In vivo Micro PIV in the Embryonic Avian Heart.

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Summary: A micro-Particle Image Velocimetry (μPIV) system is developed that enables *in vivo* PIV measurements in the heart of a chicken embryo. Long circulating, fluorescent lipid micro-spheres serve as tracer particles. The measurement system is triggered by the heart beat of the embryo to allow the collection of phase locked image ensembles. The measured velocity distribution is compared with gene expression patterns.

Motivation

Placental blood flow is expected to play a significant role in normal and abnormal human heart development. For studying this relationship experimentally, an embryonic chicken model can be used (Hogers et al. (1999)). Figure 1 displays a chicken embryo of development stage 15 (according to the staging critaria by Hamburger and Hamilton (1951)). On the right and left side of the embryo one can see vitelline vessels. The function of this vessels is comparable to the embryonic blood vessels of the mammalian placenta. It has been shown that obstructing venous flow by closing one of the vitelline veins with a clip results in severe cardiovascular malformations (Hogers et al. (1999)). 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 (Groenendijk et al. (2004)).

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 (Topper and Gimbrone Jr (1999)). *In vivo* analysis of the intracardiac flow of a zebra fish reveals shear forces beeing a key factor in the embryonic cardiogenesis (Hove et al. (2003)), though a direct relation between abnormal placental blood flow and cardiovascular malformations is missing (Hogers et al. (1999)). 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.

Materials and Methods

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 (Meinhart et al. (1999)). Fluorescence based imaging is used to distinguish between background light that is scattered by blood cells and tissue, and the signal coming from the tracer particles. Rhodamine tagged, polyethylene glycol (PEG) coated lipid-microspheres, so called "Stealth liposomes", are used as long circulating tracer particles. Stealth liposomes are coated to prevent wall adhesion and capillary blockage (Woodle and Lasic (1992)). The nominal diameter of the particles is 400 nm, but in practice also little 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 by 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. A rest of background light is stopped at a low-pass filter. An image intensified, double frame CCD-camera with a resolution of 1376 $\times$ 1040 pixel was used to capture the PIV-images.

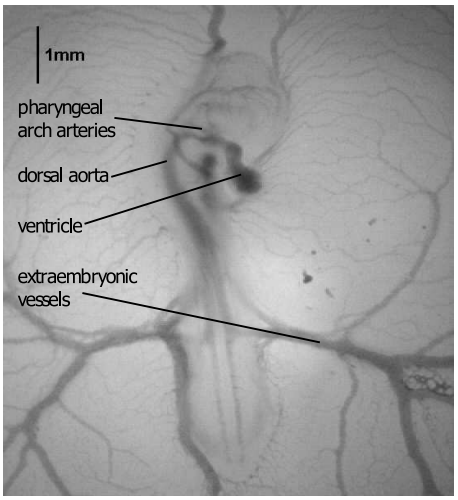

Figure 1: Chicken embryo after approximately 55 hours of incubation (Hamburger and Hamilton stage 15).

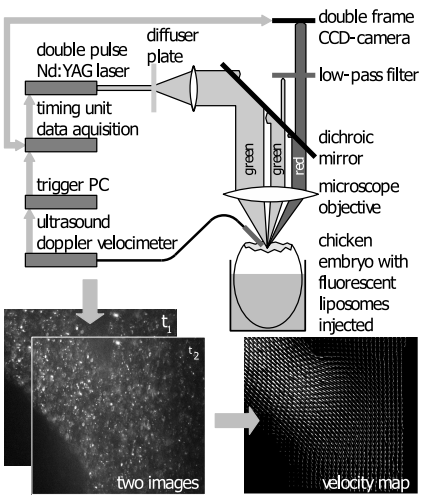

Figure 2: The μPIV set-up, using a fluorescence microscope.

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. The images were acquired using a 10× magnification objective. The flow enters from the left side of the image where the blood emerges perpendicular to the image plane. After crossing the image plane, the flow disappears again normal to the image plane in the direction of the arteries. The corresponding Reynolds number is about 0.5 at this point of the cardiac cycle which indicates that this flow is mainly determined by viscous forces, but that inertia can not be fully ignored. The dashed white lines mark the boundary of the inner lumen. Only the velocity components parallel to the image sensor are measured. This explains the decrease of velocity magnitude at the right and the left of the flow field. At the cross section indicated by the straight line, the flow is assumed to be parallel to the image plane. The third velocity component becomes zero. At this position a single velocity profile is superimposed as a continuous curve. The profile clearly shows an asymmetrical velocity distribution. This suggests the highest shear stress will appear at the inner curvature. Groenendijk et al. (2004) studied the expression of shear stress responsive genes in the cardiovascular system of chicken embryos (figure 4). The production of KLF-2 is known to be upregulated at higher shear stress (Dekker et al. (2002)). In agreement with the measured velocity profile figure 4 clearly shows high levels of KLF-2 (blue) at the inner curvature of the heart.

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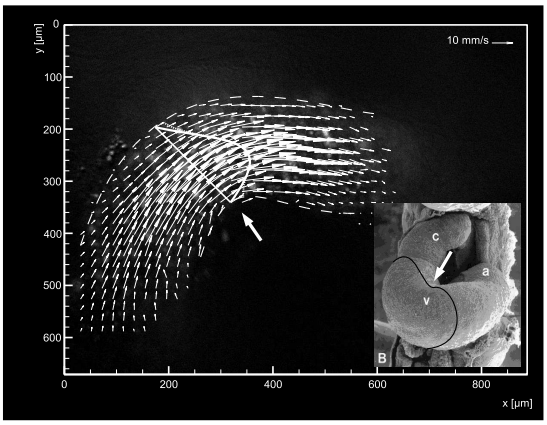

Figure 3: PIV measurement in the developing ventricle of a normal Hamburger and Hamilton stage 15 chicken embryo. The location and spatial orientation of the measurement plane relative to the looping heart is indicated in the scanning electron micrographs from Männer (2000).

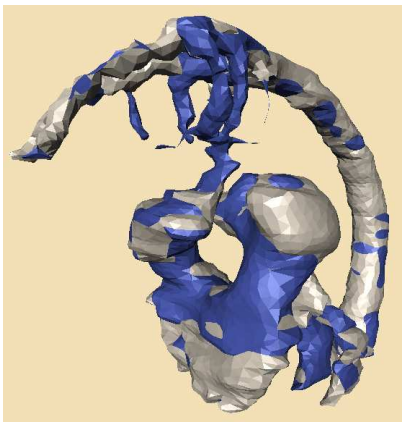

Figure 4: Localization of Krüppel-like factor-2 (KLF-2) in a Hamburger and Hamilton stage 18 embryo (approximately 60 hours of incubation) (Groenendijk et al. (2004)). The thick part of the geometry represents the developing heart. The long, curved tube is the dorsal aorta and the finger like structures in the top part of the image are the pharyngeal arch arteries.

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