Measurement of Fluorescently Labelled Red Blood Cell Velocity Using Self-Organising Maps

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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) [1] 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 [2] 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 methodology based on ***Self-Organising Maps*** [3] that track individual cells without the need of manual tracing.

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).

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.

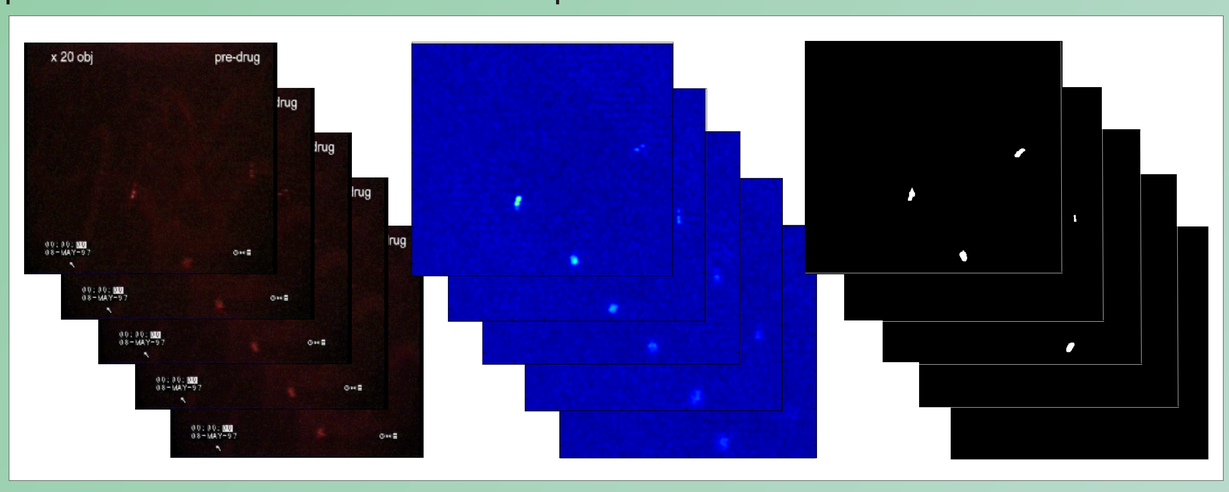

Figure 1 Movement of Fluorescently labelled Red Blood Cells shown as consecutive images. (a) The images acquired from the microscope. (b) Noise and average mean removed. (c) Data thresholded to reveal the RBC.

Methods

Self-Organising Maps

Self-Organising Maps [3] (SOM) is an algorithm with self-organising properties for a network of adaptive elements. These node elements are often termed *neurons* since their architecture is vaguely inspired on the brain interconnections and the field is called *Artificial Neural Networks*. The *neurons* receive an input signal and automatically map a set of output signals that acquire the same topological order as the input signal. Each *neuron* is densely interconnected, and receives a primary input and a great number of lateral interconnections from the outputs of other neurons.

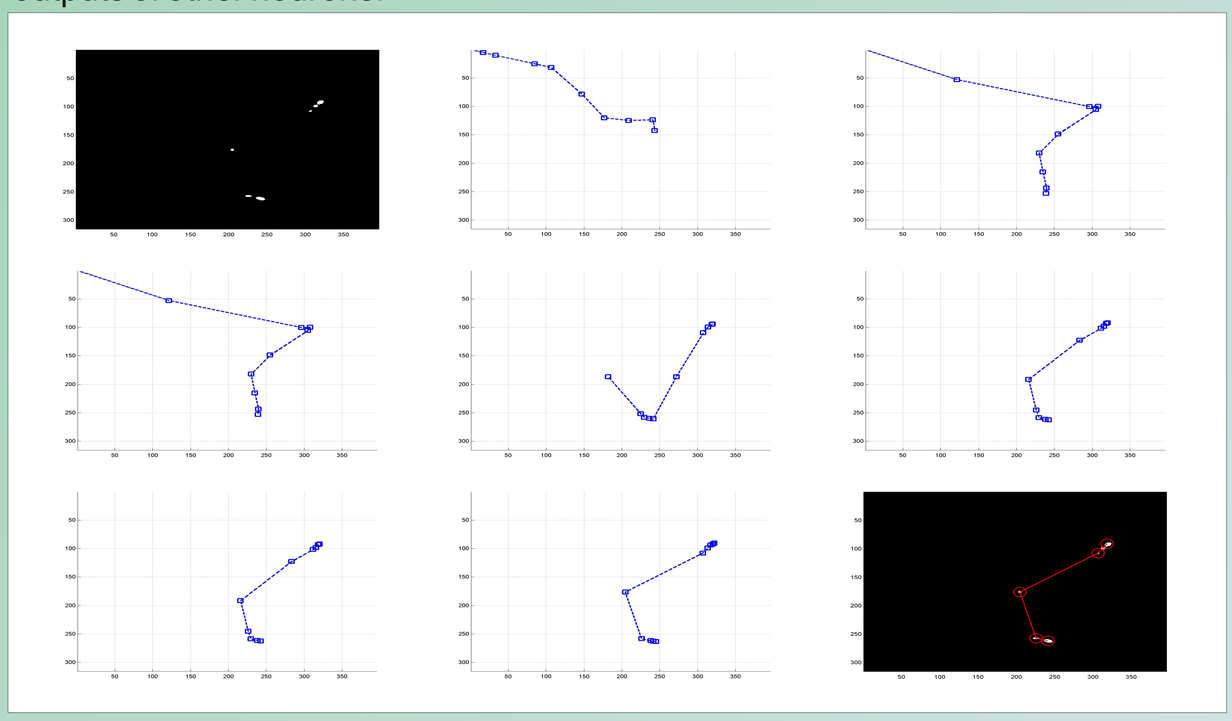

Figure 2 The process of Self-Organising of a linear SOM with 10 neurons which adapts to the RBCs that have been segmented from one frame. The SOM slowly moves towards the input data which eventually is mapped by the neurons.

The primary input will determine a "winner" node around which a cluster or neighbourhood of active neurons will be formed. The winner node with its surrounding neighbourhood will adapt to the input by modifying their spatial position (i.e. get closer to the input signal). The process continues for a number of iterations until a certain degree of adaptation is reached.

When the input is an image, certain features are extracted by the final adaptation of the neurons. The neurons will then form a set of unstructured 3D points that will be reorganised, from frame to frame, as a number of connected branches that will track the movement of the RBCs.

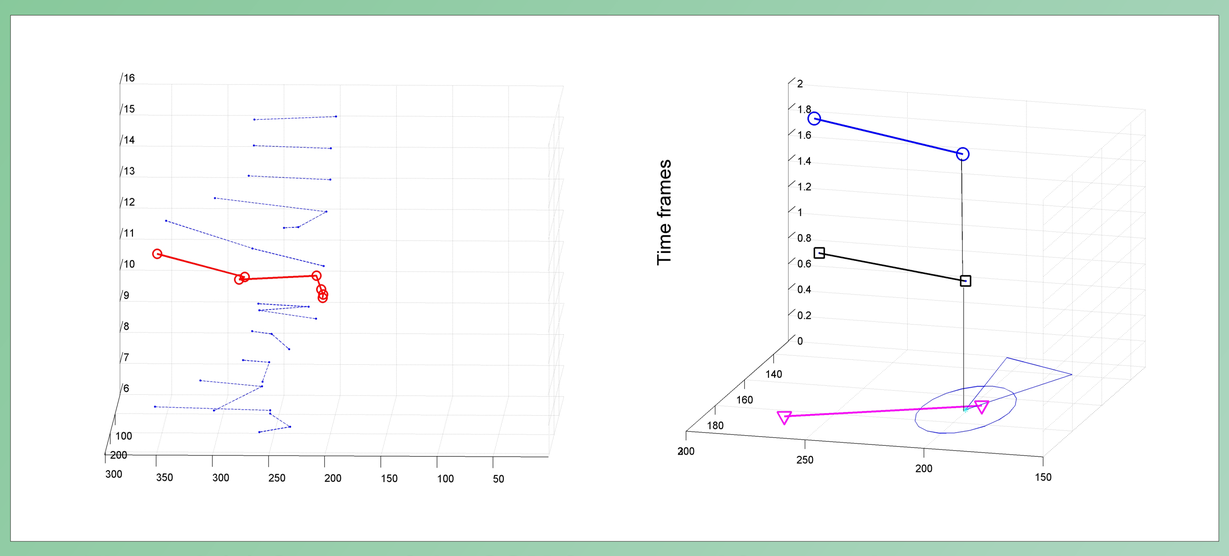

Figure 3 (a) Output of the SOM for several frames, the neurons have adapted to the topological description of the RBCs. (b) Linking process in which neurons of different time frames become a single tracking line.

The equations that define the behaviour of SOMs are presented in figure 4 where, for time t, x is the input, *i.e.* the RBCs, m_i is any node, m_c is the winner node, α is a gain factor, and N_c is the neighbourhood of the winner.

$$\|x(t) - m_c(t)\| = \min_i \|x(t) - m_i(t)\| \quad (1)$$

$$m_i(t+1) = m_i(t) + \alpha(t)\left[x(t) - m_i(t)\right] \quad i \in N_c \quad (2)$$

$$m_i(t+1) = m_i(t) \quad i \notin N_c$$

Figure 4 Equations of the Self-Organising Maps: (1) selection of winner (2) adaptation of neighbourhood around winner.

Results

Once the SOMs for each frame have been obtained, the neurons whose distance is less than a minimum level (12 pixels) are discarded assuming that they describe the same RBCs. Each neuron is linked with a *child* neuron from the next frame if it falls within a probable region defined by a cone in the direction of movement and a circle around the parent neuron (figure 3b). This process forms tracking branches shown in figure 5. The velocity is calculated by measuring the distances between neurons and dividing by the time between them.

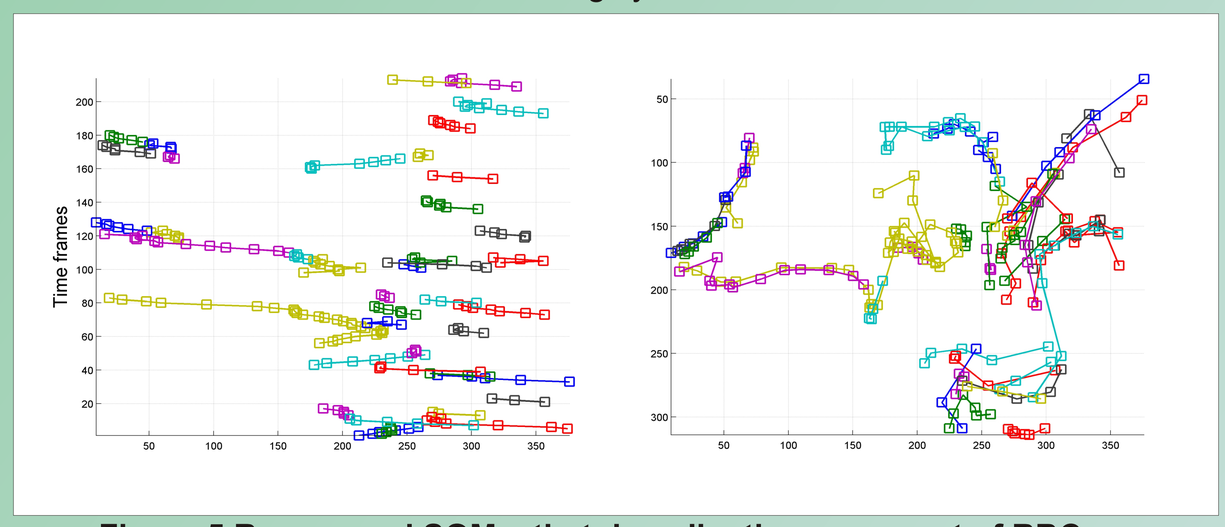

Figure 5 Rearranged SOMs that describe the movement of RBCs as tracking branches. (a) Shows the branches with time on the *y* axis and (b) shows a projection of all branches.

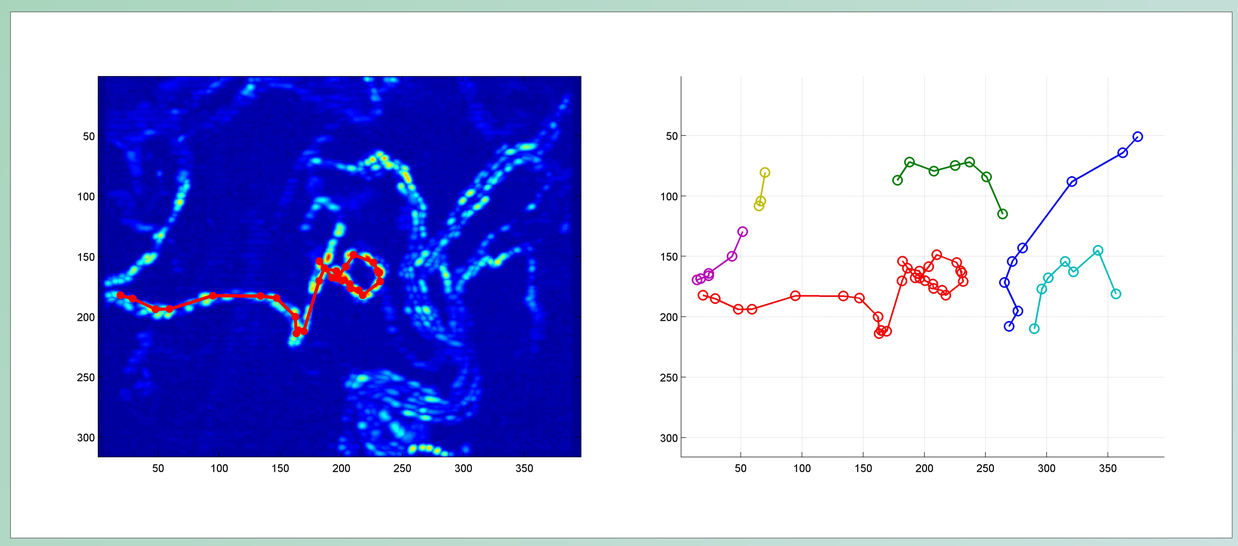

Figure 6 (a) Maximum projection of all frames and superimposed the tracking of one particularly difficult RBC. (b) Six separate tracking branches.

Conclusions

A methodology to measure velocity of RBC using SOMs has been presented. The methodology does not require hand tracing or delineation as is commonly required. Further validation is needed but the present algorithm simplifies the velocity estimation.

References

[1] Sugii Y. (2002) In vivo PIV measurement of red blood cell velocity field in microvessels considering mesentery motion, Physiol. Meas.23 403-416.
[2] Ji & Danuser (2005) Tracking quasi-stationary flow of weak fluorescent signals by adaptive multi-frame correlation. J Microsc. 220 (3) 150-167.
[3] Kohonen T (1988). Self-Organization and Associative Memory, Springer-Verlag, Heidelberg.