IN VIVO PIV MEASUREMENTS IN THE EMBRYONIC CHICKEN HEART

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Summary A Particle Image Velocimetry (PIV) system is developed that enables *in vivo* PIV measurements in the heart of a chicken embryo. Fluorescent lipid micro-spheres serve as tracer particles. Velocity distributions within the ventricle and the atrium can be resolved.

INTRODUCTION

Placental blood flow is expected to play a significant role in normal and abnormal human heart development [1]. For studying this relationship experimentally, an embryonic chicken model with manipulable extraembryonic blood flow can be used [1]. Figure 1 displays a chicken embryo after approximately 60 hours of incubation. On the right and left side of the image one can clearly see vitelline veins leading to and vitelline arteries coming from the embryo. They serve the same function as the placenta in a mammalian embryo. It has been shown that obstructing venous flow by closing one of the vitelline veins with a clip results in severe cardiovascular malformations [1]. Although the exact mechanism responsible for this is not known, it is speculated that the development is strongly linked to specific details of the wall shear stress patterns within the forming heart.

The whole vasculature including the heart is covered by a thin layer of cells, the vascular endothelium. From *in vitro* flow studies on these cells, it is known that flow induced shear stress modulates gene expression [3]. *In vivo* analysis of the intracardiac flow of a zebra fish reveals shear forces beeing a key factor in the embryonic cardiogenesis [2], though a direct relation between abnormal placental blood flow and cardiovascular malformations is missing [1]. By combining the fluorescent visualization of gene expression with a quantitative measurement of the instantaneous flow field *in vivo* using PIV, a relationship might be found.

METHOD

Due to the small dimensions of the embryonic chicken heart (about 200 μm inner diameter) a μPIV system is utilized. In comparison to a conventional PIV system, the measurement plane is defined by the limited depth of focus of the microscope objective, rather than by forming a light sheet. Fluorescence based imaging is used to distiguish between background light that is scattered by blood cells and tissue, and the signal coming from the tracer particles. Rhodamine tagged, polyethylene glycol coated lipid-microspheres, so called “Stealth liposomes”, are suitable as long circulating tracer particles and are used in the reported experiments. Stealth liposomes are coated to prevent wall adhesion and capillary blockage [4]. The nominal diameter is 500 nm, but in practice much larger agglomerates are observed.

Figure 2 shows a schematic overview of the experimental set-up. A double pulse Nd:YAG laser is illuminating the whole flow field through the objective of a Leica fluorescence microscope. The beam is widened by a diffuser plate and then reflected into the optical axis of the objective at a dichroic mirror. Background light, reflected by blood cells and tissue, is guided into the direction of illumination by the same dichroic mirror. Light with longer wavelengths, emitted by the rhodamine, passes the mirror and is imaged by the camera. The rest of background light is stopped at a low-pass filter. An image intensified, double frame CCD-camera with a resolution of 1376 x 1040 pixel was used to capture the PIV-images.

The PIV system is phase-locked to the cardiac cycle of the chicken embryo through the use of an ultrasound Doppler velocimeter. A PC calculates the measured velocity from the Doppler shifted signal in real time and triggers the timing unit of the PIV system with an adjustable delay. In this manner, it is possible to perform ensemble averaged PIV measurements at identical flow conditions to enhance the quality of the obtained velocity vector maps.

RESULTS AND DISCUSSION

Figure 3 shows the average of fifty phase-locked vector fields in the fully expanded ventricle (left) and atrium (right). The images were acquired using a 10x magnification objective for the ventricle and 5x for the atrium. Vectors outside the flow field that represent the contractile motion of the ventricular wall can be recognized.

Although the measurements resolve the flow fields at different points of the cardiac cycle well, further improvement, especially in the near wall region, is required for accurately calculating the wall shear stress from the flow profiles. The large diameter of particle agglomerates and the consequential low seeding density is accompanied by relatively extended interrogation areas of 64 by 64 pixel with the corresponding bias. Improvement is expected from the development of

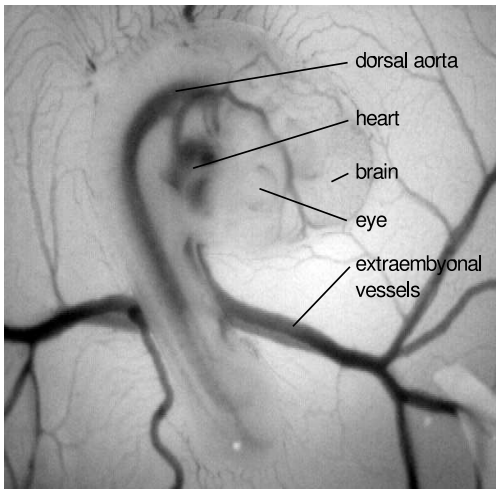

Figure 1. Chicken embryo after approximately sixty hours of incubation.

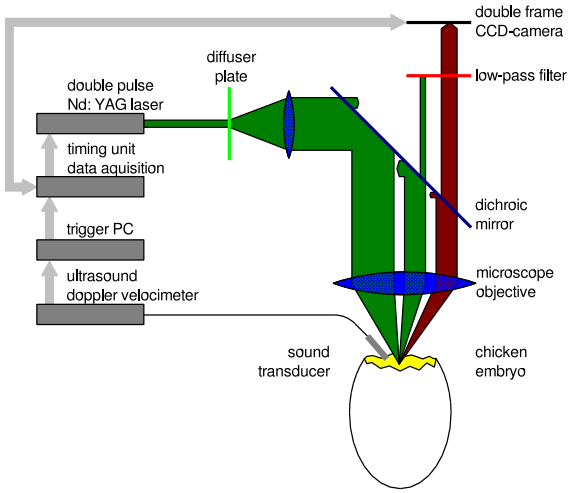

Figure 2. The μPIV set-up, using a fluorescence microscope.

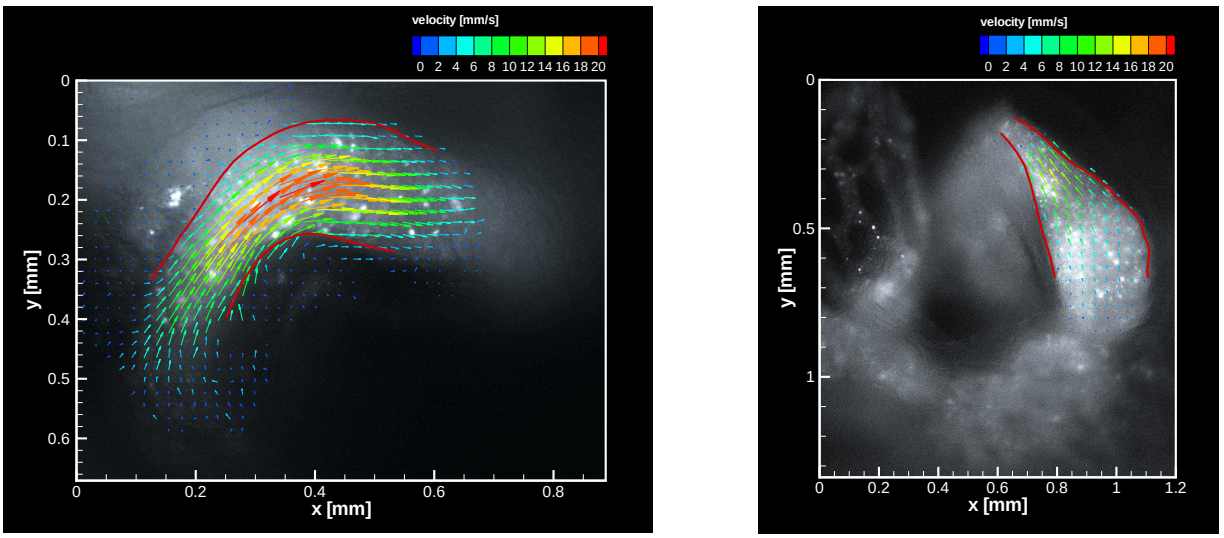

Figure 3. PIV measurement in the ventricle (left) and in the atrium (right) of a chicken embryo.

non-agglomerating particles and by excluding out of focus particles from the evaluation. The spatial resolution might be enhanced dramatically by applying a two-point ensemble correlation method [5]. In addition, a way must be found to detect the vessel wall position accurately. A value for the effective near wall fluid viscosity needs to be determined.

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