A Comparison between a Manual and Automatic Method to Characterize Red Blood Cell Trajectories

Diana Pinho*, Fernando Gayubo§, Ana I. Pereira*,£ and Rui Lima*,**

* *Polytechnic Institute of Bragança, Portugal*
§*Fundación CARTIF, Valladolid, Spain*
£*Algoritmi, University of Minho, Portugal*
** *CEFT, FEUP, Porto University, Portugal*

Abstract. The current study proposes an automatic method for the segmentation and tracking of red blood cells (RBCs) flowing through a 100 μm glass capillary. The measurements obtained with the proposed automatic method are compared with a manual tracking method using nonlinear optimization techniques.

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INTRODUCTION

Blood flow in microcirculation is crucial for the normal function of tissues and organs. Detailed study of blood cells flowing in microvessels and microchannels is essential to provide a better understanding on the blood rheological properties and disorders in microcirculation [1-5]. Image analysis plays an extremely important role to obtain information about the blood rheology. However, most of the past blood flow experimental data have been performed manually [1-3]. Although manual methods can be highly reliable, these methods are relatively time consuming and can also introduce user errors into the data. As a result, it is crucial to develop image analysis methods able to get the data automatically. The present paper presents an approach able to track the RBCs trajectories automatically. Nonlinear optimization techniques were used to compare automatic and manual methods.

MATERIALS AND METHODS

Experimental Set-up

The confocal system used in this study consists of an inverted microscope (IX71; Olympus) combined with a confocal scanning unit (CSU22; Yokogawa), a diode-pumped solid-state (DPSS) laser (Laser Quantum) with an excitation wavelength of 532 nm and a high-speed camera (Phantom v7.1; Vision Research). The laser beam was illuminated from below the microscope stage through a dry 40× objective lens. The glass capillary was placed on the stage of the inverted microscope and by using a syringe pump (KD Scientific) a pressure-driven flow was kept constant (Re ~ 0.008). More detailed information about this system can be found elsewhere [1].

The confocal images were captured around the middle of the capillary with a resolution of 640×480 pixel at a rate of 100 frames/s. Two image analyses methods were used in this study: manual method (MM) and automatic method (AM).

Manual Method (MM)

A manual tracking plugin (MTrackJ) [6] of an image analysis software (Image J, NIH) [7] was used to track individual RBC. By using MTrackJ plugin, the bright centroid of the selected RBC was automatically computed through successive images for an interval of time of 10 ms. After obtaining x and y positions, the data were exported for the determination of each individual RBC trajectory.

Automatic Method (AM)

All frames were loaded and pre-processed using Matlab [8]. The region of interest was then cropped from the images with the function *imcrop*. The median function, *medfilt2*, with one mask 5x5 pixel, was applied to eliminate most of the noise and to enhance the flowing object. In Fig. 1 we can see the result of these processing steps. In the next step, the images are subject to a segmentation filter, *Sobel*. With this segmentation it is possible to separate RBCs from the background, i.e. differentiate the area of interest (the RBCs) from the not-interest area (background image). This is possible using a *threshold* method, where a definition of one or more values of separation is enough to divide the image into one or more regions. The function *iterative threshold* was applied for the sequence of all the images.
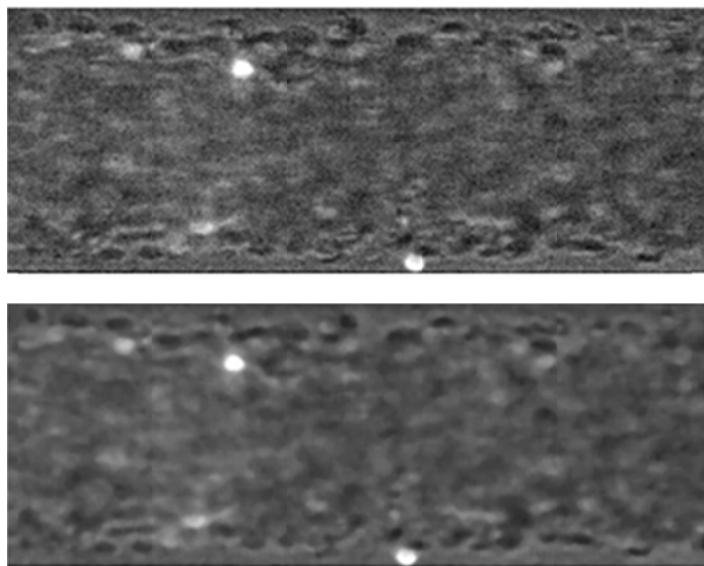

FIGURE 1. The region of interest (above) and the image filtered by using the median function *medfilt2*.

The objects are defined with the *Sobel* filter (see Fig. 2), which shows only the edge of the objects. The *Sobel* computes an approximation of the gradient of the image intensity. At each pixel point in the image, the result of the *Sobel* operator is either the corresponding gradient vector or the norm of this vector. More detailed information about this automatic method can be found elsewhere [9].
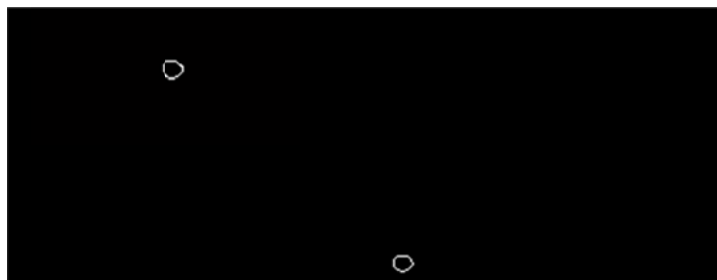

FIGURE 2. *Result of the iterative threshold method and the filter Sobel.*

RESULTS AND DISCUSSION

After obtaining series of x and y positions for both methods, data were exported for the determination of each individual RBC trajectory and to analyze the best mathematical function that approximates to the RBCs experimental flow behavior.

In this study it was measured and analyzed twenty five red blood cells at a temperature of 25 ºC. For each cell i, by using the MTrackJ plugin (MM) form ImageJ and the propose method (AM), we have obtained $\{(x_i, y_j),\ j = 1, \dots, k_i\}$ data. To compare the consistency of both methods we decided to determine the better approximation for the data by using a nonlinear optimization [10]. Hence, we consider three different functions [5] (polynomials, sum of trigonometric functions and a sum of exponential functions) defined as:

$$f_1(x,p) = \sum_{i=0}^{9} p_i x_i\ ; \quad f_2(x,a,b) = a_0 + \sum_{i=1}^{8} a_i \cos(ix) + b_i \sin(ix)\ ; \quad f_3(x,c,d,g) = \sum_{i=1}^{8} g_i e^{\left(-\left(\frac{(x-c_i)}{d_i}\right)^2\right)}.$$

where $p \in IR^{10}$, $a \in IR^9, b,\ c,\ d,\ g \in IR^8$ are the function parameters and the vector $x \in IR^{k_i}$, where i represents the cell number. To identify the functions parameters it was used the tool `cftool` present in Curve Fitting Toolbox from Matlab [11].

The error of nonlinear least squares approximation of the selected RBCs are listed in the Table 1, where **RBC** refers to the red blood cell number, $F_i = \sum_{j=i}^{ki}\left(y_i - f_i(x_i)\right)^2$ is the nonlinear least squares approximation error of the function $f_i(x,.)$, with $i = 1,2,3$, **Av** refers the error average, and **s** corresponds to the standard deviation of the errors.

Table 1 - Errors of nonlinear least squares approximation of the selected RBCs for both methods.

	Manual Method (MM)				**Automatic Method (AM)**		
RBC	F_1	F_2	F_3	**RBC**	F_1	F_2	F_3
1	0.5802	0.1998	0.4603	1	0.8738	0.2871	0.4787
2	1.2620	0.4709	0.5414	2	0.3919	0.3039	0.2493
3	0.5802	0.1717	0.4328	3	0.8912	0.6923	0.7849
4	0.9940	0.4970	1.0120	4	0.4644	0.1990	0.2304
5	1.3050	0.8490	1.2900	5	0.7941	0.5489	0.5799
6	0.1937	0.05658	0.1041	6	0.3110	0.1946	0.1991
7	0.1587	0.0754	0.0481	7	0.2538	0.1126	0.1952
8	0.4979	0.2534	0.3243	8	0.1833	0.1546	0.4099
9	0.5313	0.2296	0.2592	9	0.5139	0.4374	0.3243
10	0.8231	0.5358	0.5611	10	0.6409	0.3136	0.5406
11	0.1833	0.0834	0.1454	11	0.2259	0.1382	0.1071
12	0.4453	0.2673	0.2395	12	0.9160	0.6922	0.7696
13	0.6081	0.2685	0.5399	13	0.4071	0.2730	0.9612
14	1.6060	0.6657	1.0260	14	0.2392	0.1920	0.1821
15	0.7841	0.4323	1.4060	15	3.0220	2.0240	2.5100
16	0.6095	0.2851	1.0860	16	5.7790	4.8450	4.7570
17	1.0890	0.6595	1.6690	17	1.7780	1.6890	1.6960
18	5.2040	1.9490	11.7300	18	1.3980	0.9345	2.0040
19	0.3997	0.3997	0.2499	19	0.6008	0.3624	0.4362
20	0.5611	0.3689	0.3277	20	0.9570	0.3418	0.3859
21	0.1833	0.0965	0.1396	21	0.7467	0.3855	0.8311
22	0.9831	0.5297	0.2943	22	1.2090	0.6394	1.0780
23	1.1230	0.4937	0.4924	23	0.5624	0.2787	0.2345
24	2.9160	1.5330	1.8520	24	6.1240	2.5090	3.3190
25	4.9850	1.3170	2.0980	25	2.4780	0.9938	1.3230
Av	1.144	**0.5080**	1.1330	**Av**	1.2700	**0.7820**	0.9360
s	1.3230	**0.4670**	2.2830	**s**	1.5690	**1.0440**	1.1310

The results, from Table 1, indicate that the function $f_2(x,a,b)$ (sum of trigonometric functions) was the best approximation, to the motion of the RBCs flowing in microchannels, for both methods. This is confirmed by the

value of the error average. Another important aspect is the fact that the standard deviation of the errors is small when we use the function f_2.

CONCLUSIONS

The present study indicates that the data obtained from the proposed automatic method (AM) is equivalent to data obtained from the manual method (MM) as the function that best approaches is the same, i.e., the function f_2 (sum of trigonometric functions). Hence, the proposed automatic method is a promising way to track blood cells flowing in microchannels.

ACKNOWLEDGMENTS

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