In vivo PIV measurements in the embryonic chicken heart

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Abstract

A PIV system is developed that enables *in vivo* PIV measurements in small blood vessels with periodically moving boundaries such as the heart of a chicken embryo. Fluorescent lipid micro-spheres serve as tracer particles. The velocity distribution within the ventricle and the atrium of an embryonal chicken heart can be resolved.

1 Introduction

For answering miscellaneous medical and biological questions it it essential not only to measure the average blood velocity in a vessel, but also to obtain information about the spatial velocity distribution. An example is the question whether placental flow conditions can have an influence on the human embryonic heart development [4]. For studying this relationship experimentally, an embryonic chicken model with manipulable extraembryonic blood flow can be used [4]. Figure 1 displays a chicken embryo after approximately 60 hours of incubation. The extraembryonic vessels on the right and on the left side of the embryo serve the same function as the vessels in the mammalian placenta. It has been shown that obstructing venous flow by closing one of this vessels with a clip results in severe cardiovascular malformations [4]. It is speculated that the heart development is strongly linked to specific details of the wall shear stress patterns within the forming heart. The wall shear stress acts mainly on the vascular endothelium, that is a thin layer of cells lining the whole vasculature including the heart. From *in vitro* flow studies on these cells, it is known that flow induced shear stress modulates gene expression [5], though a direct relation between abnormal placental blood flow and cardiovascular malformations is missing [4].

Particle Image Velocimetry (PIV) is a non invasive whole field velocity measurement technique. PIV has already been used for *in vivo* flow field measurements in mesentery vessels of rat [1, 2] as well as in the embryonic heart of a zebrafish [3]. By combining the fluorescent visualization of gene expression with a quantitative measurement of the instantaneous flow field *in vivo* using PIV, a relationship between placental blood flow and cardiogenesis might be found.

2 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 a microscope objective, rather than by forming a light sheet. Instead of the naturally existing blood cells, fluorescent liposomes with a nominal diameter of 500 nm are used as tracer particles (figure 3). The liposome surface is coated with polyethylene glycol (PEG) molecules to prevent wall adhesion [6]. The use of fluorescent tracer particles allow one to distinguish between the light that is emmitted by the tracer particles and background light that is scattered by the surrounding tissue.

The experimental set-up is shown in figure 2. 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.

The laser illuminates the 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

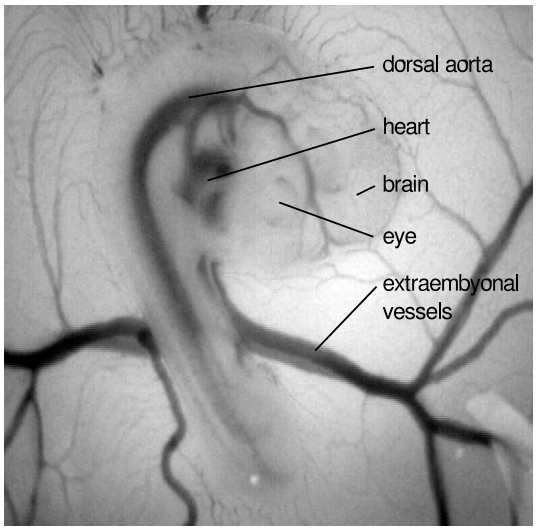

Figure 1: Chicken embryo after approximately sixty hours of incubation.

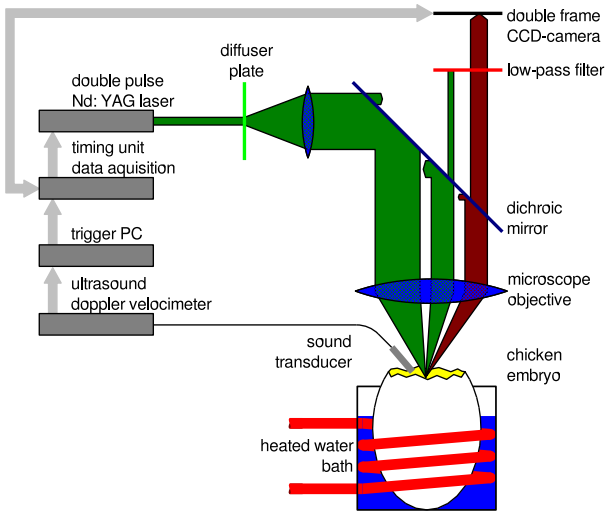

Figure 2: A μPIV set-up, using fluorescent stealth liposomes as tracer particles.

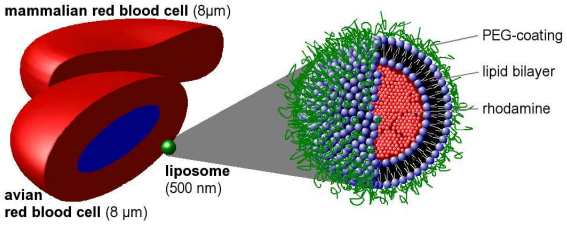

Figure 3: PEG coated liposome.

wavelengths, emitted by the fluorescent liposomes, passes the mirror and is imaged by a double frame CCD camera. The rest of background light is stopped at a low-pass filter.

3 Results and Discussion

Figure 4 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. From the velocity distributions at different points in the cardiac cycle velocity profiles are extracted. This profiles are approximated with a quadratic fit under the assumtion of a quasi one dimensional flow. From the derivative of the fit the wall shear stress is estimated. Assuming the near wall viscosity beeing 4 cp, stresses are found to be around 2 Pa.

Although a first number for the wall shear stress could be given, this must be regarded as an order of magnitude approximation. 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 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 [7]. 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.

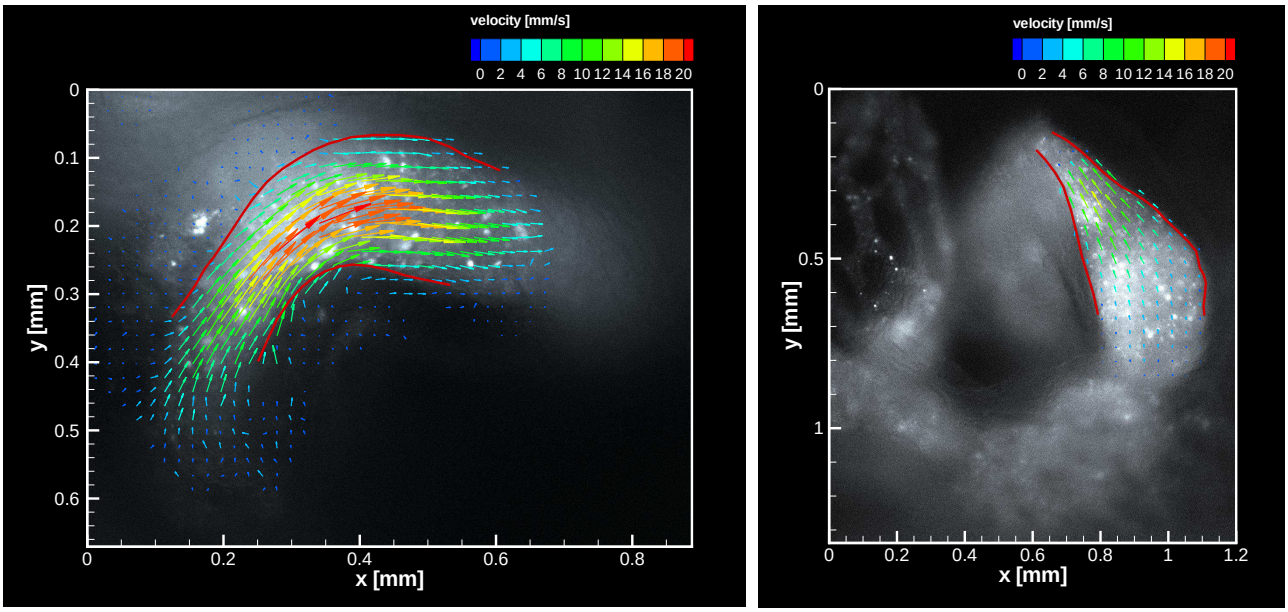

Figure 4: PIV measurement in the ventricle (left) and in the atrium (right) of a chicken embryo.

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