TRACKING LEUKOCYTES FROM *IN VIVO* VIDEO MICROSCOPY USING MORPHOLOGICAL ANISOTROPIC DIFFUSION

Scott T. Acton[1] *and Klaus Ley*[2]

[1]Department of Electrical and Computer Engineering
[2]Department of Biomedical Engineering and Cardiovascular Research Center, School of Medicine
University of Virginia, Charlottesville, Virginia 22904
acton@virginia.edu

ABSTRACT

The study of inflammatory disease hinges upon the behavior and movement of leukocytes and their interaction with the endothelium. We put forth a method for tracking leukocytes *in vivo*, whereas tracking has been demonstrated previously only for *in vitro* experiments. The tracker is based on the enhancing capability of morphological anisotropic diffusion, a partial differential equation model for adaptively filtering imagery that retains structures of interest. Morphological anisotropic diffusion excels over standard diffusion in the ability to preserve objects of a certain shape and scale, and it improves upon standard morphological filters in terms of edge preservation and adaptive smoothing. We use the video frames enhanced by morphological diffusion for edge-based registration and background removal in the tracking process.

1. INTRODUCTION

The analysis of leukocyte rolling is a fundamental part of the investigation of inflammatory diseases such as arthritis and multiple sclerosis. *Rolling* denotes the downstream movement of a leukocyte (white blood cell) in a shear flow field while the cell is in continuous or intermittent contact with the vessel wall. Several salient features of leukocyte rolling are relevant to inflammation including rolling leukocyte flux, leukocyte rolling velocity, leukocyte rolling acceleration and rolling leukocyte volume fraction. In this paper, a method for automated tracking for data collection within the study of rolling leukocytes *in vivo* (within a living subject) is demonstrated. Previously, limited success has been achieved with automated cell tracking only for *in vitro* experiments in a controlled flow chamber. A major advance in tracking is pursued here to track rolling leukocytes *in vivo*, which has not been possible with existing video processing techniques. Current *in vitro* studies utilize tedious manual (frame-by-frame) recording of cell position for tracking.

The leukocyte tracking system utilizes shape-sensitive morphological anisotropic diffusion for enhancement, for background registration and background removal, and for leukocyte detection. Combined with adaptive template matching and cell position prediction, successful tracking of leukocytes *in vivo* is demonstrated for the first time. We are exploring the viability of the approach using video recordings of rolling leukocytes observed by intravital microscopy in the mouse cremaster muscle, the rat mesentery, and the mouse carotid artery with and without fluorescent labeling. We are investigating the diffusion-based approach in comparison with existing trackers used with *in vitro* studies (*viz.*, the centroid and correlation trackers). For the study of rolling leukocytes *in vivo*, the diffusion-based tracking system allows the computation of the velocity and acceleration of many leukocytes at many points in time and space, which is likely to result in new discoveries regarding the molecular mechanism of inflammation. Automated tracking will improve the study of leukocyte rolling dramatically, allowing an increase in experimental throughput and removing possible investigator bias. Cell tracking will not only accelerate and enhance analysis, but will also enable the generation of new biomedical knowledge not available through existing technology.

2. TRACKER OVERVIEW

Compared to *in vitro* leukocyte tracking in a flow chamber, tracking of leukocytes *in vivo* is a significantly more challenging problem. The first challenge encountered with *in vivo* video microscopy is the movement of the entire vessel. For example, in our mouse cremaster studies, abrupt movements are encountered with mouse respiration. The movements cause loss of track, erroneous feature computation (e.g., velocity measurement) and motion blur. We propose a diffusion-based registration system that extracts the vessel boundaries using an edge detection algorithm and then registers the boundaries to counteract the movement. The proposed edge detection method is based on a morphological anisotropic diffusion technique.

A unique challenge associated with the *in vivo* video microscopy (in contrast to *in vitro* microscopy) is the extreme level of noise and clutter. Because we know the approximate scale and shape of the objects of interest (the rolling leukocytes), we can apply morphological diffusion methods of video enhancement. Morphological anisotropic diffusion permits the extraction of the rolling leukocytes and the removal of off-scale clutter and noise from the video sequence without the boundary degradation encountered with standard morphology. As a result, a robust tracking procedure can be implemented

0-7803-6725-1/01/$10.00 ©2001 IEEE

that outperforms standard centroid-based and correlation-based tracking algorithms.

Although effective tracking of leukocytes *in vivo* requires several video processing problems to be solved, this paper focuses on the use of morphological anisotropic diffusion for enhancement of the imagery used in leukocyte tracking.

3. ALGORITHMIC DETAILS

3.1 Morphological Anisotropic Diffusion

The backbone of the leukocyte tracking system is formed by morphological anisotropic diffusion [2]. The diffusion process is utilized in the background registration and removal process, the video enhancement process and the deformable template matching process.

With anisotropic diffusion, a partial differential equation (PDE) model is used to smooth the imagery and to enhance certain features such as edges. The innovation of anisotropic diffusion is the introduction of an adaptive diffusion coefficient $c(\mathbf{x})$ that encourages intra-region smoothing over inter-region smoothing. If $c(\mathbf{x})$ is constant at all locations $\mathbf{x}$ (where $\mathbf{x}$ is a vector representation of 2-D position), then smoothing progresses isotropically. If $c(\mathbf{x})$ is allowed to vary according to the local image gradient, we have anisotropic diffusion. A basic anisotropic diffusion PDE is given by

$$\frac{\partial I_t(\mathbf{x})}{\partial t} = \mathrm{div}\{c(\mathbf{x})\nabla I_t(\mathbf{x})\}. \qquad (1)$$

For discrete-domain diffusion, we use [1]

$$[I(\mathbf{x})]_{t+1} = \left[I(\mathbf{x}) + (\Delta T) \sum_{d=1}^{\Gamma} c_d(\mathbf{x}) \nabla I_d(\mathbf{x}) \right]_t, \qquad (2)$$

where t is an integer-valued iteration number, Γ is the number of directions in which diffusion is computed (typically $\Gamma = 4$), $\nabla I_d(\mathbf{x})$ is the simple difference in direction d at location $\mathbf{x}$ and ΔT is the time step ($\Delta T \leq \frac{1}{4}$ for stability).

The difference between the typical implementation of anisotropic diffusion and the morphological approach lies in the computation of $c(\mathbf{x})$. Morphological anisotropic diffusion allows the preservation of certain shapes at certain scales. For example, we can use a circular structuring element $\mathbf{B}$ with a given diameter to preserve nearly circular objects above a certain scale. In the process, the morphological filter eliminates noise and objects of irrelevant shape that degrade the tracking performance. Because the scale and shape of the leukocytes are known *a priori*, morphological diffusion is successful in processing images containing leukocytes as the features of interest. In morphological anisotropic diffusion, the diffusion coefficient is computed using

$$c(\mathbf{x}) = \exp\left\{ -\left[\frac{|\nabla\{[(\mathbf{I} \circ \mathbf{B}) \bullet \mathbf{B}](\mathbf{x})|}{k} \right]^2 \right\} \qquad (3)$$

where $\mathbf{I} \circ \mathbf{B}$ is the morphological opening of $\mathbf{I}$ by structuring element $\mathbf{B}$ and $\mathbf{I} \bullet \mathbf{B}$ is the closing. In (3), k is an edge strength parameter that is set according to the minimum difference in intensity between the vessel wall and the rolling leukocyte.

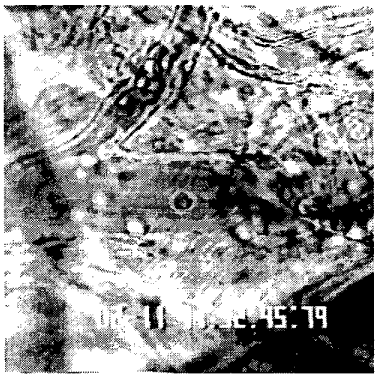

Fig. 1: A video microscopy frame showing rolling leukocytes in a mouse cremaster venule.

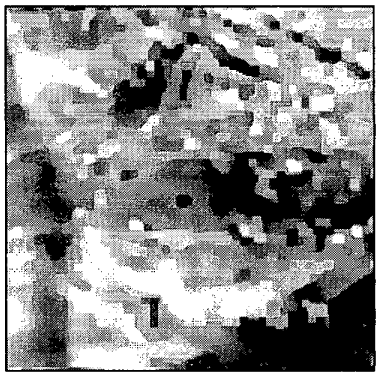

Fig. 2: After morphological diffusion w/ 5x5 struct. element.

Fig. 3: After open-close filter w/ 5x5 struct. element.

The advantage of morphological anisotropic diffusion over traditional anisotropic diffusion is that the smoothing process is truly scalable – the structuring element $\mathbf{B}$

determines the minimum connected component size within the image level sets. A combination of morphology and diffusion has already been successfully utilized in the design of sampling conditions for diffused images [3]. Here, this potent combination is used to effectively extract objects of interest (leukocytes) for tracking. Compared to traditional morphology, the advantage of morphological anisotropic diffusion is found in the boundary preservation that is inherent to the PDE approach. Where standard open and close filters enforce the geometry of the structuring element on the boundaries of the image objects (e.g., corner rounding), the morphological diffusion operation allows the detail in the boundaries to be retained. Consider the difference between the morphological diffusion result in Fig. 2 and the open-close result (using the same structuring element) in Fig. 3. The diffusion method eliminates the background noise/clutter while enhancing the leukocyte profiles.

The edge detection process associated with morphological anisotropic diffusion uses the morphological Laplacian operator. Here, Laplace's equation, $\nabla^2 f(\mathbf{x}) = 0$, is replaced by

$$\nabla^2_{morph} I(\mathbf{x}) = S(\mathbf{x}) - I(\mathbf{x}) = 0, \quad (4)$$

where **S** is defined as the mean between the dilation and erosion with respect to structuring element **B**:

$$\mathbf{S} = \frac{\mathbf{I} \oplus \mathbf{B} + \mathbf{I} \ominus \mathbf{B}}{2}. \quad (5)$$

By locating zero crossings (solutions of eq. (4)), we detect thin, closed contours of the minimum scale dictated by **B**.

3.2 Background Registration and Removal

In the leukocyte tracking process, we first use the edges to perform image-to-image registration for the video microscopy. Once edges (e.g., venule boundaries) are detected, we can register the background to a fixed position by correlating the edge template of the current frame with the edge template of the initial frame in the video sequence. The edge correlation (matching the vessel boundaries) enables background registration and removal.

A major challenge in tracking cells *in vivo* is prompted by the movement and clutter involved with the imaging background. For example, in mouse cremaster studies, the background moves with each respiration (nearly twice per second). In terms of image processing, the background is shifting by more than ten pixels in such frames, which precludes tracking and accurate data collection.

We have implemented edge-based automated registration using morphological anisotropic diffusion and the morphological Laplacian. The registration software fixes the position of background features over time by maximizing the correlation of coarse-scale edges between the images (given that the vessels do not move). By using edges, we minimize the registration error due to subtle variations in intensity. Intensity-based registration systems are unsuccessful within *in vivo* microscopy due to the lack of reliable intensity patterns and the shifts in contrast over time. Given a registered video sequence, we can time-average the video frames to obtain an estimate of the background. Then, we subtract the background from the video frames, leaving only the moving objects in the foreground. Background subtraction improves the robustness of the tracker, as items in the background are avoided.

Background removal also facilitates enhancement of the potential target leukocytes for tracking. Fig. 4 provides an example of enhancement after background removal. The image in Fig. 4(b) contains the features of interest (the leukocytes) and does not contain the background. Further improvement is obtained by enhancing the foreground image of Fig. 4(b) using morphological anisotropic diffusion, as shown in Fig. 4(c). If we utilize standard morphology, we lose objects of interest (consider the dark leukocyte on the right of Fig. 4(a)) and we distort the leukocyte boundaries (notice the diamond-like appearance of the leukocytes in Fig. 4(d)).

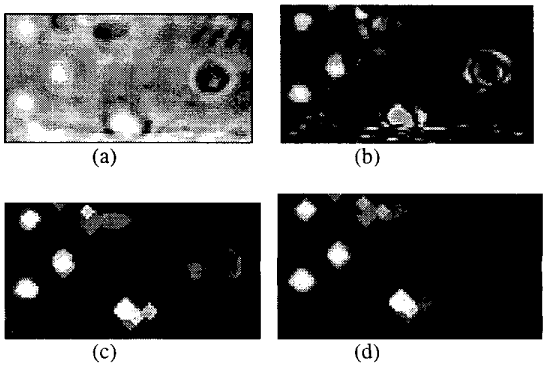

Fig. 4: (a) A subimage from Fig. 1; (b) The difference image computed after edge-based registration showing 6 leukocytes; (c) the enhanced image using morphological anisotropic diffusion with a 5x5 circular structuring element; (d) the enhanced image using the open-close filter with a 5x5 circular structuring element.

3.3 Adaptive Template Matching and Position Prediction

To accommodate changes in cell shape and appearance encountered with rolling leukocytes, we use adaptive template matching techniques. The adaptive templates utilize the enhanced imagery computed via morphological anisotropic diffusion and are robust despite shape changes and contrast changes. We assume that the initial target cell location is given (selected by the operator), and we build a template over time for each leukocyte according to its intensity profile. In this technique, future target profiles are estimated by a weighted average of previous target observations. Given a template T_k and an observation O_k, we can form a new template by allowing

$T_{k+1} = \gamma T_k + (1-\gamma)O_k$. An example of adaptive template matching is shown in Fig. 5. At the bottom of Fig. 5, a 1-D graph showing the profile of the adaptive template and the profile of the observed cell is given.

For the case of cell occlusion (by muscle tissue, for example), we include an estimator/predictor (based on the Kalman filter) to "coast" the track position through the occlusion [4]. The powerful predictive process not only allows coasting, but also allows a reduction in the search area for reacquiring the cell in subsequent frames.

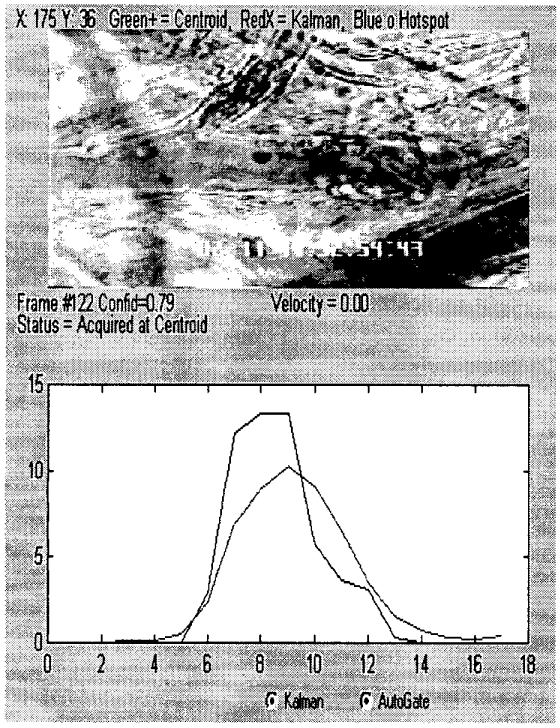

Fig. 5: An example frame from the leukocyte tracking process. The lower curve in the graph is the adaptive template profile and the upper curve is the observed target profile.

Automated tracking of rolling leukocytes enables improvement in the repeatability and throughput of intravital experiments, as compared to manual (frame-by-frame) analysis. The automated tracker also decreases the influence of potential investigator bias. We are in the process of validating the automated tracker by comparing the results to that of manual tracking. An example comparison of displacement curves is given in Fig. 6 for a rolling leukocyte tracked using transillumination of a venule in the mouse cremaster muscle. Early results indicate that automated tracking can replace the tedious manual analysis.

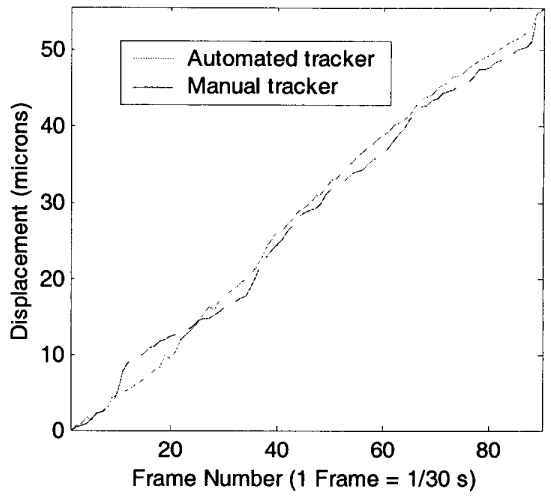

Fig. 6: A comparison of leukocyte displacement for the automated tracker (top solid line) and the manual tracking method (bottom dashed line). Typical deviation is less than one micron.

4. ACKNOWLEDGEMETS

Scott T. Acton is supported in part by the U.S. Army Research Office. Klaus Ley is in part supported by NIH R01 HL 64381.

5. REFERENCES

[1] S.T. Acton, "Diffusion-based edge detectors," in the *Handbook of Image and Video Processing*, Academic Press, pp. 433-447, 2000.

[2] C.A. Segall and S.T. Acton, "Morphological anisotropic diffusion," *Proc. of the IEEE Int. Conference on Image Processing*, Santa Barbara, Ca., October 26-29, 1997.

[3] C.A. Segall, S.T. Acton and A.K. Katsaggelos, "Sampling conditions for anisotropic diffusion," *Proc. of the SPIE Symposium on Visual Communications and Image Processing*, San Jose, January 23-29, 1999.

[4] C.A. Segall, W. Chen, and S.T. Acton, "Video tracking using the morphological pyramid," *Journal of Electronic Imaging*, vol. 8, pp. 176-184, April, 1999.