Wall shear stress measurements using μPIV in the outflow tract of a chick embryo

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Abstract In order to study the role of blood-tissue interaction in the developing chick embryo heart, detailed information about the hemodynamic forces is needed. In this study we present measurements of the three-dimensional distribution of the wall shear stress in the outflow tract of an embryonic chick embryo. The data is obtained in a two-step process: first, the three-dimensional flow field is measured using scanning microscopic Particle Image Velocimetry. Subsequently, the location of the wall is determined by analyzing slices of the velocity data perpendicular to the mean flow direction. In each slice, a polynomial fit is used to describe the flow field. The location of the wall and the wall shear stress can be derived from these fits. This results in a three-dimensional reconstruction of the geometry and detailed information about the wall shear stress in the outflow tract.

1. Introduction

The cardiovascular system already functions before it has completely developed. During this time, the blood flow affects the development of the primitive heart and blood vessels, hereby strongly coupling form and function. This coupling stems from the interaction between hemodynamic forces and cell responses. In particular, gene expression in the endothelial cells – the cells that line the inside of blood vessels – has been found to be controlled by the wall shear stress (Reneman et al., 2006).

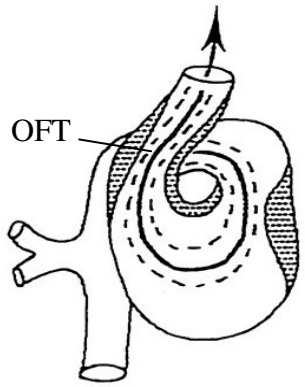

Figure 1: a schematic representation of the embryonic chick heart at stage 17 (52-65 hours of incubation). The outflow tract is indicated with "OFT"; (Based on Hogers et al., 1997)

Recent studies using chick embryos, a commonly used model system for human development, have shown that an alteration of flow in the heart leads to defects/malformations in the cardiovascular system later on (see e.g. work using the "venous clip model", Hogers et al., 1997). In this study we focus on the flow pattern in the so-called "outflow tract" (see Figure 1). This is the last segment of the embryonic heart, which at the stage under investigation (HH 17-18, approximately 3 days of incubation) resembles a looped, contracting tube. Changes in gene expression (and eventually birth defects) are most significant in this part of the heart after alteration of the cardiac inflow by an extra-embryonic venous clip. The aim of this study is to document the changes in the flow pattern – and thus wall shear stress in the outflow tract (OFT).

2. Experimental Techniques

Experiments are performed "in ovo" by carefully opening an incubated chick egg (as described in detail by Vennemann et al., 2006, 2007 and Poelma et al., 2008). The wall shear stress (WSS) is derived using a two-step process: first, the in-plane velocity is determined by means of scanning micro Particle Image Velocimetry (PIV). As tracer particles, bio-inert 1 micrometer PEG-coated polystyrene particles containing a fluorescent dye are used, which are injected in an extra-embryonic blood vessel. At each scanning location, 500 image pairs are recorded on a epifluorescent microscope at typically 18x magnification. They are sorted using a quick-and-dirty phase estimation (see also Poelma et al., 2008). Depending on their phase, they are divided in 10 equally spaced bins. By assuming periodicity of the flow, the image pairs within each bin can then be used in a correlation averaging PIV algorithm. This in-house algorithm uses grid-refinement with a final spatial resolution of 48x48 pixels (50% overlap), corresponding to a vector spacing of 17 micrometers.

The measurement is then repeated at a slightly lower z-location using the computer-controlled translation stage of the microscope. The spacing between the planes is chosen to be 12 micrometers, comparable to both the expected in-plane resolution and the correlation depth. This process yields a three-dimensional measurement of the velocity in the outflow tract for each step in the cardiac cycle. This data is subsequently used in the second step: in slices perpendicular to the mean flow direction, a 2D polynomial fit is used to find the wall location and the wall shear stress. More details about this step are given in the next section.

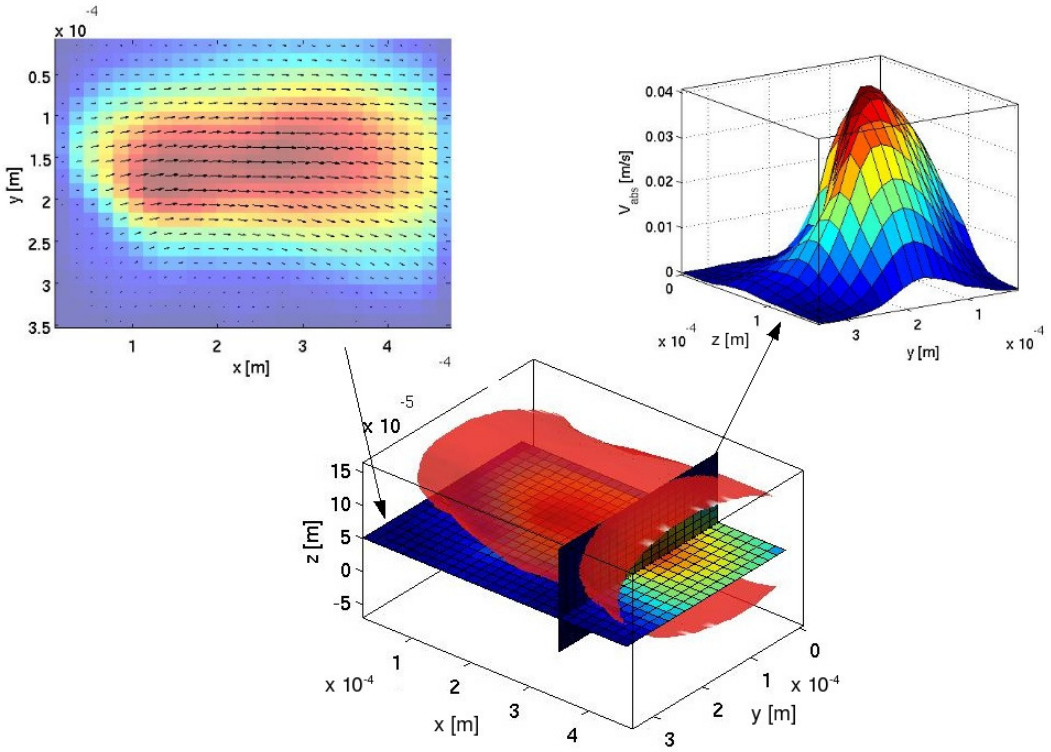

Figure 2: schematic representation of the reconstruction of the OFT geometry. *Top left:* one of the original PIV measurements that is 'stacked' to form the 3D measurement (*bottom*). From this data set, slices in the y-z plane (*top right*) are used to find the location of the wall and the value of the wall shear stress. The resulting geometry is shown as the red isosurface (*bottom*).

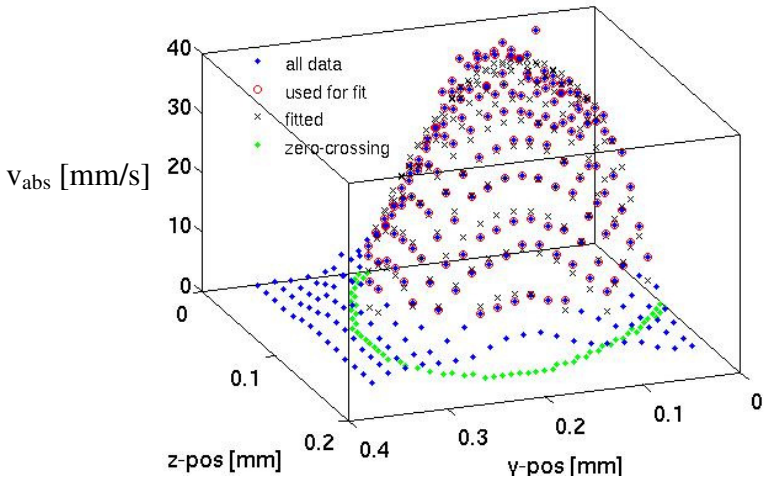

Figure 3: a slice in the y-z plane (similar to Fig. 2, top right) and the fitting of a polynomial function; from the entire data set (*blue dots*), only data above a certain threshold are used (*red circles*). The fit result is shown as the *black crosses*, while the zero-crossing of the fitted function is indicated by the *green dots* (in the $v_{abs} = 0$ plane).

3. Determination of the wall shear stress

Each measurement series of 500 image pairs results in one velocity field for each of the 10 steps in the cardiac cycle. As the measurements are performed in 14 planes in total, this results in a large 4-dimensional matrix containing the velocity information. From these matrix, the geometry of the OFT and the local wall shear stresses need to be determined. Here we will only discuss the results a systole, i.e. during the contraction of the heart, which results in maxima in flow and WSS.

Many different approaches could be used to find the geometry of the OFT. The most straightforward would be to find an isosurface of the magnitude of the velocity, $v_{abs} = 0$. Assuming that the flow velocity only reaches zero at the wall due to the no-slip condition, this isosurface would coincide with the wall locations. In practice, the measurement will contain noise and the isosurfaces obtained were highly irregular. This could be overcome somewhat by either smoothing the data or using a non-zero level for the isosurface (e.g. 5% of the maximum velocity). However, both result in an "underestimated" geometry (i.e. a geometry that is smaller than reality). Due to the fact that not the entire OFT was measured, this method also yielded truncated geometries.

As alternative, a segmental analysis was performed on slices perpendicular to the mean flow directions. The flow direction was more or less aligned with the horizontal (x) axis, so no interpolation was needed to obtain these slices. Each of the slices (in the y,z-plane, see also Figure 3) showed a relatively smooth distribution of the flow velocity. A 2D polynomial fit was used to describe the data in each slice. Subsequently, this polynomial function is used to find the wall location (which corresponds with the zero-crossing of the function) and the wall shear stress. The latter can easily be calculated from the derivative of the function at the location of the zero-crossings. An example of this fitting process is shown in Figure 3. Not all the data is used for the fit: only the data that is above a certain threshold is taken into account. This improves the stability of the fitting process (velocity measurements in the wall, which are usually zero, should obviously be ignored) and avoids the use of under-resolved gradients near the wall. In practice, using data than was above 20% of the maximum in the slice gave good results. The choice for the fit function is a compromise between stability/robustness and flexibility. For example, a 2D parabola would generally give good results and useful zero-crossings (i.e. a circle in the $v_{abs}=0$ plane). In reality the flow profile might be skewed, something that obviously is not taken into account, so that only symmetric WSS patterns will be obtained. On the other hand, choosing a function with to many terms will lead to non-realistic zero-crossings. A compromise between an overly restrictive function and stability of the fitting process led to the use of a second order polynomial of the form:

$$v_{abs}(y,z,\ x=const) = c0 + c_1\, y + c_2\, y^2 + c_3\, z + c_4\, z^2 + c_5\, yz; \qquad (1)$$

Typical discrepancies between the fit result and the measured data (expressed as the standard deviation of the differences) are 3-5%. An example of the fitting process (showing original data, data used for fitting, the fitted result and the extrapolated zero-crossing) is given in Figure 3.

4. Results and Discussion

An example of a reconstructed OFT is shown in Figure 4 (*left*): the surface represents the wall of the OFT, while the color coding represents the shear rate; the latter can be multiplied with the viscosity to find the wall shear stress. There is some uncertainty about the exact value of the viscosity of blood, in particular of the developing embryonic chick. Usually, a value 3-4 times that of water is used. For the current study, we are more interested in the spatial distribution, rather than the absolute values, of the wall shear stress. Therefore, we chose to keep the data in shear rate format. The right-hand figure shows a slice in the *x,y*-plane (i.e. one of the original PIV measurements) at the centerline of the OFT. Again, the color-coding of the wall location shows the distribution of the WSS.

Measurements have been performed on a number of embryos, containing a control group and a group with restricted vitelline vein flow. Typically, a measurement takes up to half an hour, including preparation (injection of tracer, etc.) and a total data acquisition time of 10 minutes.

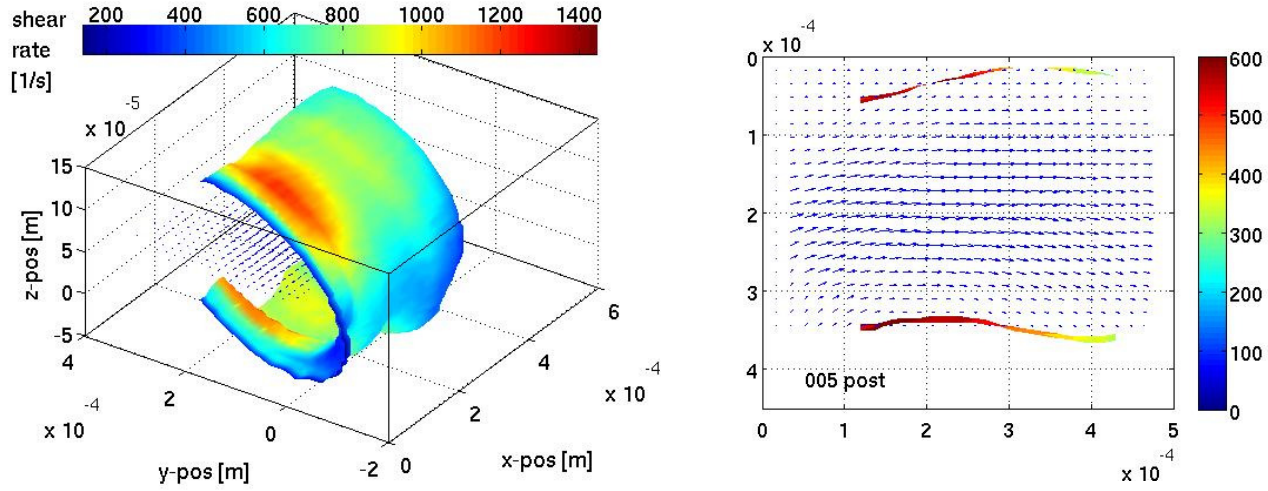

Figure 4: ***(left)*** **a reconstructed segment of the walls of the outflow tract, color-coded with the shear rate;** ***(right)*** **a velocity field at the midplane of the outflow tract, with reconstructed wall location and shear rate.**

5. Conclusions and Outlook

Using scanning microscopic PIV, the geometry of the OFT and the local wall shear stress has been determined for a number of embryos. While the technique uses some approximations (the most critical being the description of the flow field as a 'stack' of 2nd order, 2D polynomial fits), it is a step forward compared to either visualizations or the conventional estimation of the wall shear stress from the mean velocity and diameter. The biological interpretation of the results is currently in progress, for an indication of the biomedical framework, we refer to Hierck et al. (2008).

In the current experiment, the flow direction is more or less aligned with the horizontal axis of the measurement frame-of-reference (see e.g. Figure 4, *right*). This allowed a reconstruction of the OFT geometry in a “slice-by-slice” manner. For more complex geometries (such as more curved segments or bifurcations), this approach is obviously no longer possible. In that case, more sophisticated techniques need to be used. Most techniques require some knowledge of the flow topology in order to perform (local) fitting. Currently, we are evaluating algorithms to automate the

detection and description of this flow topology, by e.g. finding the medial axis or the use of non-uniform rational B-spline (NURBS). These techniques are required to avoid the need for (manual) operator input.

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