SPATIOTEMPORAL SEGMENTATION FOR VALIDATION OF ROLLING LEUKOCYTE TRACKING DATA

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ABSTRACT

Processing of bulk microscopy video data requires automated tracking of rolling leukocytes in the hundreds to compute a rolling velocity distribution, which is an indispensable descriptor in inflammation research and anti-/pro-inflammatory drug testing. However, for any automated tracking method to be successful, an automated validation process must exist to accept or reject the output of tracking. In this paper, we propose an automated validation technique that first generates a spatiotemporal image from the cell locations output by a tracking method; then, it segments the spatiotemporal image to detect the presence or absence of a leukocyte by employing an edge-response filter followed by an active contour method. The proposed direction sensitive edge-response filter, the maximum absolute average directional derivative (MAADD), computes the magnitude of the mean directional derivative over an oriented line segment and chooses the maximum of all such values within a range of orientations of the line segment. Our validation experiments show that the proposed method is successful in 93% of the trials using manual tracking, in 83% using correlation tracking and in 84% using active contour tracking method.

1. INTRODUCTION

The velocity distribution of rolling leukocytes (activated white blood cells moving significantly slower than the blood flow) is a critical tool for inflammation studies and for anti-/pro-inflammatory drug testing [5]. Hundreds of leukocytes must be tracked from intravital microscopy videos to compute the velocity distribution. Automated methods are preferred since manual tracking methods are extremely tedious, time consuming, and often prone to bias. The efficacy of such automated tracking methods is typically measured by comparing the tracking outputs to "ground truth," *i.e.*, manual measurements, as for example, see [1],[8]. Since the goal of any automated tracking technique is to avoid the manual data collection, the automated tracker outputs must be validated as a part of an automated data acceptance process in the absence of ground truth data.

The aim of this paper is to propose an automated validation method that accepts or rejects a spatial track-path obtained by joining the leukocyte center locations output by a tracking method. The proposed validation technique is based on spatiotemporal image analysis [9]. A 2D spatiotemporal domain is conceived by the spatial track-path and time (video frame number) as two orthogonal axes. A 2D spatiotemporal image is created by interpolating the intravital video intensity on the aforementioned spatiotemporal domain. Given this spatiotemporal image, the hypothesis of our validation method is: *if the tracker is able to follow a rolling leukocyte, then the spatiotemporal image will have a "trace" of that rolling leukocyte and the tracker computed centers will be lying within the trace*. A rolling leukocyte "trace" may be defined as a thin, elongated, inclined stripe on the spatiotemporal image with two continuous edges that delineate the stripe from its background. A trace indicates that a rolling leukocyte was present in the video sequence over a period of time. To validate a track-path we detect the leukocyte trace, if any, and check if the tracker computed centers lie within the detected leukocyte trace.

In detecting the leukocyte trace we utilize an active contour or snake- [4] based segmentation method preceded by a filtered edge-response from the spatiotemporal image. The proposed nonlinear edge-response filter, the maximum absolute average directional derivative (MAADD), computes the magnitude of the mean directional derivative over an oriented line segment; then, the operator chooses the maximum of all such values within a range of orientations of the line segment. The active contour then uses the output of the MAADD, a filtered image gradient magnitude, as the external force for capturing the cell trace.

2. PROPOSED METHOD

The proposed validation method is comprised of three essential steps – (a) generating a spatiotemporal image from tracker output cell center coordinates, (b) obtaining filtered edge-response from spatiotemporal image via MAADD, and (c) detecting a leukocyte trace within the spatiotemporal image by active contour based segmentation applied on the MAADD edge-response. In this section we elaborate the technical details of these steps.

2.1. Spatiotemporal Image Generation

In order to obtain the spatiotemporal image containing leukocyte trace, we create a path in 3D by joining the cell location coordinates through time (using the x, y coordinates and video frame number). Figure 1 (left) shows such a path in 3D. Figure 1 also shows the spatial track-path, which is the 2D projection of the 3D path on the image domain. Let the coordinates of the starting point (cell location at the beginning of tracking) of a spatial track-path be (x_1,y_1). Let $d(x,y)$ denote the distance of any point (x,y) on the spatial track-path from (x_1,y_1) measured along the spatial track-path. We create a 2D spatiotemporal image where the horizontal axis corresponds to the distance $d(x,y)$, and the vertical axis corresponds to time, *i.e.*, the image frame number. The gray values for this spatiotemporal image are obtained from the video sequence– if the tracker output at time instant (frame number) t is (x_t,y_t), then the voxel (3D pixel) value at location (x_t,y_t,t) from the video is assigned to the pixel location $(d(x_t,y_t),t)$ on the spatiotemporal image.

A practical construction method for the spatiotemporal image considers at every discrete point on the spatial track-path a straight line orthogonal to the image plane. The maximum number image frames in the sequence is the height of each of these 1D image columns. Placing the 1D image columns side-by-side in the same order as they stand on the spatial track-path a spatiotemporal image is obtained where two orthogonal axes are the number of image frames and the distance measure $d(x,y)$. In this case $d(x,y)$ represents number of discrete points on the spatial track-path starting the count at (x_1,y_1). Figure 1 (right) shows a spatiotemporal image formed on the spatial track-path. In this case the tracker was able to track the leukocyte. Consequently, a leukocyte trace with unbroken edges appears. The Figure also shows the overlaid locus $(d(x_t,y_t),t)$ of the tracker for t running from 1 through the maximum number of frames in the sequence. The overlaid locus $(d(x_t,y_t),t)$, which we refer to as spatiotemporal track-path, is seen to be intersected by the leukocyte trace throughout its stretch.

We exhibit some example spatiotemporal images in Figure 2. The leftmost image in Figure 2 shows a spatiotemporal image corresponding to a leukocyte appearing darker than the background. The second from left image in Figure 2 shows a change of contrast – the leukocyte first appears darker than the background, then it gradually turns brighter. In both these cases the spatiotemporal track-path is seen to be completely inside the unbroken leukocyte traces. In the third image from left in Figure 2, the tracker has tracked a leukocyte for a while, then it has jumped to another leukocyte (shown by an arrow on the image) and has tracked the latter cell for the rest of the sequence. This loss of the correct cell is visible in the spatiotemporal image– the leukocyte trace is broken. The fourth image from left in Figure 2 depicts a situation where the tracker follows the leukocyte, but it is significantly off the leukocyte center throughout. The fifth image from left in Figure 2 shows the tracker being able to track the leukocyte for some time, then moving away (shown by an arrow) from the leukocyte for the rest of the sequence. The rightmost image in Figure 2 shows an extreme case where the tracker loses the leukocyte in the beginning of the sequence, and consequently no trace is observed in the spatiotemporal image.

2.2. MAADD Pre-filtering

Once a spatiotemporal image is generated, the subsequent tasks are to detect the leukocyte trace (if existing) and then to verify if the tracker computed cell centers (or equivalently the spatiotemporal track-path) are within the trace. In Figure 3 we show the gradient magnitudes of the spatiotemporal images of Figure 2. We observe that images are corrupted by mostly horizontal streaks. Also the trace edges appear to be broken in some places in Figure 3. These two characteristics of spatiotemporal images are the principal impediments towards segmentation by conventional methods, *e.g.*, the watershed method [10]. Thus before extracting the trace, we suppress noise and bridge the gaps within the trace borders via the following filter:

$$g(x,y) = \max_{\theta_1 \le \theta \le \theta_2} \left| \frac{1}{2R} \int_{-R}^{R} \frac{dI}{dp}(x + r\cos(\theta), y + r\sin(\theta))\, dr \right| \quad (1)$$

For each $\theta \in [\theta_1,\theta_2]$, the filter (1) first computes the magnitude of image directional derivative $\frac{dI}{dp}$ averaged over a (straight) line segment of length $2R$ ($r \in [-R,R]$) and orientation θ. The directional derivative is computed along a direction p perpendicular to the line segment, *i.e.*, p makes an angle $\theta + \pi/2$ or $\theta - \pi/2$ with the x axis. The filter then chooses the maximum of all the averaged directional derivative magnitudes; hence, this is essentially a nonlinear order statistic filter. We refer to the filter (1) as the maximum absolute average directional derivative (MAADD). MAADD suppresses unwanted edges in the spatiotemporal images as illustrated in Figure 4. One prominent example of edge-bridging and unwanted streak suppression is the fourth image from left in Figure 4. Since the leukocyte traces extend diagonally in a spatiotemporal image, we first compute the angle (ω) the diagonal of the image makes with the base of the image. Then we set $[\theta_1,\theta_2]$ as $[\omega-\pi/8, \omega+\pi/8]$, noting by extensive observation that a leukocyte trace edge direction in a microvenule does not exceed the angular range of $\pi/4$.

2.3. Active Contours for Trace Detection

After we obtain the MAADD edge-enhanced image of a spatiotemporal image, we employ a region growing method

to delineate the leukocyte trace. The region growing method is a natural choice in this case because we assume that the initial point of the spatiotemporal track-path to be correct being at the beginning of tracking. Using this initial point as a "seed," we grow a region guided by the MAADD edge-response to delineate an entire leukocyte trace. However, region growing techniques such as the watershed method [10] fail to delineate the entire trace, because of its high sensitivity to feeble ridges formed on the edge surface. For this reason, we execute region growing via a parametric active contour that can be regularized or tuned to ignore weak edges [7].

The parametric active contour is a deformable contour, $(X(s),Y(s))$ parameterized via $s\in[0,1]$ the movement of which on the image plane is governed by the following partial differential equation (PDE) [4]:

$$\frac{\partial X}{\partial t}(s,\tau) = \alpha\frac{\partial^2 X}{\partial s^2}(s,\tau) - \beta\frac{\partial^4 X}{\partial s^2}(s,\tau) + u(X(s,\tau),Y(s,\tau))$$
$$\frac{\partial Y}{\partial t}(s,\tau) = \alpha\frac{\partial^2 Y}{\partial s^2}(s,\tau) - \beta\frac{\partial^4 Y}{\partial s^2}(s,\tau) + v(X(s,\tau),Y(s,\tau)). \quad (2)$$

First two and the third term in the right hand side of (2) express respectively the internal and the external force. α and β are non-negative weights respectively for resistance to stretching and resistance to bending. $(u(x,y),v(x,y))$ is the 2D vector field playing the role of the external force. In (2) the active contour is treated as a function of (pseudo) time τ, and the desired contour position is given by the steady state solution of (2) starting from an initial contour.

To increase the capture range of a snake, Xu and Prince propose a 2D force field called gradient vector flow (GVF) via diffusion of the gradient forces [11]. GVF attracts the initial contour towards an edge from a large distance. When the initial active contour is arbitrarily located inside the target object, imposing a Dirichlet boundary condition (BC) on the GVF PDE can effectively grow the snake to delineate the target object that GVF alone (without the Dirichlet BC) cannot always achieve [6], [7]. A snake from a "seed" initial location can be grown within the framework of the following Enhanced GVF (EGVF) PDE framework:

$$\mu\nabla^2 u - (g_x^2 + g_y^2)(u - g_x) = 0,$$
$$\mu\nabla^2 v - (g_x^2 + g_y^2)(v - g_y) = 0, \text{ when } (x,y)\in(\Omega\setminus C)$$
$$(u,v) = \lambda\mathbf{n}_{\partial C} \text{ when } (x,y)\in\partial C,$$
$$\nabla u.\mathbf{n}_{\partial\Omega} = 0, \text{ and } \nabla v.\mathbf{n}_{\partial\Omega} = 0, \quad (3)$$

where Ω denotes the image domain, C denotes the domain enclosed by the initial or seed active contour (assumed to be a circle), $\Omega\setminus C$ denotes the set difference of Ω and C, $\partial\Omega$ and ∂C are respectively the boundaries of Ω and C, and $\mathbf{n}_{\partial C}$ and $\mathbf{n}_{\partial\Omega}$ are unit outward normal to the boundaries ∂C and $\partial\Omega$ respectively, μ is a non-negative parameter controlling the smoothness of the vector field, λ is a positive parameter (Dirichlet BC), and g is an edge strength indicator.

After we compute g via (1), we utilize it in solving (3). Once we obtain the EGVF field (u,v) from (3), we utilize the vector field in (2) for snake evolution. The initial contour (a circle) is taken at a bottom corner of a spatiotemporal image that is specified by the starting point of a spatiotemporal track-path. The segmentation process is achieved in multiple stages, as the single seed growth has been observed to be insufficient to capture long and thin leukocyte traces. Once the active contour stops evolving a new seed point is chosen at the segmentation boundary point, which is (1) above the previous seed point, and (2) has minimum MAADD value among all the boundary points of the growing segment. Then a new EGVF field is constructed based on the new seed and the active contour from the new seed is grown. This process is allowed to continue until one of the following happens:

(C1) the growing segment reaches the top spatiotemporal image border (see Figure 5 for example) or

(C2) the growing segment no longer includes any trace edge. This situation is detected as soon as the MAADD value at the growing segment falls below a threshold T_g (see Figure 6 for example).

In order to select the threshold value T_g we assume that the leukocyte trace has a step edge of height μ, and the image noise is uncorrelated zero-mean Gaussian noise. It is then possible to show that the MAADD has a normal distribution $N(\mu,\sigma)$, when the MAADD is computed at trace edge; for off-trace-edge MAADD is $N(0,\sigma)$ distributed. The standard deviation σ is related to the noise standard deviation and the segment length ($2R$) employed in MAADD. We then apply the likelihood ratio test [2], which sets the threshold value: $T_g = \mu/2$. μ is estimated by the mean MAADD values on the trace edge obtained from the initial few stages of the growing segmentation. Segmentation results for images in Figure 4 are given in Figure 7.

3. VALIDATION TRIALS

Validation experiments are performed on the outputs of three trackers – (a) manually marked cell centers, (b) active contour [8] tracking, and (c) correlation tracking [1]. Tracking data set consists of 75 rolling leukocytes (each 91 frames long, 3 seconds duration, 30 frames per second) from *in vivo* animal experiments. To minimize jitter introduced by the breathing of the living subject, video frames are registered [3] prior to performing the validation experiments. If a tracker computed cell center is within one cell radius of the manually detected cell center, then the frame is considered 'tracked' [8]. When a tracker is able to

track a leukocyte on at least 61 frames (2 seconds), the tracker output can be accepted for leukocyte velocity computation [8]. Table 1 illustrates performance of the validation trials. The proposed validation technique accepts 70 manual tracks out of 75. Out of 49 acceptable tracks produced by the snake tracker, the proposed validation method (correctly) accepts 40 and (wrongly) rejects 9. On the other hand, out of 26 unacceptable tracks produced by the snake tracker, the validation method correctly rejects 23 and wrongly accepts 3. Similar interpretations are there for the third row of Table 1 with correlation tracking method. To demonstrate the efficacy of MAADD in the proposed validation technique, we apply the same active contour segmentation method without using it: we replace $g(x,y)$ in (3) by $f(x,y) = |\nabla I(x,y)|^2$. Table 2 shows the validation performance without using MAADD that is observed to be inferior compared to the performance shown in Table 1.

Table 1. Validation results by the proposed method

Method	Valid Paths Accepted	Invalid Paths Rejected	Invalid Paths Accepted	Valid Paths Rejected	Total Percentage of Correct Validation
Manual	70	0	0	5	93
Snake	40	23	3	9	84
Correlation	10	52	10	3	83

Table 2. Validation performances without MAADD.

Method	Valid Paths Accepted	Invalid Paths Rejected	Invalid Paths Accepted	Valid Paths Rejected	Total Percentage of Correct Validation
Manual	29	0	0	46	39
Snake	22	22	1	30	59
Correlation	4	59	4	8	84

4. REFERENCES

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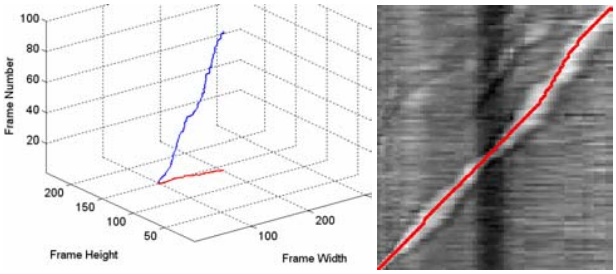

Figure 1. (left) Tracker output in 3D and projected 2D spatial track-path. (right) Spatiotemporal image, and overlaid spatiotemporal track-path.

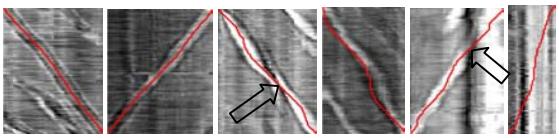

Figure 2. Different spatiotemporal images and overlaid spatiotemporal track-path (for descriptions see the text).

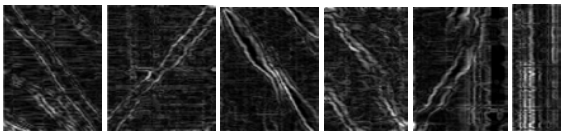

Figure 3. Gradient magnitudes of image in Figure 2.

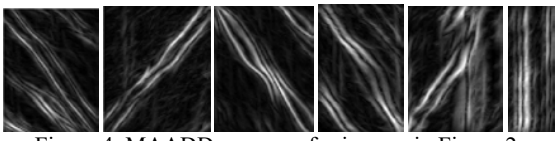

Figure 4. MAADD response for images in Figure 2.

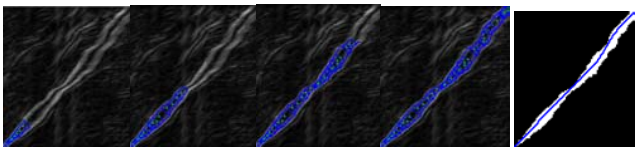

Figure 5. Segmentation stopped by criterion (C1).

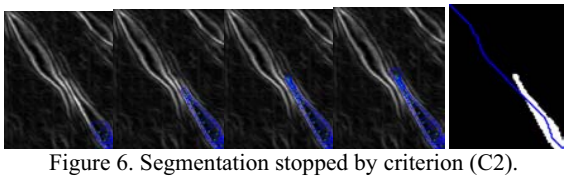

Figure 6. Segmentation stopped by criterion (C2).

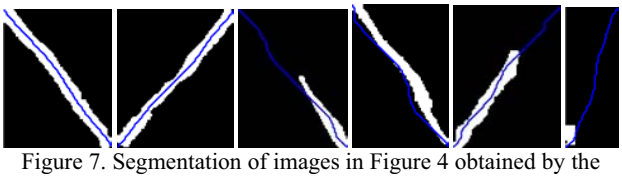

Figure 7. Segmentation of images in Figure 4 obtained by the proposed active contour method.