis. To better understand the relationship between hemodynamics and genetic programming, detailed quantitative measurements of these hemodynamic forces must be made. In this paper we attempt to use DPIV[3], normally used at a much larger scale, to map the flow inside the embryonic heart in zebrafish (*Danio rerio*) and quail (*Coturnix coturnix japonica*).

## II. METHODOLOGY

The first set of experiments were done on 4.5 day post-fertilization (dpf) zebrafish embryo. The fish were mounted in ultra low gelling temperature agarose, and imaged with an inverted Zeiss Microscope. A Dalsa High speed CCD camera acquired the images at 440 frames per second. Using the blood cells as particles, in-house software was used to perform the DPIV analysis on the images.

Another set of experiments was done on quail embryos, stages 10-16. Due to the embryo's location inside the egg, the blood cells alone could not be used as markers. Cyto 11, a fluorescent dye from Molecular Probes, was injected into the embryo's blood stream where it stained the blood cells. A Hamamatsu Image intensifier in conjunction with a Yokogawa CSU Real Time Spinning Disk Confocal attachment and a Princeton Instruments I-Pentamax CCD camera were used to acquire the images. These images were processed with the previously mentioned DPIV software.

## III. RESULTS

Our data show that DPIV can be performed at a scale smaller than ever before, and in the process have provided insight into embryonic hemodynamics.

### *A. Zebrafish*

DPIV data showed the presence of a jet and adjacent vortices between the ventricle and bulbous during systole in the 4.5 dpf embryo. Fig. 1, shows some representative results. Blood was calculated to reach velocities up to 0.5 cm/s resulting in a Reynolds number of approximately 0.02 and a shear stress of 75 dynes/cm$^2$. Velocity and vorticity profiles agree with those of similar flows.

### *B. Quail*

The quail embryo was situated in the egg such that its heart was not clearly visible. Instead, DPIV analysis was performed on some extra-embryonic vessels, showing that fluorescent labeling of cells can also be used. The DPIV data agreed with visual observations of the highly pulsatile nature of the flows.

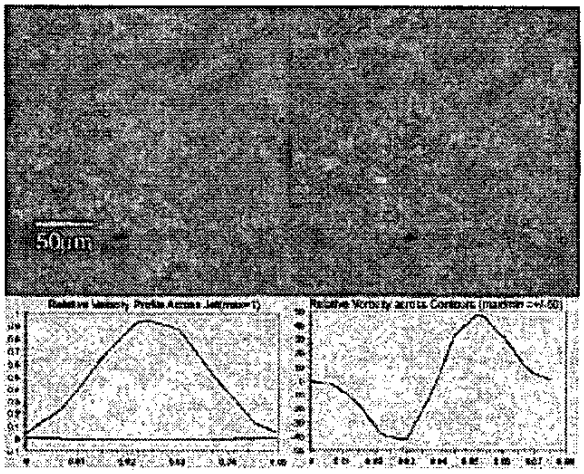


Fig. 1. Superposition of DPIV results during systole over original image with the corresponding profiles beneath. The heart chambers, going from left to right are the bulbous, the ventricle and the atrium. Profiles were obtained by crossing contours and vectors starting at the bottom.

## IV. DISCUSSION

Our work is a preliminary step in performing quantitative in-vivo flow studies. The results above show, for the first time, the presence of a jet and resulting vortices in an embryonic zebrafish heart, a heart that could fit inside a human hair. The implications of a jet at such a low Reynolds number implies that extremely high shear rates are experienced by the heart at these stages. Reference [4] and others have shown that the endothelial cells, which line the heart and the rest of the vasculature, are sensitive to shear stress. The shear stress calculated from the results above, are well above the range detectable by endothelial cells, and imply that shear stress may play an important role in triggering much of the genetic programming responsible for heart development.

The work with the quail embryos also bodes well for further studies. The use of fluorescent labeling may help to improve imaging in areas where there is insufficient contrast. Using confocal microscopy techniques in conjunction with fluorescent labeling allows for much finer optical sectioning, and hopefully more resolution in the velocity measurements.

It should be noted that fluorescent labeling increases the amount of equipment needed for imaging by a large degree. Due to the fact that most high-speed cameras have low sensitivity in the range of fluorescent dyes, methods of increasing the overall brightness, such as image intensifiers, are required.

## V. CONCLUSION

Digital Particle Image Velocimetry (DPIV) has been shown to be a useful tool for quantitative in-vivo flow measurements. These type of measurements may help in understanding the link between hemodynamic forces and genetic programming in cardiogenesis. Eventually this understanding could be used to detect and treat the precursors of heart defects at an early stage in development. Using a high-speed CCD camera and different microscopy techniques, we were able to acquire images to be used in DPIV. Our results give us insight into the hemodynamics of two different embryonic systems.

## REFERENCES

[1]S.P.M O'Brien, T. Wheeler, and D.J.P. Barker, "Fetal programming influences on development and disease later in life" London: RCOG Press 1999.

[2]D.J.P. Barker,"Fetal origins of cardiovascular and lung disease," New York: Marcel Dekker Inc., 2000.

[3]M. Gharib and D. Dabiri, "Digital particle image velocimetry," in *Flow visualization techniques and examples*, A.J. Smits and T.T Lim Eds. London: Imperial College Press, 2000, pp.123-147.

[4]J.N. Topper and M.A. Gimbone Jr., "Blood flow and vascular gene expression: fluid shear stress as a modulator of endothelial phenotype," *Mol. Med. Today*, 5, pp. 40-46, 1999.