Red Blood Cell Tracking and Velocity Measurement with a Keyhole Model of Movement

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Introduction

Two main image-based techniques for measuring red blood cell (RBC) velocity have been used, both based on the mathematical technique of cross correlation. ***Particle Image Velocimetry*** (PIV) is a *2D cross correlation* in which an investigating window determines the probable displacement of the investigated region between two consecutive images. The size of the window is a limitation and most studies are limited to single vessels or at most a bifurcation. ***Kymograph*** methods require user intervention to determine a line of analysis over which *1D correlation* or slope analysis determines the velocity in a single straight vessel. In this work we propose a proper tracking algorithm based on a **Keyhole model of probable movement** of cells.

Materials

Red Blood Cells

RBCs were obtained by cardiac puncture from donor anaesthetised BDIX rats into a heparinized syringe and fluorescently labelled with DiI (D-282 Molecular Probes; Cambridge Bioscience).

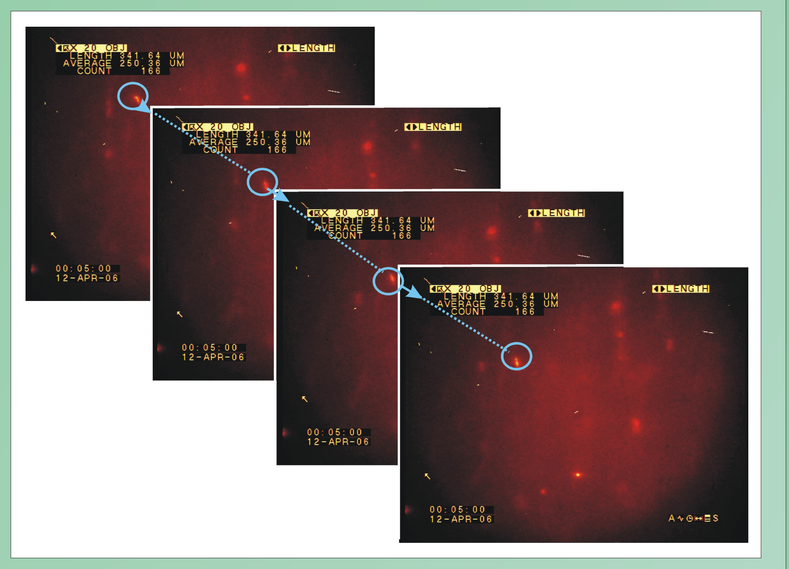

Figure 1 A tracking algorithm should follow the path of single RBCs from frame to frame

Intravital microscopy

Intravital microscopy was carried out on tumours growing in transparent 'window chambers' implanted into the dorsal skin flap of male BDIX rats under Hypnorm and midazolam anaesthesia. Fluorescently labelled RBCs (<0.2ml/200g) were injected into a cannulated tail vein. Intravital microscopy was performed with an inverted Nikon Diaphot 200.

Methods

Pre-processing and keyhole model

Pre-processing removes noise and artefacts from the images. First, the mean image from the whole set is removed from each frame, then frames are smoothed by removing even fields and averaging neighbouring elements. Cells are obtained by thresholding and their centroids are obtained. The tracking algorithm is based on a keyhole model that describes the probable movement of a segmented cell between contiguous frames of a video sequence. When a history of movements exists, past, present and a predicted landing position of the cells define two regions of probability with a keyhole shape. This keyhole model is used to determine if cells in contiguous frames should be linked to form tracks and also as a post-processing tool to join split tracks and discard links that could have been formed due to noise or uncertainty. When there is no history, a circular region around the centroid of the parent cell will be used as region of probability.

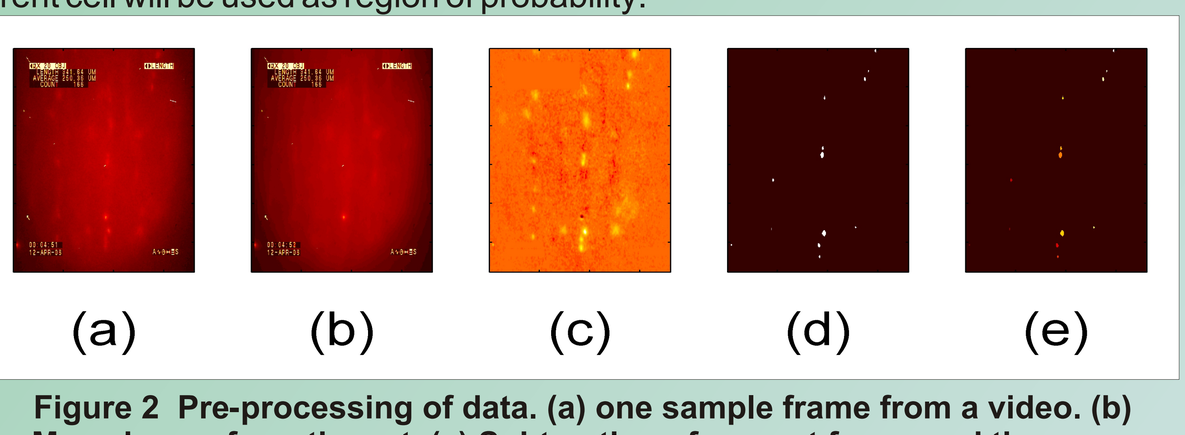

Figure 2 Pre-processing of data. (a) one sample frame from a video. (b) Mean image from the set. (c) Subtraction of current frame and the mean image (d) Thresholding of the difference. (e) Labelling of separate cells from which centroids are obtained.

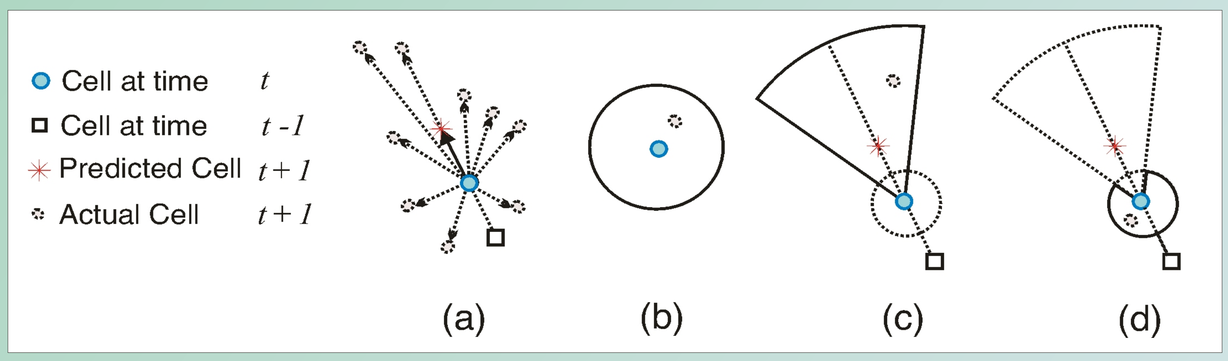

Figure 3 RBC keyhole movement model. (a) It is assumed that between consecutive frames a RBC can move towards any direction and with any distance. (b) Without movement history, the only assumption possible is that its landing prediction will be within a circular region. (c, d) A landing position is predicted assuming constant velocity and direction, this creates two probable regions, a wedge (c) and a circle (d) which when combined create a keyhole model.

Post-processing joins tracks and discards links that could have been formed due to noise or uncertainty. Outliers are removed based on the distribution of the average velocities of the tracks. Since the position and time of each cell is recorded, a wealth of statistical measures can be obtained from the tracks.

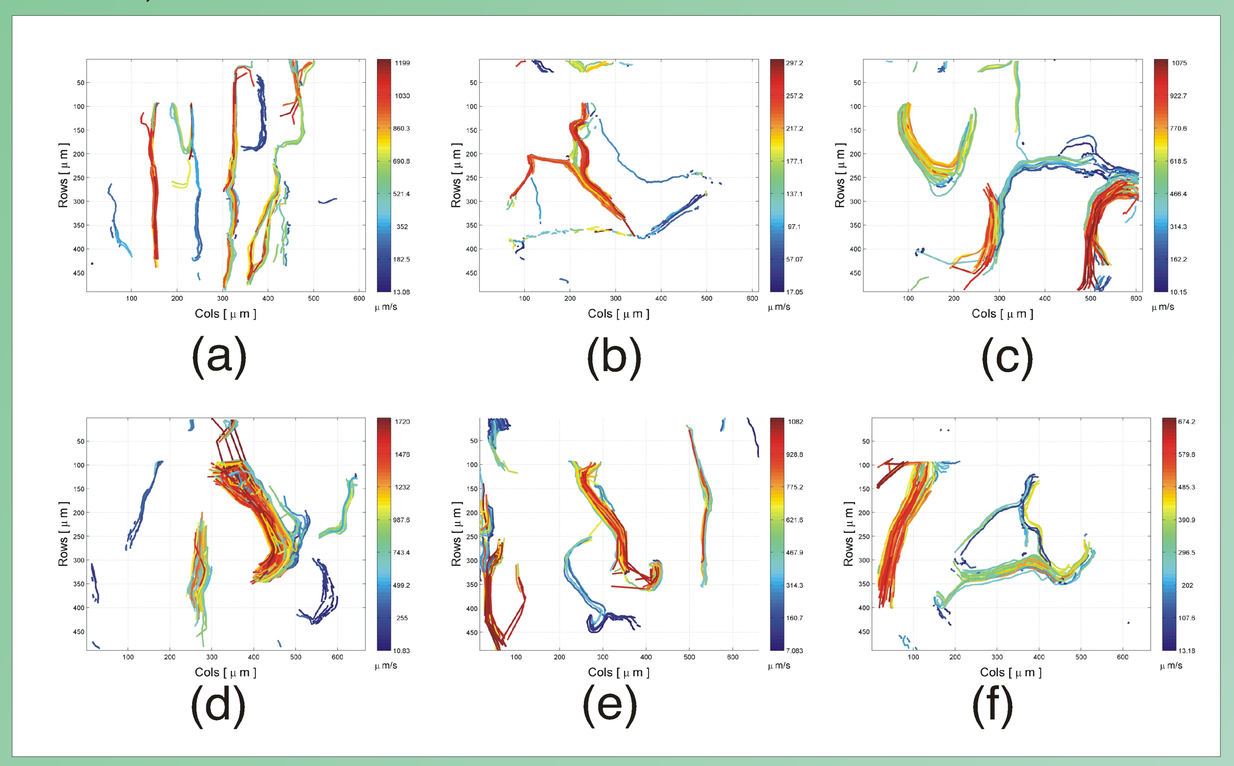

Figure 4 Tracks obtained for 6 different tumours. Each individual RBC track is presented as a line with colours representing the velocities. It can be seen that the velocity in some vessels is consistently faster (red) than in others (blue).

Results

The algorithm was tested on two sets of experiments. First, the vasculature of six tumours with different geometries was analysed; average velocities ranged from 86 to 372 [μm/s], with maximum and minimum track velocities 13 and 1212 [μm/s], respectively. Second, a longitudinal study of velocities was performed after administering a vascular disrupting agent to two tumours and the time behaviour was analysed over 24 hrs. On one of the tumours there is a complete shutdown of the vasculature while in the other there is a clear decrease of velocity at 30 mins and at 6 hours there is a recovery of the tumour blood flow.

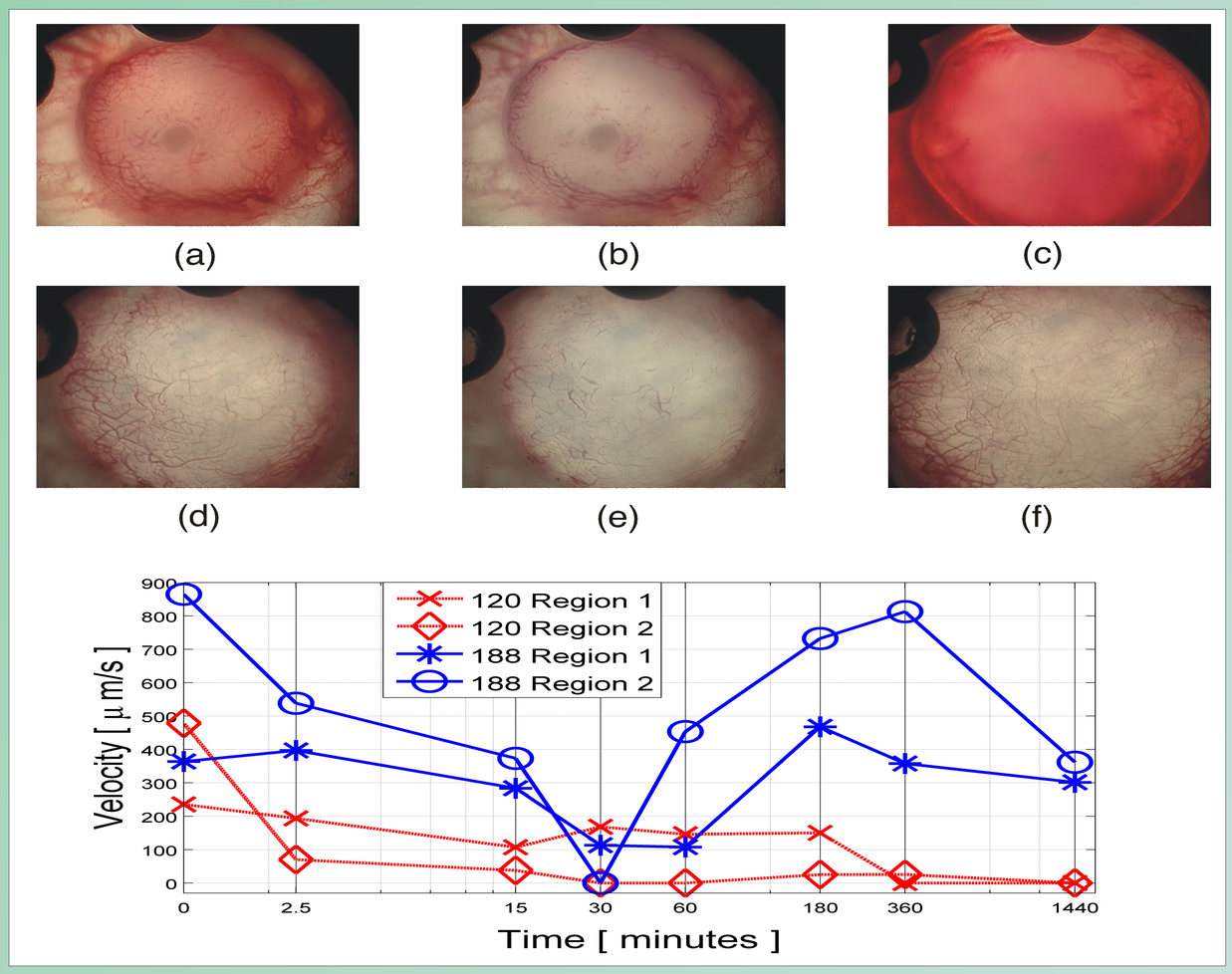

Figure 5 Light microscopy of the whole tumours at different time points. Top row: VEGF120 tumour at (a) 0 minutes, (b) 60 minutes and (c) 24 hours. Middle row: VEGF188 tumour at (d) 0 minutes, (e) 60 minutes and (f) 24 hours. The VEGF120 presents shutdown of the vessels and then haemorrhage while the VEGF188 presents shutdown of vessels and then recovery of vasculature. Bottom Row: Time curves for the velocities of 2 regions in a tumour expressing VEGF188 (solid lines) and 2 regions in tumours expressing VEGF120 (dashed lines). The velocities were evaluated at before the drug CA-4-P was administered and 2.5, 15, 60 minutes 3,6 and 24 hours after administration. While the VEGF120 presents a rapid decrease in the velocity and a complete shutdown by 6 hours, the VEGF188 presents again a decrease in flow and then a recovery up to levels similar to the initial state.

Conclusions

A tracking algorithm has been presented. The algorithm relies on a keyhole model that describes the probable movement of a red blood cell (RBC) through the vasculature of the vessels in tumours. The algorithm requires minimal user intervention and is able to track simultaneously RBCs in several straight or tortuous vessels without cross-correlation or manual identification of the cells.