# Medical Bio Trial Use Proposal: Medical Bio Trial Use Proposal Report

**Proposal Number:**2009B1910
**Experiment Title:** Phase-contrast imaging of cardiac flow and shear in zebrafish
**Project Leader:** Andreas Fouras, Monash University
**Beamline:** BL20XU

## Aims and background

This study aimed to develop a new quantitative measure of in-vivo blood flow using the penetrating and high intrinsic contrast afforded by Phase Contrast X-ray Imaging coupled with the accurate and quantitative fluid dynamics information provided by Particle Image Velocimetry (PIV). The ability to image blood flow using such a technique would find widespread application in the study of vascular diseases. Specific aims of this experiment were:

- Development of the imaging methodology towards more clinically meaningful and complex mammalian models such as mice.
- Application of the method to achieve both morphological and functional measurements of the cardiac flows and shear stresses within zebra-fish at key stages of their development;

## Methodology

Successful PIV of cardiac flow rates require high resolution, high speed image sequences. Previous beam-time experience has shown us that 0.7 micron pixels are optimal. At these resolutions, low exposure times are necessary to freeze the motion of blood in any single image. At this resolution, exposure times under 1 millisecond are necessary. Imaging was performed at both the upstream and downstream hutch of BL20XU to optimise the flux available and hence minimise the exposure times at very high spatial resolutions. To enable rapid imaging, a high speed image intensified detector, high speed shutter and timing apparatus were utilized to rapidly and accurately trigger data acquisition. The Hamamatsu BM3 was used as an X-ray converter. A high-speed shutter has been developed and was a vital component in these experiments for reducing dose and protecting optics from the high flux beam.

## Experimental Results

The first experiment targeted the collection of in vitro measurements. This allowed for baseline data to be collected and for any final optimisation of imaging parameters, such as propagation distance. It was found that the downstream hutch provided insufficient flux for imaging at physiological flow-rates. The upstream hutch proved more appropriate as the higher brightness enabled a 10x reduction in exposure time, allowing exposure times of 100 microseconds. The high-speed detector provided the capability of frame rates greater than 2000 fps. The quality and coherence of the beam in the upstream hutch was insufficient for imaging of red blood cells directly, which require a highly coherent beam to exploit the small phase difference between them and the surrounding plasma and tissue. The addition of ultrasound contrast agents to blood enabled measurement of pulsing blood flow at physiological flow-rates through in vitro vascular models (See Figures 1 and 2).

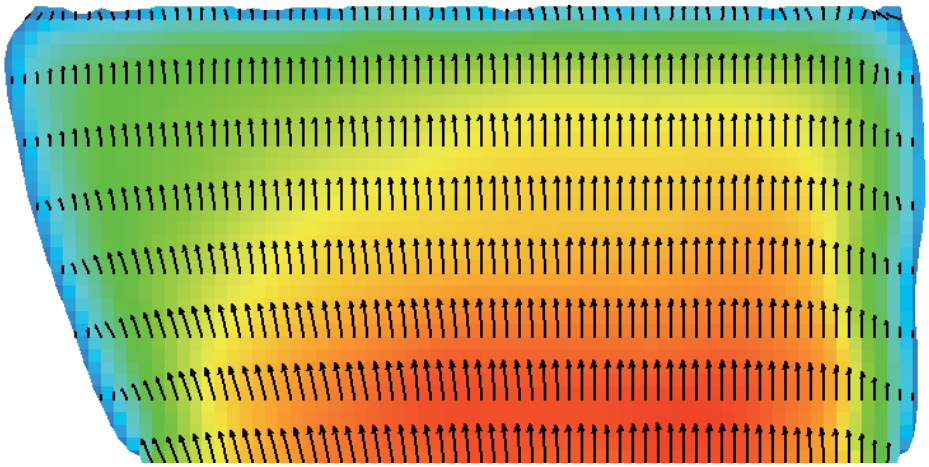

Figure 1: Instantaneous velocity measurement of pulsatile blood flow at physiological flow rate within a realistic stenosis model using X-ray PIV. Vectors show the velocity and contours show the vertical component of the velocity. Velocity measurements are captured at 2000 frames per second with 100us exposures.

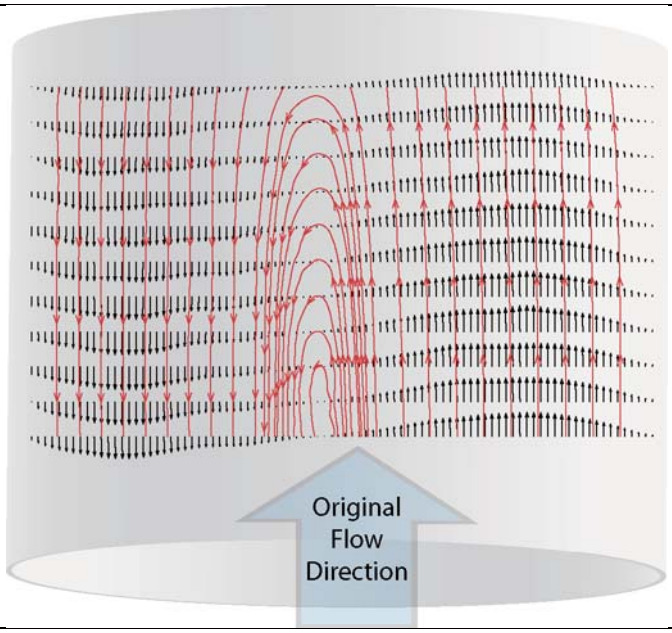


Figure 2: X-ray PIV results of recirculating flow in a glass capillary, captured with the use of the linear VCA shutter. The schematic of the capillary is overlaid with the velocity vector field and streamlines, as were calculated through X-ray PIV analysis of the fluid flow. The experiment was conducted at SPring-8 (Japan) using blood, on the BL20XU beamline, and is only possible with reduced dose to the sample to prevent clotting. The analysed images were captured at 3ms exposures, at a frame rate of 300fps.

## Current and Future Challenges

Experiments were successful in applying PIV to high-speed phase contrast imaging. Results show accurate measurement of blood flow through in vitro models at physiological flow-rates. This represents a significant improvement in the maximum flow-rates capable of being measured using this technique, and has advanced the technology to a point where application to in vivo cardiovascular flows in mammalian models is possible.

Application of this technique to zebrafish models proved difficult. The high flux needed for in vivo imaging resulted in reduced beam quality, which rendered image quality inadequate for high resolution wall shear stress measurement. Results indicate that the addition of contrast agents will improve signal to noise ratio enabling application of PIV to phase contrast imaging of zebrafish cardiac flows. Due to the very small size of the zebrafish, we were unable at this time to incorporate these contrast agents into our *in vivo* experiments. These experiments were therefore unsuccessful. Future experiments planned will incorporate contrast agents, allowing physiological flow-rates to be resolved.

## Status of publications

Two publications are under preparation using data taken during these experiments:

Chua, C.S., Higgins, S., Hourigan, K. & Fouras, A. (2010) *An asynchronous high-speed synchrotron shutter* (Submitted to *Journal of Synchrotron Radiation*)

Jamison, R.A., Dubsky, S., Higgins, S., Siu, K.K.W., Hourigan, K. & Fouras, A. (2010) *Physiological pulsatile blood flow within a realistic in-vitro stenosis model* (under preparation for submission to *Experiments in Fluids*)