Measurement of Leukocyte Motions in a Microvessel Using Spatiotemporal Image Analysis

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Abstract— This paper describes a method for recognizing and measuring the motion of each individual leukocyte in microvessel from a sequence of images. A spatiotemporal image is generated whose spatial axes are parallel and vertical to vessel region contours. In order to enhance and extract only leukocyte traces with a turned velocity range even under noisy background, we use a combination of a filtering process using Gabor filters with sharp orientation selectivity and a subsequent 3D spatiotemporal grouping process. The proposed method is shown to be effective by experiments using image sequences of two kinds of microcirculation, rat mesentery microvessels and human retinal capillaries.

Index Terms—Leukocyte, Gabor filter, Microvessel, Spatia axis.

I. INTRODUCTION

There have been considerable improvements on imaging techniques of microcirculation for the last few decades. These imaging techniques have made possible visualization of the motion of blood cells in microvessels. For the purpose of both clinical use and physiological investigation, a key problem is to measure the velocity and flux of blood cells[1]~[5]. In spite of the improvements of the imaging techniques, there are few well-developed techniques for analyzing a large amount of image sequences obtained using these techniques. Therefore, the quantitative analysis of microcirculation has been quite limited. Although several image processing systems have been developed, these systems only deal with tasks which can be performed using simple image processing techniques such as the measurement of erythrocyte velocity[6], platelet adhesion[7], and arteriolar vasomotion[8] using differential operation, frame subtraction, and edge detection, respectively.

In this study, we deal with the problem of recognizing and measuring the motion of each individual leukocyte in microvessels. In order to measure the velocity of leukocytes, each leukocyte must be recognized and segmented out because each of leukocytes is flowing separately. In the previous work, these measurement have been performed manually, for example, by counting the number of video frames[1]~[5], which places limits on their accuracy, reproducibility and the amount of data can be collected.

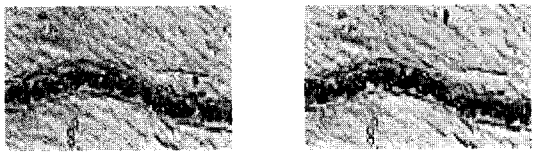
In this paper, we extend the method so as to be applicable to the recognition of leukocyte motion with an arbitrary specified velocity range even under noisy background, and show the potential usefulness of the method for different kinds of image sequences of microcirculation.

II. SPATIONTEMPORAL IMAGE ANALYSIS FOR EXTRACTING MOVING LEUKOCYTES

2.1 Spatiotemporal Image

Fig. 1 shows two frames of a microscopic image sequence of a rat mesentery microvessel with moving leukocytes. The image size is 400x200 pixels. The frame interval of the sequence is 1/30sec. The sequence consists of 100 frames. It is difficult to find leukocytes from only one frame, whereas we can observe moving leukocytes that adhere to microvessel walls from continuous video images (although it is troublesome to accurately identify and enumerate all the leukocytes). Fig. 2 shows another example of a microcirculation image (411x249 pixels), which was taken from fluorescent angiography by a laser scanning ophthalmoscope in order to measure microcirculation of the human ocular fundus. In this case, it is hard to observe the leukocyte flow (although it is visible) even from continuous video images because a leukocyte is imaged only as a small and noisy fluorescent dot and its velocity is fast relative to the video rate.

The basic approach to measuring such motions is based on the method of spatiotemporal image analysis[9].



(a)23th frame (b)46th frame

Fig. 1 Two frames in an image sequence of rat mesentery microvessels

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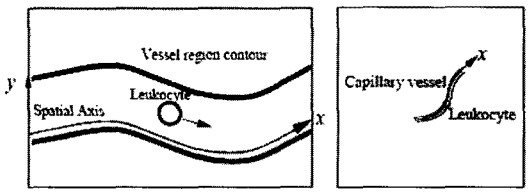
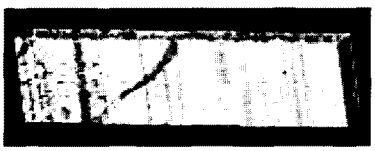
We generate a 3D spatiotemporal image whose spatial axes are parallel and vertical to the flowing direction of blood cells (Fig.3(a)).

Fig.2 Fluorescent angiographic image of human ocular fundus taken by a scanning laser ophthalmoscope



(a) (b)

Fig.3 Generation of a spatiotemporal image from the image sequence of microcirculation



(a)

(b)

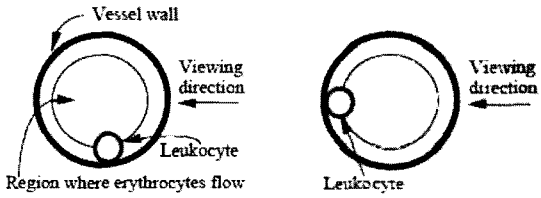
Fig.4 Generated spatiotemporal images
(a)Spatiotemporal image just along an automatically extracted vessel wall contour
(b)Spatiotemporal image along a curve 10 pixels apart from the vessel wall contour

Fig. 4 shows the spatiotemporal images generated from the image sequence shown in Fig. 1. The leukocyte trace in Fig. 4(a) is relatively easy to see in the image, whereas the ones shown in Fig. 4(b) are difficult to detect because they are obscured by erythrocytes. The leukocytes that we are trying to extract are adhering to the tube wall, while erythrocytes flow through the central portion Fig. 5 shows the cross section of a vessel; the region which erythrocytes pass through is shaded. If a vessel is so narrow that only one leukocyte passes through, we generate a 2D spatiotemporal image whose spatial axis is taken along the extracted vessel (Fig.3(b)). Fig. 6 shows the spatiotemporal image obtained from the image sequence of the human ocular fundus. Its spatial axis is the curve which is shown as a white line in Fig. 2. The trace of a leukocyte manages to be observed as a sequence of noisy florescent dots in the middle of Fig. 6. The sequence of fluorescent dots is almost horizontal because the velocity of a leukocyte is high relative to the video rate. The previous method is not suitable for such a case because the enhancement of leukocyte traces is performed only to the diagonal direction in a spatiotemporal image.

2.2 Spatiotemporal Filter for Enhancement of Leukocyte Traces

Given a spatiotemporal image as shown in Fig. 4 or Fig. 6, we try to enhance components originated only from leukocyte motion and suppress other components. In designing a filter for leukocyte enhancement, we consider the following constraints:

-The apparent shape and size of leukocytes are known to some extent.
-The minimum and maximum velocities of leukocytes are limited.



(a) (b)

Fig.5 Axial cross-section of a vessel and viewing direction (a)Leukocyte whose motion is visualized as motion along contours of a vessel region (b)Leukocyte obscured by erythrocytes

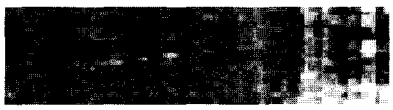


Fig.6 Generated spatiotemporal image

The apparent shape of a leukocyte in the images of a rat mesentery microvessel is a small ring-like shape (as shown in Fig.3). So, the leukocyte traces are imaged as lines with a limited thickness in the 2D spatiotemporal images whose spatial axis is parallel to vessel contours. In Fig. 4(b), there are three traces originated from leukocyte motion. Furthermore, the orientation of traces have a limited range of angles dependent on the minimum and maximum velocities of a leukocyte. Also, in Fig. 6, the trace of a leukocyte can only be observed as a sequence of very noisy fluorescent dots, but we can

assume that the orientation of traces have a limited range of angles dependent on the minimum and maximum velocities of a leukocyte. In the following, we design a filter which can effectively enhance the leukocyte traces.

We use Gabor filters having sharp orientation selectivity in order to deal with noisy background and an arbitrary specified range of velocities. Gabor filters have been utilized for optical flow computation[10] and texture analysis [11]. Here, we use Gabor filters in order to recognize and segment out moving objects, i.e. leukocytes, from a noisy image sequence mainly based on motion information.

We consider the 2D Gabor function give by

$$h(x,t) = g(x',t') \bullet \exp(2\pi j w_{xo} x') \quad (1)$$

Where $(x',t') = (x\cos\phi + t\sin\phi, -x\sin\phi + t\cos\phi)$ are rotated coordinates, and where

$$g(x,t) = \left(\frac{1}{2\pi\lambda\sigma^2}\right) \bullet \exp\left(-\frac{x^2 + (t/\lambda)^2}{2\sigma^2}\right) \quad (2)$$

The Gabor filter in the frequency domain is represented by

$$H(w_x, w_t) = \exp\{-2\pi^2\sigma^2[(w_x' - w_{xo}')^2 + (w_t')^2\lambda^2]\} \quad (3)$$

where $(w_x', w_t') = (w_x\cos\phi + w_t\sin\phi, -w_x\sin\phi + w_t\cos\phi)$. We use a Gabor filter with relatively large σ and large $\lambda(\lambda > 1)$ so as to make a filter have sharp orientation selectivity. Also, we use only real components of the Gabor filters because our aim is to enhance dark or brightlines.

Although we assume that the minimum and maximum velocities of leukocytes are limited, the range of the velocity cannot be covered with only one Gabor filter having sharp orientation selectivity. So, we use multiple Gabor filters at different angles between the minimum and maximum angles dependent on the range of velocities of leukocytes. We use the maximum or minimum values among these filter outputs at multiple angles as the final output of filter. If we enhance brightlines, we use the maximum value. Otherwise, we use the minimum value. When we deal with a 3D spatiotemporal image, we apply 3D Gabor filters. We use the 3D Gabor function given by

$$H(w_x, w_y, w_t) = \exp\{-2\pi^2\sigma^2[(w_x' - w_{xo}')^2 + (w_y')^2 + (w_t')^2\lambda^2]\} \quad (4)$$

where $(w_x', w_y', w_t') = (w_x\cos\phi + w_t\sin\phi, w_y, -w_x\sin\phi + w_t\cos\phi)$, Currently, we consider rotation only around w_y-axis of coordinates. However, the performance of leukocyte enhancement may be improved by considering rotation having more degrees of freedom.

2.3 Extracting and Grouping Leukocyte Trace Regions

We perform thresholding operations for filtered spatiotemporal image. We use two threshold values. We select the first threshold value so as to extract regions originated only from true leukocyte traces even if extracted regions do not cover wide area of true regions. On the other hand, we select the second threshold value so as to cover the regions originated from true leukocyte traces as much as possible even if some false regions are extracted. Among the regions extracted using the second threshold, we select only the regions connected with the regions extracted using the first threshold. These regions are regarded as the candidate regions of leukocyte traces.

In order to grouping and selecting leukocyte trace regions, we use the constrains that the velocity of each leukocyte is almost uniform, and the size of leukocytes is know to some extent. We can assume that the leukocyte traces have elongate shape with almost uniform orientation and known diameter in the 3D spatiotemporal image. So, the candidate regions which originated from the same leukocyte trace can be expected to form an elongate cluster having a restricted extent around its principal axis. We try to find such clusters from 3D candidate regions extracted using the thresholding operations.

Our clustering method is based on region growing. First, we select the largest region as an initial region. We use this region as a seed region to merge the regions which belong to the same leukocyte traces. We approximate the shape of leukocytes by the principal axis of the regions. Next, we select a region to be merged with the seed region. We select a candidate region which satisfies the condition that the maximum distance to the principal axis from the candidate region is the shortest of all the regions. If the maximum distance is smaller than a threshold, we merge the region with the seed region and recomputed the new principal axis based on the seed region and the merged region to repeat this region growing process. Otherwise, we stop this region growing process and find a next seed region to start another region growing process. Finally, we can obtain several clusters which have elongate shapes. Each cluster can be regarded as an individual leukocyte trace. The velocity of a leukocyte can be computed from the direction of the principal axis of the cluster.

III. EXPERIMETAL RESULTS

3.1 Rat Mesentery Microvessels

Fig. 7 shows the output images of the spatiotemporal filter for the spatiotemporal images generated from the image sequence of rat mesentery microvessels. The original images and the generated spatiotemporal images are shown in Fig. 1 and Fig. 4, respectively. We applied the set of 3D Gabor filters shown in Equation (4). The angels of Gabor filters were $30°$,$40°$, and $50°$. The parameters in Equation (4) were set at $w_{xo} = 0.1$, $1/2\pi\sigma = 0.02$ and $\lambda = \sqrt{2}$. We took the minimum of their outputs as the final output. In Fig. 7, the output of the spatiotemporal filter effectively enhanced three leukocyte traces in spite of heavy noisy.

(a)

(b)

Fig. 7 Filtered spatiotemporal images (a)Filtered result corresponding to Fig. 4(a). (b)Filtered result corresponding to Fig. 4(b)

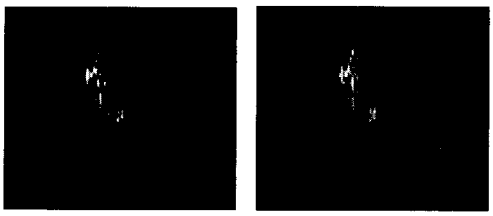
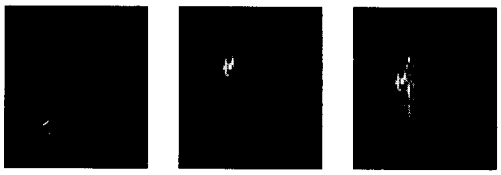
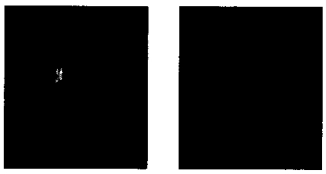


Fig.8 Candidate regions of leukocyte traces extracted by thresholding the spatiotemporalfiltered image

We performed the thresholding operations described in 2.3 for the output of the spatiotemporal filter. Fig. 8 is a stereo display of 3D regions extracted by thresholding. We applied the clustering method to the 3D regions shown in Fig. 8. We could obtain five clusters as shown in Fig. 9. In the image sequence, there were four moving leukocytes, and four traces shown in Fig. 9(a)~(d) corresponded to these four leukocytes. Although a cluster shown in Fig. 9(e) was a false region, it can be easily regarded as noise because it was very small compared with other clusters. By using the 3D spatiotemporal clustering method, we could extract four individual leukocyte motions.



(a) (b) (c)

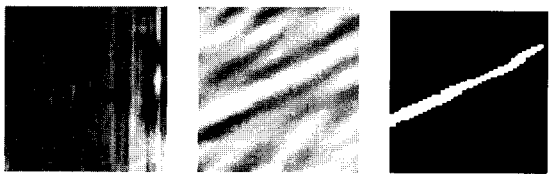


(d) (e)

Fig.9 Results of 3D spatiotemporal clustering method

3.2 Human Retinal Capillaries

Fig. 10 shows the results of the spatiotemporal filter for the image sequence of human retinal capillaries in fluoresce in angiography. The original images and the generated spatiotemporal images are shown in Fig. 2 and Fig. 6, respectively. First, we applied the Gaussian filter to the spatiotemporal image, and sub sampled along the vertical axis to enlarge it vertically. Next, we applied the set 2D Gabor filters shown in Equation (3) to the vertically enlarged spatiotemporal image shown in Fig. 10(a). The angles of Gabor filters were $50°$, $55°$, and $60°$. The parameters in Equation (3) were set at $1/2\pi\sigma = 0.02$, and $\lambda = \sqrt{2}$. We took the maximum of their outputs as the final output. In Fig. 10(b), the output of the spatiotemporal filter effectively enhanced a leukocyte trace in spite of fast motion of a fluorescent dot. After the thresholding operations, we could extract a leukocyte trace as shown in Fig. 10(c).



(a) (b) (c)

Fig.10 Results for human ocular fundus angiographic images (a)Vertically enlarged spatiotemporal image (b)Spatiotemporal filtered image (c)Extracted leukocyte trace

IV. CONCLUSIONS

We have described a method for automatically extracting leukocytes in a microvessel. We regarded the recognition of moving leukocytes as the problem of extracting leukocyte traces in a spatiotemporal image. We have used a set of Gabor filters having sharp orientation selectivity in order to effectively enhance leukocyte traces under noisy background. Furthermore, the 3D spatiotemporal clustering method have been developed to identify each individual leukocyte motion. We have shown experimentally that the method is effective for mage sequences of two different kinds of microcirculation. We believe that the proposed method can be extended from the particular problem addressed here so as to deal with general moving object recognition problems under noisy background.

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REFERENCES

[1] G.W.Schmid-Schonbein, S.Usami, R.Skalak, and S.Chien, "The interaction of leukocytes and erythrocytes in capillary and postcapillary vessels", Microvas. Res., vol. 19, pp. 45-70, 1980

[2] H.Komatsu, A.Koo, and P.H.Guth, "Leukocyte flow dynamics in the rat liver microcirculation", Microvas. Res., vol. 40, pp. 1-13, 1990

[3] U.H.von Andrian, J.D.Chambers, L.M.Mcevoy, R.F.Bargaze, K.E.Arfors, and E.C.Butcher, "Two-step model of leukocyte-endothelial cell interaction in inammation: distinct roles for lecam-1 and the leukocyte 2 integrins in vivo", Proc. Natl. Acad. Sci. USA New York, vol. 88, pp. 7538-7542, 1991

[4] S.Wolf. O.Arend, H.Toonen, B.Bertram, F.Jung, and M.Reim, "Retinal capillary blood flow measurement with a scanning laser ophthalmoscope," Ophthalmology, vol. 98, no. 6, pp.996-1000, 1991

[5] T.Tanaka, K.Muraoka, and K.Shimizu, "Fluorescein fundus angiography with scanning laser ophthalmoscope," Ophthalmology, vol. 98, no. 12, pp.1824-1829, 1991

[6] B.P.Fleming, B.Klitzman, and W.O.Johnson, "Measurement of erythrocyte velocity by use of a periodic differential detector," American Journal of Physiology, vol. 249, (Heart Circ. Pysiol. 18)H899-H905, 1985

[7] N.Tateishi, M.Okazaki, and T.Shiga, "Determination and kinetics of platelet adhesion onto material surfaces," Journal of Membrane Science, vol.41, pp.315-322, 1989

[8] C.Y.J.Yip, S.Y.Aggarwal, K.R.Diller, and S.C.Bovik, "Simultaneous multiple site arteriolar vasomotion measurement using digital image analysis," Microvaslular Research, vol.41, pp. 73-83, 1991

[9] R.C.Bolles and H.H.Barker, "Epipolar plane image analysis: A technique for analyzing motion sequences," Proc. 3rd Int. Symposium on Robotics Research, pp. 41-48, 1986

[10] D.J.Heeger, "Optical flow from spatiotemporal filters," Proc. 1st ICCV, pp.181-190, 1987

[11] S.C.Bovik, M.Clark, and W.S.Geisler, "Multichannel texture analysis using localized spatial filters," IEEE Trans. On PAMI, vol.12, no.1, pp.55-73,1990

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