

## A Multi-timestep Framework for Accurate Erythrocyte Image Velocimetry

Simon Tupin, Adele Lam, Joseph van Batenburg-Sherwood  
Department of Bioengineering, Imperial College London, London, UK

Experimental measurements of microscale blood flow are needed to provide insight into blood behaviour in the microcirculation and its effects on the endothelium, particularly in cases where the properties of the erythrocytes (red blood cells) are altered in complex ways, such as malaria and diabetes. Using microfluidics, it is possible to fabricate channels that mimic idealised sections of the microvasculature, with sequentially bifurcating vessels. We are developing systems that will enable accurate characterisation of both blood flow and the endothelial cell responses to changes in erythrocyte mechanical properties.

Micro particle image velocimetry ( $\mu$ PIV) can be used to measure velocities with high spatial resolution in such microchannels, but the wide range of velocities in bifurcating networks makes accurate measurement a challenge. In the present study, we develop a multi-timestep approach to  $\mu$ PIV acquisition of flow in branching networks that mimic an idealised section of an arteriolar network. Image pairs are captured in a sequence of increasing timestep ( $\Delta t$ ) values, and an optimal  $\Delta t$  is selected locally to increase accuracy.

A custom  $\mu$ PIV system designed specifically for microscale blood flow experiments was developed. The setup comprises an inverted microscope equipped with two illumination methods: a dual head pulsed Nd:Yag laser to image fluorescent microparticles and a red LED to image erythrocytes. An sCMOS camera was used to record pairs of images at a resolution of  $0.325 \mu\text{m}/\text{px}$  with a 20X objective. The system is controlled using a custom LabVIEW program that enables interleaved was used to perfuse either  $0.5\mu\text{m}$  diameter fluorescent particles suspended in glycerol solution or human erythrocytes suspended in an Optiprep and phosphate-buffered saline solution at a haematocrit of 25% in a  $50\mu\text{m}$  squared cross section bifurcating microchannel.

Figure 1a shows the optimised velocity field measured in the microparticles experiment, with velocity magnitudes that vary by more than an order of magnitude. The selected  $\Delta t$  values are shown in Figure 1b, and vary both between and within branches. The developed framework was then applied to erythrocytes (Figure 1c). The velocities varied significantly throughout the channel, with clear asymmetries that arise due to nonuniform haematocrit distributions [1].

The multi-timestep approach developed in this study was found to be effective for the velocity measurement in branching geometries exhibiting large velocity gradients. The accuracy of the near wall velocity was notably improved, which is particularly important when estimating the shear stress acting on the channel walls, or the endothelium for the vascularised channels we are developing (Figure 1d).

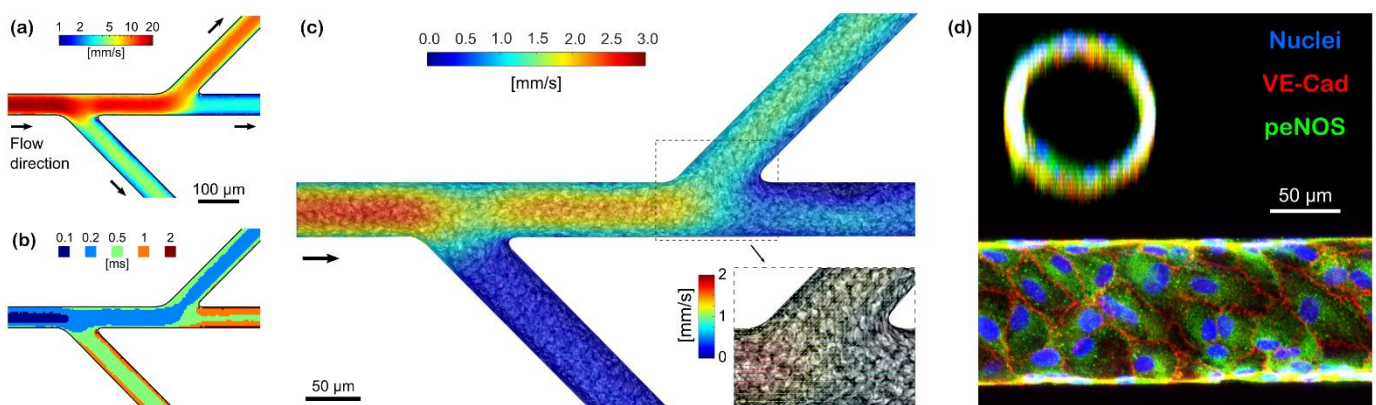


Figure 1: Multi-timestep approach applied to microparticle experiment (a: optimised velocity field; b: local  $\Delta t$  selection) and (b) erythrocytes experiment. (c) Vascularised microchannel.

**Reference** [1] van Batenburg-Sherwood, J., Balabani, S. Continuum microhaemodynamics modelling using inverse rheology. *Biomech Model Mechanobiol* **21**, 335–361 (2022).