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Automatic Tracking of Red Blood Cells in Micro Channels using OpenCV

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Abstract. The present study aims to developan automatic method able to track red blood cells (RBCs) trajectories flowing through a microchannel using the Open Source Computer Vision (OpenCV). The developed method is based on optical flux calculation assisted by the maximization of the template-matching product. The experimental results show a good functional performance of this method.

Keywords: Red blood cells.Blood flow. Tracking. Optical flow. Template-matching. OpenCV.

INTRODUCTION

Several studies in clinical observations and experiments studies, abnormal microscopic blood flow behaviors are often associated with several disorders and diseases, such as hypertension, anemia, diabetes, cancer and malaria[1-4]. Therefore, microcirculation has attracted extensive interests and broad studies have been conducted with the help of the advances in experimental and computational techniques to provide a better understanding on the blood disorders in microcirculation. An automatic cell tracking method can be used to acquire some cell features that can change along the trajectories making it possible to diagnose several microvascular diseases.

In this study, it is presentedan automatic tracking method able to compute automatically red blood cells (RBCs) trajectories using OpenCV[5],in orderto minimize user errors and shorten handling time when compared to the traditional manual methods.

MATERIALS AND METHODS

Experimental Set-up

The physiological fluid examined was composed of Dextran 40 (Dx40) containing ~1% of human RBCs. The blood samples were submitted to washing and centrifuging processes and were stored hermetically at 4ºC until the experiments were performed at temperature of ~37ºC. All procedures were carried out in compliance with the guidelines of the Ethics Committee on Clinical Investigation of Tohoku University.For the microfluidic experiments, the microchannels were placed on the stage of an inverted microscope (IX71, Olympus, Japan) and the temperature of the stage was adjusted by means of a thermo plate controller (Tokai Hit, Japan) to 37ºC. The flow rate of the working fluids was controlled by using a syringe pump (KD Scientific Inc., USA).The images of the flowing RBC's were captured using a high speed camera (Phantom v7.1, Vision Research, USA) and transferred to a computer to be analyzed.Detailed description of the samples preparation and high-speed video microscopy system can be found elsewhere[6, 7].

The data obtained from experiments are the digital video sequences captured at the frame rate of 1000 frames/s with an exposure time of 20 μs, with frame intervals of 1000 μs and with resolution of static images 800 x 600 pixels each.

The microchannel containing the hyperbolic contraction was produced in polydimethylsiloxane (PDMS) using standard soft-lithography techniques from a SU-8 photoresist mold.More detailed description can be found elsewhere[6].

The geometry and dimensionsof the micro-fabricated channel analyzed ispresent in Figure 1.

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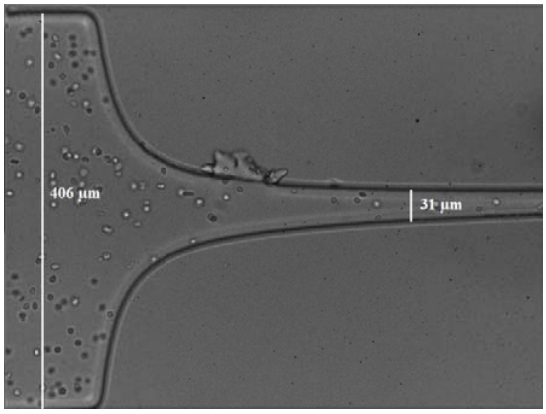

FIGURE 1. Geometry and dimensionsof the PDMS hyperbolic microchannel.

Trackingalgorithm

The algorithm first step consists in applying a Gaussian filter in order to reducesome artifacts and some noisein the images.To get better results, emphasizing the cells from the background,it was slightlyincreasedthe contrast of the all images in the video sequence.Then, a RBC is selected using the computer mouse, clicking in the center of the cell toobtaining the initial cell position *x* and *y* (pixels). From thesecoordinates itis drawnand fixed a rectangle at that instance and extracted the respective region of interest (ROI). This ROI is the template thatcontains only the selected cell and will be used by the template-matching mechanisms to search the cell exact position.

To estimate the cell shifts that occurred between two consecutive frames of a video sequence, at the first stage, the optical flow method[8]was used. The goal is to locate the cell in the next frames. This process is repeated frame by frame using the present cell position and estimating the next cell position on the next frame.

The function *calcOpticalFlowPyrLK*, in OpenCV, allows the calculation an optical flow for a low feature set using the iterative Lucas-Kanade method with pyramids. Optical flow method introduces additional conditions for estimating the actual flow. The optical flow is calculated inside a sub-window that is overlapped and referred to the cell center.

Then, for convenience, that sub-window in each frame is dynamically parameterized becoming a narrow rectangle as the cell is evolving inside the microchannel. This allows avoiding neighbor's cells that are getting close to the tracking cell. Simultaneously, it expands the sub-windows horizontally to make it possible to trackthe accelerated motion cell.

The optical flow process could present some estimation errors. To correct it, a search method based on template-matching is used.

The function *matchTemplate*(method=CV_TM_CCOEFF_NORMED)[9](OpenCV)compares the initial template given by the manuallyselected cell to the sub-window regionextracted in each frame. The process consists in calculating anormed correlation, for each pixel, between the sub-windows and the cell template. The global maximum of the correlations corresponds to a best match and to a true cell location. This process is described by equations(1),(2)and(3).

$$T'(x',y') = T(x',y') - 1/(w \cdot h) \cdot \sum_{x'',y''} T(x'',y'') \tag{1}$$

$$I'(x+x',y+y') = I(x+x',y+y') - 1/(w \cdot h) \cdot \sum_{x'',y''} I(x+x'',y+y'') \tag{2}$$

$$p = \arg\max_{x,y} \frac{\sum_{x',y'} (T'(x',y') \cdot I'(x+x',y+y'))}{\sqrt{\sum_{x',y'} T'(x',y')^2 \cdot \sum_{x',y'} I'(x+x',y+y')^2}} \tag{3}$$

Where p is the detected position of the cell, T is the cell template, I is the sub-window containing a fraction of the image frame, x, y, x', y', x'', y'' are the index values of T, I, T' and I'. Where w, h are the width and the height of the sub-window.

The template matching process can evenproduce errors. Heuristicswere added to improve the error correction process. When the position of the cell in the current frame, estimated by optical flow, is preceding the obtained position in the previous frame, this mean that cell was moving backwards (which is not valid) this position is rejected and the template matching is performed beyond that position. At the other error case, the correction is carried out based on the cell distance for two consecutive frames, as the cell motion is accelerated, the past distance covered between two frames should be shorter than the distance for the next frames. In this case, the template matching process is attempted beyond the last distance correctly computed.

RESULTS AND DISCUSSION

After selected the desired cell and obtaining the initial position x and y, the algorithm is run and it obtained the trajectory of the cell in microchannel.

In this study it was analyzed RBCs flow behavior in a hyperbolic microchannel. In Figure 2, we can see the result of the application of the proposed algorithm in twoindividual labeled RBC's. In these studies it was able to successfully obtain the complete RBC trajectory without errors.

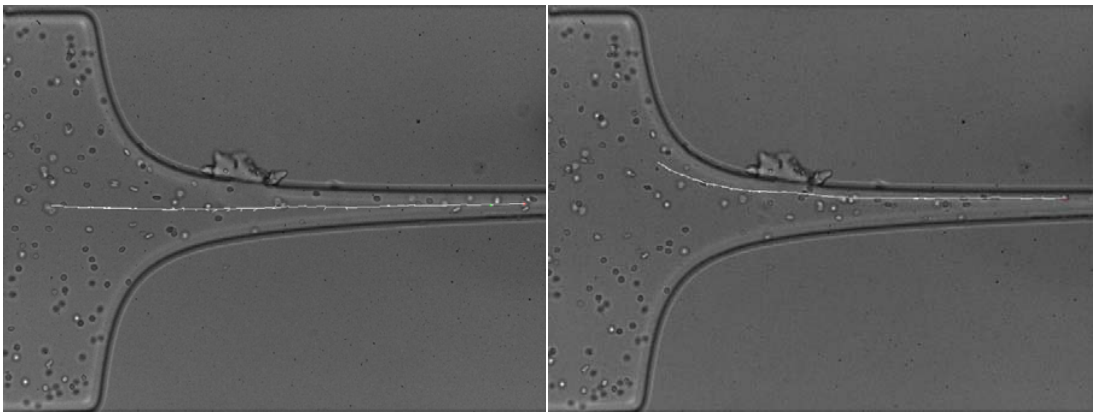

FIGURE 2. Trajectories of individual labeled RBC's with application of the proposedautomatic tracking method using openCV. A: RBC in initial position x = 71, y = 295 (pixels) and B:RBC in initial position x = 162, y = 231 (pixels).

The average time of dataprocessingwas0.64s/frame in a Pentium P6100@ 2GHz microprocessor.

We used the optical flow estimator as locater of the RBC in the next frame. That estimator can produce wrong positions. The incorporation ofthe template matching process performs correction on this error.

The average correction done by the template matching process on the error derived by the optical flow estimator is presented in Table 1.

TABLE1. Average correction (pixels) of the template matching on the error of the optical flow process estimator.

Labeled RBCs	Correction to Position X	Correction to Position Y
RBC A	11.8	0.5
RBC B	13.9	0.4
RBC C	19.7	0.5

The velocityresults for each RBCalong the microchannel, given by the proposed method, in the three selected RBCs, are presented in Figure 3.

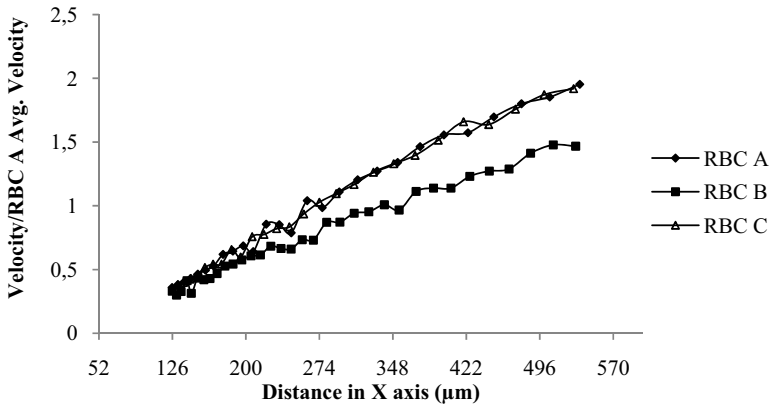

FIGURE 3. Relative velocities to RBC A average velocity of three individual labeled RBCs determined by the proposed tracking method using OpenCV.

In Figure 3, it is possible to observe that the velocity of the three RBCs increases as the cells travel through the hyperbolic microchannel, as expected. It is also shown that the RBC B has a lower velocity when compared with the remaining cells.

CONCLUSIONS

In this work, an automatic tracking of red blood cells trajectories was successfully implemented, using OpenCV.

The data processing time using this automatic method is short and the right cells trajectories are obtained correctly for the majority of the cells(76%). The position correction method was suitable to increase the performance of the optical flow process. Hence, the proposedautomatic method might be a potential way to trackRBCs trajectories through the hyperbolic microchannel.

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