An Improved Method for Velocity Estimation of Red Blood Cell in Microcirculation

Wen-Chen Lin, Tung-Ju Lin, Cheng-Lun Tsai and Kang-Ping Lin

Abstract— **This paper presents a coarse-to-fine combined method for dealing with large displacement situations caused by low speed of frame rate in microscopic video sequences. Motion image estimation method utilizes the modified block matching method based on image warping to perform a wide range of changes in the amount of search comparison, and then using the optical flow method to fine adjustment pixel by pixel, to complete the overall precision of the estimation. In the evaluation experiment, we have compared both current optical flow and proposed method by testing them with simulated vessel images, the results of the estimation is quite accurate.**

I. Introduction

Dynamic observation of micro-vascular mechanisms is provided to understand the disease and the relationship of the physiological function in microcirculation. The information of blood flow of microcirculation presents an major role in health evaluation and angiopathy prevention [1]-[3]. The related researches in the field of blood flow measurement that have opened up wide range of applications in biomedical field [4],[5]. Several technologies of measures for estimating blood flow information that have been used include cross correlation, space-time images, Hough transform and optical flow. Some studies have been applied to measure blood flow in vivo [6] and majorly applied to the images obtained from nail-fold capillaries [7]. The authors also demonstrated that the optical flow method is superior to cross correlation or space-time image methods for estimating RBC velocity in simulated micro-vessel images [8]. However, optical flow method for estimating RBCs velocity is limited by insufficient image resolution, low imaging frame rate, and high noise level [9]. In particular, the blood flow images are usual acquired by low sampling rate. Using a CCD camera with a frame rate of 30 fps is basic specification to consider budget savings. Therefore, frame to frame image with large displacements could be caused by using of low speed of frame rate. To evaluate the accuracy and feasibility of applying large motion estimation is challenge. In recent years, some other methods for estimating RBCs flow have also been applied, and can roughly be divided into two categories.

Optical flow Algorithm (OFA) is pixel-based representation to calculate the motion between two image frames. Each pixel has a motion vector, which has smoothness constraint determined from the nearly pixels. The OFA can only estimate the amount which is very accurate to very small displacement in frame to frame image. If objects movement is large or no features are overlapping, the OFA could not have a reliable velocity flow field.

A Block-Matching Algorithm (BMA) is a technque of pointing mapped blocks in a sequence of images for the purposes of motion estimation. The whole anchor image was split into many small blocks. Each block has a motion vector to determine whether a searched block in anchor image matches the search block in target image. The advantage to BMA is that the larger search window gets motion vector. The disadvantage of BMA is an expected loss of accuracy.

Based on the above, we propose a coarse-to-fine warping scheme for dealing with large displacement situations caused by low frame rate in in vivo microscopic video sequences. The approach is combined with block-matching, image warping and optical flow to solve above issues for velocity estimation.

II. Method

In velocity estimation process, the block diagram consists of block-matching, image warping and optical flow (see Fig. 1). In the first process, the BMA is performed by the way of a small block matching that the major purpose is to solve the changes of large displacement. Image warping is the second process to use thin plate splines (TPS) deformation as a more corresponding motion frame than the reference frame. By this way, we could use a coarse-to-fine warping scheme for dealing with large displacement situations. Block-match based motion estimation with TPS warping provides flow vectors to allow getting a suitable velocity field. Flowchart of Block-matching algorithm shows in Fig.2. Finally, we also use the OFA to do adjustment pixel by pixel, to complete the overall precision of the velocity estimation (see Fig.3).

A. Block-matching Algorithm

The basic idea of BMA is defined as flowing. Each current frame is divided into N equal-size blocks, called source blocks. Each source block is associated with a search region in the reference frame. The objective of is to find a candidate block in the search region best matched to the source block [10],[11]. The search region for mapping to a

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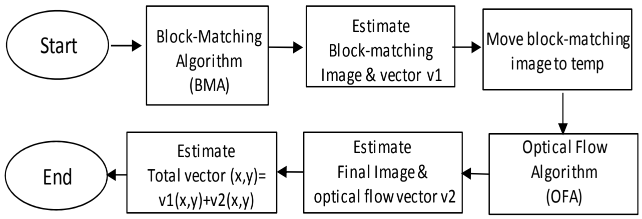


Figure 1. A flowchart of velocity estimation.

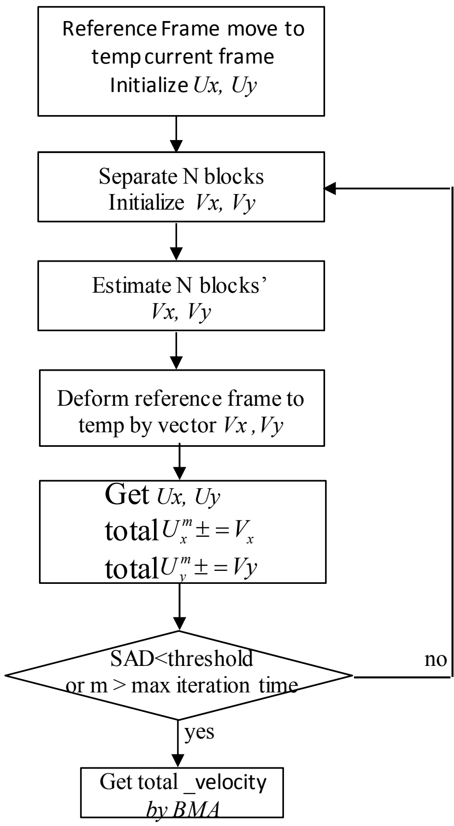


Figure 2. A flowchart of Block-matching algorithm

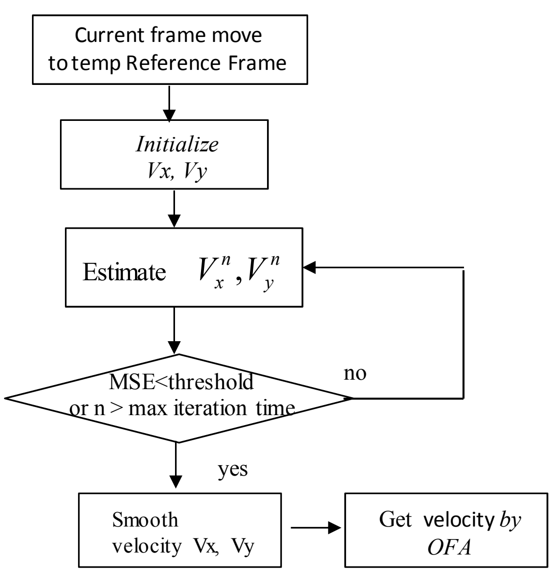


Figure 3. A flowchart of optical flow algorithm

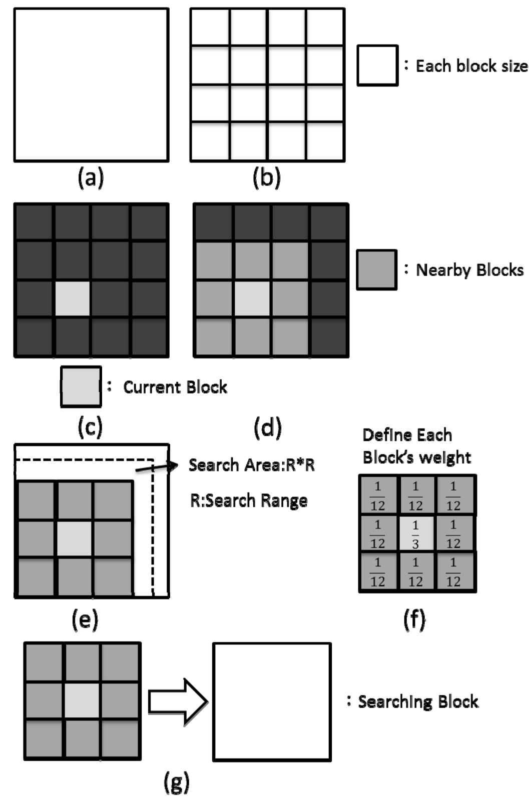


Figure 4. definition the searching block that the weight is generated.

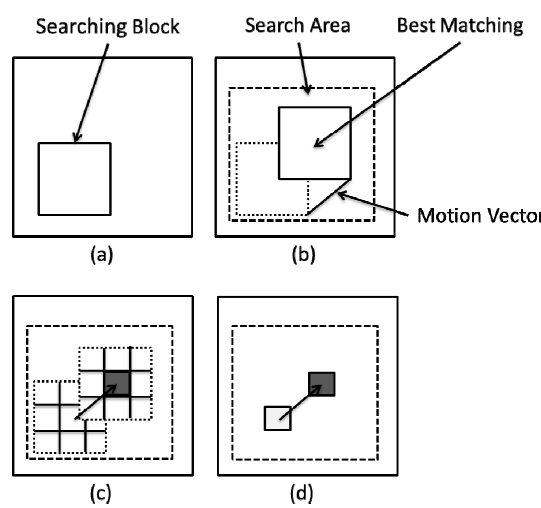


Figure 5. Search window and Block-matching motion estimation

proper macro block is constrained up to *R* pixels. The *R* is defined as one of the searching parameter. If larger motions appear, then the method requires a larger *R*. We also define the weight of searching block as shown in Fig. 4 and Fig. 5. The matching of each source is based on a cost function. There are many useful cost functions with the most common and less computationally time is Sum of Absolute Difference (SAD) given by equation (1). The relative distances between a source block and its candidate blocks are called motion vectors.

$$SAD = \sum_{i=1}^{N} |C_i - S_i|) \quad (1)$$

where N is the size of the bock, C and S are the pixels being compared in candidate block and source block, respectively.

B. Thin Plate Splines (TPS) warping

The theorem of TPS is to match the image information obtained from one image to the corresponding another image [12]. TPS can always map the corresponding region information exactly, and keep the whole image deforming energy in minimum. The two sets of image information can usually be defines as P_i and h_i. The $P_i=(x_i,y_i)$ and $h_i=(X_i,Y_i)$ are the control points belonged to the original image and targeted image, respectively. The matching transformation Φ, which matches each coordinate point in original image to targeted image based on the equation (2), can be obtained after the mapping approach. The mapping coefficients of the TPS transformation are depended on the numbers of the selected searching region and the mapping points shown in the equation (2).

$$\Phi(P) = a_0 + a_x x + a_y y + \sum_{i=1}^{n} \omega_i U(|P - P_i|) \quad (2)$$

$$W = (\omega_1 \ \ldots \ \omega_n \ a_0 \ a_x \ a_y)^T = L^{-1} M \quad (3)$$

Where $P=(x,y)$ are the location of the images. To obtain TPS mapping transformation, the most important step is to estimate the parameters ($w_1, w_2, \ldots, w_n$; a_0, a_x, and a_y) by the equation (3), which defines the mapping transformation of the corresponding points between original image and targeted image. When a location (x,y) of arbitrary point P and the distance between two arbitrary points shown as ($|P-P_i|$) in original image are substituted into equation (2), the corresponding location of the point in targeted image can be determined.

C. Optical flow algorithm

Based on Horn and Schunck method [13],[14], an approach to constrain optical flow field is developed. For Horn and Schunck method, it calculates optical flow within gradient of images by iterating the equation (4) and (5).

$$\hat{v}_x^{(k+1)}(X,t) = \bar{v}_x^{(k)}(X,t) - \frac{\partial f}{\partial x}\left(\frac{\frac{\partial f}{\partial x}\cdot \bar{v}_x^{(k)}(X,t) + \frac{\partial f}{\partial y}\cdot \bar{v}_y^{(k)}(X,t) + \frac{\partial f}{\partial t}}{\alpha^2 + \left(\frac{\partial f}{\partial x}\right)^2 + \left(\frac{\partial f}{\partial y}\right)^2} \right) \quad (4)$$

$$\hat{v}_y^{(k+1)}(X,t) = \bar{v}_y^{(k)}(X,t) - \frac{\partial f}{\partial y}\left(\frac{\frac{\partial f}{\partial x}\cdot \bar{v}_x^{(k)}(X,t) + \frac{\partial f}{\partial y}\cdot \bar{v}_y^{(k)}(X,t) + \frac{\partial f}{\partial t}}{\alpha^2 + \left(\frac{\partial f}{\partial x}\right)^2 + \left(\frac{\partial f}{\partial y}\right)^2} \right) \quad (5)$$

where k is the number of iterations, the overbar denotes weighted local averaging (excluding the present pixel), and all partials are evaluated at the point (x, y, t). The initial velocities are usually taken as zero.

III. Result and discussion

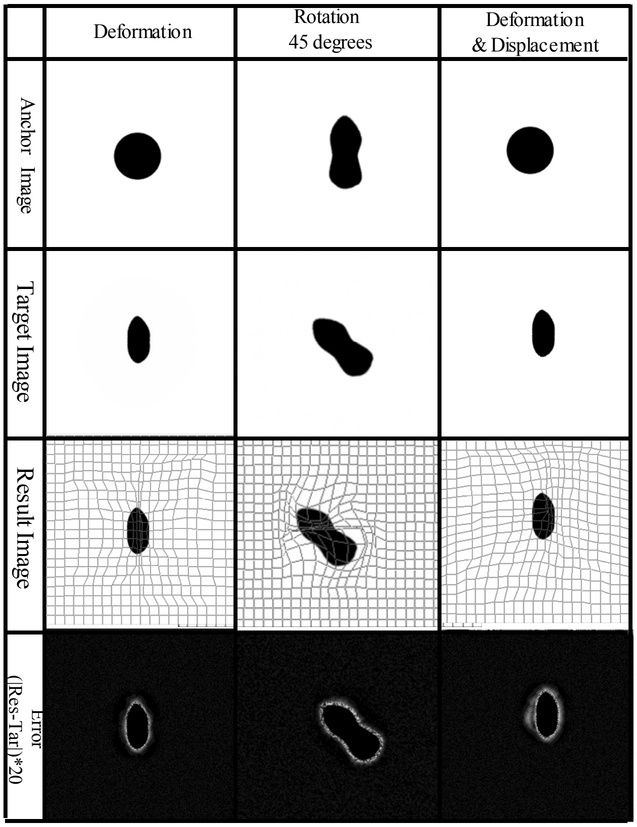
In the evaluation experiment, both current optical flow method and proposed method were applied to compare the performance by testing them on simulated vessel images. The accuracy is evaluated by calculating the mean value and standard deviation of the error results (Table 1). Simulations of various micro-vessels were considering in different size and diameter, where (a)-(c) the diameter of the micro-vessel is one, two and four times of the RBC size, respectively, (d)-(e) micro vessel changes from narrow to wide and vice versa (f)-(g), and two vessel shapes, (f) branching (g) clip. The calculation accuracy of different flow patterns and vessel shapes are examined respectively. Based on the comparison, the use of proposed method, which is superior to an optical flow method, is proposed for measuring RBC velocity.

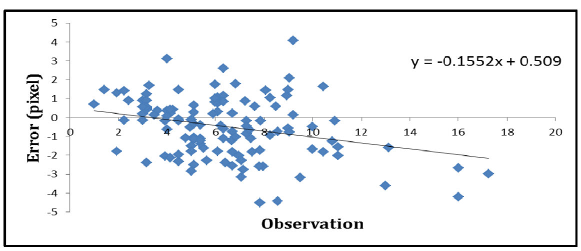
In addition, RBCs exhibit different types of motions and different deformed shapes in response to local flow conditions [15]. The simulations of RBC consist of deformation, rotation and displacement were applied to evaluate our proposed method. The results can be seen from Fig. 6. The RBC's motion estimation does not have any impact with deformed shape and large displacement.
Furthermore, our proposed method has been used to measure the microcirculation blood flow in vivo. Fluorescent microscopy video of the mouse brain microcirculation was acquired by CCD in a special resolution of 1.42μm and frame rate of 30 frames per second. The results of RBCs velocities estimation for the long straight vascular estimated flow velocity 204 ~ 285 μm /sec, the tortuous vessels estimation flow velocity of 327~ 444 μm / sec. In our verification (see Fig. 7), the algorithm error compared with the human eye, RBC's velocity estimation has average error of 1.25 ± 0.95 pixel in a long straight microvessel, and has average error of 1.8 ± 1.35 pixel in tortuous microvessel, the results of the estimation is quite accurate. The results compared with the real rate described in the literature are matched. It is confirmed that the present method can accurately estimate the blood flow velocity in microvessel.

Table 1. The average error between two methods, Mean ± S.D. (pixel/frame)

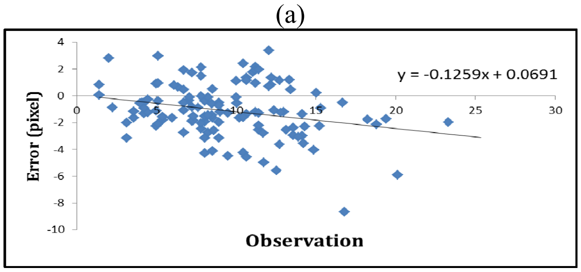
Measurement / Shape pattern	Optical flow[8]	Propose Method
(a)	0.72±1.16	0.47±0.51
(b)	1.05±1.17	0.66±0.55
(c)	1.12±1.19	0.68±0.61
(d)	1.28±1.17	0.68±0.45
(e)	1.07±1.16	0.82±0.44
(f)	1.22±1.17	0.78±0.65
(g)	1.16±1.18	0.70±0.45
Total error	1.09±1.17	0.68±0.52

Figure 6. The RBC's motion estimation based on different deformed shapes.





(a)



(b)

Figure 7. Error assessment between proposed method and human eye observation (a) in long straight microvessel and (b) in tortuous microvessel.

IV. CONCLUSION

This contribution proposes a method for motion estimation of RBCs in microcirculation to improve the accuracy and feasibility of applying large motion estimation. According to the obtained results, it can be found that our proposed method has a potential to solve optical flow issues with large displacements caused by low speed of frame rate. About advancing application, we will further focus on the image processing algorithm to provide automatic blood flow velocity measurement.

REFERENCES

[1] S. Bertuglia, P. Leger, A. Colantuoni, G. Coppini, P. Bendayan and H. Boccalon, "Different flowmotion patterns inhealthy controls and patients with Raynaud's phenomenon," Technol Health Care, 7: 113-123, 1999.

[2] E. Bonacci, N. Santacroce, N. D'Amico, and R. Mattace., "Nail-fold capillaroscopy in the study of microcirculation in elderly hypertensive patients," Arch. Gerontol. Geriatr., suppl. 5: 79-83, 1996.

[3] M. R. Cesarone, L. Incandela, A. Ledda, De Sanctis M T, R. Steigerwalt, L. Pellegrini, M. Bucci, G. Belcaro and R. Ciccarelli, "Pressure and microcirculatory effects of treatment with lercanidipine in hypertensive patients and invascular patients with hypertension," Angiology, 51: 53-63, 2000.

[4] David J. Chaplin, Peggy L. Olive, and Ralph E. Durand, "Intermittent Blood Flow in a Murine Tumor: Radiobiological Effects", Cancer research, 47: 597-601, 1987.

[5] Arthur M. Iga, Sandip Sarkar, Kevin M. Sales, Marc C. Winslet and Alexander M. Seifalian, "Quantitating Therapeutic Disruption of Tumor Blood Flow with Intravital Video Microscopy," Cancer Research, 66: 11517-11519, 2006.

[6] W.-C. Lin, C.-C.Wu, T.-C. Huang, W.-C. Lin, B.Y. Chiu, R.-S. Liu, and K.-P. Lin, "Red blood cell velocity measurement in rodent tumor model: an in vivo microscopic study," Journal of Medical and Biological Engineering, vol. 32, no. 2, pp. 97–102, 2012.

[7] C.-C. Wu, W.-C. Lin, G. Zhang, C.-W. Chang, R.-S. Liu, K.-P. Lin, and T. -C. Huang, "Accuracy evaluation of RBC velocity measurement in nail-fold capillaries," Microvascular Research, vol. 81, no. 3, pp. 252–260, 2011.

[8] T.-C. Huang, W.-C. Lin, C.-C. Wu, G. Zhang, and K.-P. Lin, "Experimental estimation of blood flow velocity through simulation of intravital microscopic imaging in micro-vessels by different image processing methods," Microvascular Research, 80(3):477–483, 2010.

[9] You, S. , Ataer-Cansizoglu, E. Erdogmus, D. Massey, M. Shapiro, N., "Microvascular blood flow estimation in sublingual microcirculation videos based on a principal curve tracing algorithm," 2012 IEEE INTERNATIONAL WORKSHOP ON MACHINE LEARNING FOR SIGNAL PROCESSING, Sept. 23–26, 2012.

[10] Bernd Kitt, Benjamin Ranft, and Henning Lategahn, "Block-Matching based Optical Flow Estimation with Reduced Search Space based on Geometric Constraints," 2010 13th International IEEE Annual Conference on Intelligent Transportation Systems Madeira Island, Portugal, Sept. 19-22, 2010.

[11] Djamal Boukerroui, J. Alison Noble, Michael Brady, "Velocity Estimation in Ultrasound Images: A Block Matching Approach," Information Processing in Medical Imaging Lecture Notes in Computer Science, Vol. 2732:586-598, 2003.

[12] Y.-C. Tsai, H.-D. Lin, Y.-C. Hu, C.-L. Yu and K.-P. Lin, "Thin-Plate Spline Technique for Medical Image Deformation," Journal of Medical and Biological Engineering, 20(4): 203-210, 2000.

[13] M. Manjunatha and M. Singh, "Computerised visualisation from images of blood flow through frog mesenteric microvessels with multiple complexities," Medical and Biological Engineering and Computing, 40: 634-640, 2002.

[14] M. Manjunatha, Swaroop S. Singh, and Megha Singh, "Blood flow analysis in mesenteric microvascular network by image velocimetry and axial tomography," Microvascular Research, 65: 49-55, 2003.

[15] Jae Hong Jeong , Yasusiko Sugii, Motomu Minamiyama, Koji Okamoto, "Measurement of RBC deformation and velocity in capillaries in vivo," Microvascular Research, 71: 212-217, 2006.