A Monte Carlo approach to rolling leukocyte tracking in vivo

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Abstract

Tracking the movement of rolling leukocytes in vivo contributes to the understanding of the mechanism of the inflammatory process and to the development of anti-inflammatory drugs. Several roadblocks exist that hinder successful automated tracking including the moving background, the severe image noise and clutter, the occlusion of the target leukocyte by other leukocytes and structures, the jitter caused by the breathing movement of the living animal, and the weak image contrast. In this paper, a Monte Carlo tracker is developed for automatically tracking a single rolling leukocyte in vivo. Based on the leukocyte movement information and the image intensity features, a specialized sample-weighting criterion is tailored to the application. In comparison with a snake-based tracker, our experiments show that, as the noise intensity level increases, the performance of the snake tracker degrades more than that of the Monte Carlo tracker. In cases, where the leukocyte is observed in contact with the vessel wall, the Monte Carlo tracker is less affected by the image clutter. From tracking within 99 intravital microscopic video sequences, the Monte Carlo tracker exhibits superior performance in the reduced localization error and the increased number of frames tracked when compared with the centroid tracker, the correlation tracker and the GVF snake tracker.

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1. Introduction

During the early stages of inflammation, leukocytes roll along the endothelium of postcapillary venules. Tracking the movement of rolling leukocytes contributes to the understanding of the mechanism of the inflammation and to the development of drugs that treat diseases such as Crohn’s disease, multiple sclerosis, heart disease, and arthritis (Hafezi-Moghadam et al., 2001; Jung et al., 1998; Kunkel et al., 2000). Conventionally, leukocyte movement is tracked manually and this requires tens of hours of user-interactive image processing work after each experiment. Automated tracking methods could replace this time-consuming and tedious work.

In our intravital small animal experiments, the leukocytes are imaged using transilluminated specimens. Video recordings are made via a CCD (charge-coupled device) camera coupled with an intravital microscope, which has a water immersion objective SW25/0.6 numerical aperture modified for telescopic imaging (Ley, 2001; Ley et al., 1995). The video frames have been recorded at a spatial resolution of 320 × 240 pixels, where the pixel-to-micron ratio is 2.47 pixels/μm horizontally and 2.34 pixels/μm vertically, and the temporal resolution is 30 frames per second. The experiments involve both wild type and E-selectin knockout mice. Fig. 1 shows several selected frames of microscope observation in a postcapillary venule of the cremaster muscle of a mouse (Ley, 2001). Tracking rolling leukocytes in vivo is challenging. Difficulties include the background movement, the severe image noise and clutter, the cell deformation and contrast change, the occlusion of the target leukocyte by other leukocytes and structures and the jitter caused by the breathing movement of the living animal (Acton et al., 2002).

Currently, there are several approaches to cell tracking. The centroid tracker computes the “center of mass (image
intensity)" in a sub image to estimate the center position of a leukocyte (Ghosh and Webb, 1994), while the correlation method locates the cell through template matching (Kusumi et al., 1993). Zimmer et al. proposed a parametric active contour method, which modifies the edge map in the GVF (gradient vector flow) snake (Xu and Prince, 1998) using both the relative homogeneity of the background to better localize weak target boundary parts and the additive repulsive coupling between contours to maintain contour separation between cells in contact (Zimmer et al., 2002). These three trackers are able to track leukocytes in vitro (in a flow chamber).

Some image processing methods have been applied in tracking leukocytes in vivo, resulting in enhanced centroid trackers and enhanced correlation trackers. Background registration suppresses the background movement (Acton et al., 2002). Morphological anisotropic diffusion mitigates the effect of the noise and clutter (Acton and Ley, 2001). The adaptive template matching is applied to deal with the leukocyte deformation and contrast change. In the case of occlusion, the leukocyte position can be predicted by the Kalman filter (Ray et al., 2002). Although these enhanced trackers are more robust than the standard centroid tracker or the standard correlation tracker, unacceptably large localization errors result from their use (Goobic et al., 2001).

Sato et al. demonstrated that the leukocyte motion in vivo could be extracted through analyzing the spatiotemporal image processed by the orientation-selective filter and the global optimization for grouping the leukocyte trace segments (the moving leukocytes are extracted as traces in the spatiotemporal images) (Sato et al., 1997). But this method only tracks leukocytes rolling near the vessel wall. (Here, when we refer to "vessel wall" (see Fig. 1), we are identifying the upper and lower visible borders of a trans-illuminated vessel in a 2D image.) More recently, a snake (active contour) tracker was developed by Ray et al. (2002). It captures the leukocyte through minimizing an energy function, defined on the basis of internal energy, external energy, shape, size, position, and sampling of the contour. Under most circumstances, the snake tracker is able to successfully track rolling leukocytes in vivo. However, when the leukocyte rolls near the vessel wall, a strong vessel wall edge may cause the active contour to capture the vessel wall instead of the target.

Generally speaking, previous work on visual tracking can be classified into deterministic tracking and stochastic tracking (Zhou et al., 2004). Stochastic tracking often reduces to an estimation problem, in which a stochastic state transition model is built to approximate the evolution of the target state, normally including the displacement or the velocity. An observation model establishes the relationship between the target state and the image intensity observation, and the tracking process is the estimation of the current state given the available observations. The Kalman filter is a well-known solution for tracking, but it is restricted to linear dynamics and Gaussian noise (Kalman, 1960). The extended Kalman filter (EKF) is only suitable for nonlinear models in which the local linearization assumption holds. The unscented Kalman filter (UKF) performs better than the EKF in the nonlinear models but still assumes the noise processes to be Gaussian (Julier and Uhlmann, 1997). Particle filter uses a set of samples to approximate the posterior density of the target state (Doucet, 1998; Doucet et al., 2001; Gordon et al., 1993). It is able to accommodate nonlinear motion models and non-Gaussian noise. Isard and Blake first applied particle filter in visual tracking and proposed the CONDENSATION (conditional density propagation) algorithm for tracking in a densely cluttered image (Isard and Blake, 1998; Isard, 1998).

In this paper, we develop a Monte Carlo tracker to track a single rolling leukocyte in vivo. Based on the leukocyte movement information and the image intensity features, a specialized sample-weighting criterion is tailored to rolling leukocytes observed in vivo. In comparison with a
snake-based tracker, our experiments show that, as the noise intensity level increases, the performance of the snake tracker degrades more than that of the Monte Carlo tracker. In cases, where the leukocyte rolls near the vessel wall, the Monte Carlo tracker is less affected by the image clutter due to the vessel wall edge. While tracking 99 in-vitral microscopic video sequences, the Monte Carlo tracker exhibits superior performance in the reduced localization error and the increased number of frames tracked when compared with the centroid tracker, the correlation tracker and the GVF snake tracker.

The paper is organized as follows. In Section 2, we briefly review the CONDENSATION algorithm. Section 3 develops the Monte Carlo tracker. We have a discussion about the Monte Carlo tracker in Section 4 and demonstrate the tracking results in Section 5. Section 6 concludes the paper.

2. Background

In this section, we briefly review the CONDENSATION algorithm.

In frame $i+1$, let $X_{i+1}$ be the target state and $Z_{i+1}$ be the image intensity observation. The stochastic state transition model $p(X_{i+1}|X_i)$, assumed to be a Markov chain, is built via learning from training sequences. The observation model $p(Z_{i+1}|X_{i+1})$ is defined to make the maxima in $p(Z_{i+1}|X_{i+1})$ correspond to the measured image intensity features. The density of $p(X_{i+1}|Z_{i+1})$ (where $Z_{i+1} = \{Z_1, Z_2, \ldots, Z_{i+1}\}$) can be propagated according to Isard and Blake (1998):

$$p(X_{i+1}|Z_{i+1}) = k_{i+1}p(Z_{i+1}|X_{i+1})p(X_{i+1}|Z_i),$$

where

$$p(X_{i+1}|Z_i) = \int p(X_{i+1}|X_i)p(X_i|Z_i) \, dX_i,$$

and $k_{i+1}$ is a normalizing constant.

Based on the factored sampling (Grenander et al., 1991), the CONDENSATION algorithm approximates $p(X_{i+1}|Z_{i+1})$ by a sample set $\{s^{(m)}_i, n^{(m)}_i\}$, where $s^{(m)}_i$ is the sample $m$, $n^{(m)}_i$ is its weight, $M$ is the sample size, and $m = 1, 2, \ldots, M$. Suppose the sample set $\{s^{(m)}_i, n^{(m)}_i\}$ approximates $p(X_i|Z_i)$. We generate $\{s^{(m)}_{i+1}, n^{(m)}_{i+1}\}$ to approximate $p(X_{i+1}|Z_{i+1})$ using following steps:

1. Resample the sample set $\{s^{(m)}_i, n^{(m)}_i\}$ by drawing a sample $s^{(m)}_{i+1}$ with probability $n^{(m)}_i$, $m = 1, 2, \ldots, M$;
2. Draw a sample $s^{(m)}_{i+1}$ from the state transition model $p(X_{i+1}|X_i = s^{(m)}_i)$, $m = 1, 2, \ldots, M$;
3. Weight $s^{(m)}_{i+1}$ by the image intensity observation

$$n^{(m)}_{i+1} = \frac{p(Z_{i+1}|X_{i+1} = s^{(m)}_{i+1})}{\sum_{j=1}^M p(Z_{i+1}|X_{i+1} = s^{(j)}_{i+1})}, \quad m = 1, 2, \ldots, M.$$

The target state is estimated by

$$\hat{X}_{i+1} = E_{p(X_{i+1}|Z_{i+1})}(X) \approx \sum_{j=1}^M n^{(j)}_{i+1} s^{(j)}_{i+1}.$$

3. The Monte Carlo tracker

In the Monte Carlo tracker, “the target state” is the position of the leukocyte center, which implies $X_{i+1} = (X_{i+1,1}, Y_{i+1,2})$. We generate the sample $s^{(m)}_{i+1}$ to represent $X_{i+1}$ by use of the previous target leukocyte movement information. To weight $s^{(m)}_{i+1}$, a local image intensity measurement method is developed on the basis of the leukocyte image intensity properties.

3.1. Sample generation

In the CONDENSATION algorithm, the sample $s^{(m)}_{i+1}$ is generated from the state transition model $p(X_{i+1}|X_i)$, which is built through learning. In our case, the leukocyte moves in a stochastic way due to the heterogeneity within the rolling leukocyte population as well as to the non-uniform distributions of adhesion receptors and the irregular leukocyte surface morphology (Damiano et al., 1996). Learning from some training sequences cannot obtain a motion model representing the movement of all the leukocytes. In the Monte Carlo tracker, we predict the leukocyte position using the movement of previous steps, and then generate the sample $s^{(m)}_{i+1}$ around the prediction.

The horizontal direction in the microscopic image is set to be parallel with the blood flow direction in the vessel and the leukocyte moves almost horizontally. The elapsed time between two consecutive frames in the video sequence is 1/30 s. In this short time period, the leukocyte vertical movement is negligible and its horizontal movement does not change dramatically. The leukocyte position is predicted by following equations:

$$\bar{x}_{i+1} = \bar{x}_i + 2(\bar{x}_i - \bar{x}_{i-1}) + (1 - z)(\bar{x}_{i-1} - \bar{x}_{i-2}),$$

$$\bar{y}_{i+1} = \bar{y}_i,$$

where $(\bar{x}_{i+1}, \bar{y}_{i+1})$ is the predicted leukocyte position in frame $i+1$, $(\bar{x}_i, \bar{y}_i)$ is the estimated position in frame $i$, and $z$ is a constant.

The sample $s^{(m)}_{i+1} = (x^{(m)}_{i+1}, y^{(m)}_{i+1})$ is generated around $(\bar{x}_{i+1}, \bar{y}_{i+1})$:

$$x^{(m)}_{i+1} = \bar{x}_{i+1} + \bar{R} \cos \bar{z},$$

$$y^{(m)}_{i+1} = \bar{y}_{i+1} + \bar{R} \sin \bar{z}, \quad m = 1, 2, \ldots, M,$$

where $\bar{R}$ is a random number with normal distribution $N(0, \sigma_\theta^2)$ and $\bar{z}$ is a random number with uniform distribution on the interval $[0, 2\pi]$.

3.2. Local image intensity measurement

Leukocytes are almost spherical in shape and their radii measure approximately 4 μm (Damiano et al., 1996). In 2D intravital microscopic video sequences, they appear almost
circular with radii ranging from 5 pixels to 8 pixels and display two image intensity properties:

(a) The image intensity changes significantly near the leukocyte boundary.
(b) The image intensity is relatively constant inside the leukocyte.

On the basis of these two properties, we develop a method to measure the local image intensity features \( Z \) for a given location \( (x, y) \).

Suppose \( (x, y) \) is the position of the leukocyte center. By performing radial edge detection around \( (x, y) \), we can detect the leukocyte boundary. We construct several line segments extending radially from \( (x, y) \) with coordinates \( (l_{0,x}(k), l_{0,y}(k)) \) (as shown in Fig. 2a),

\[
\begin{align*}
l_{0,x}(k) &= x + r(k) \cos \theta, \\
l_{0,y}(k) &= y + r(k) \sin \theta, \\
r(k) &= r_1 + \frac{k}{K} (r_2 - r_1), \\
\theta &= \frac{k_1}{N_1} 2\pi, \\
k_1 &= 0, 1, \ldots, N_1 - 1,
\end{align*}
\]

(7)

where \( N_1 \) is the number of line segments, \( K + 1 \) is the number of points on each line, \( \theta \) gives the orientation of the line segment, and \( r_1 \) and \( r_2 \) are pre-specified values delimiting the length of the line segments. Note that (7) defines line segments for detecting potential cell boundaries, where (6) gives a locus of samples around the predicted leukocyte position. The one-dimensional edge detection operator (Blake and Isard, 1998) is applied on each line segment

\[
 e_0(k) = |l'_0(k-2) + 2l'_0(k-1) - 2l'_0(k+1) - l'_0(k+2)|,
\]

(8)

where \( l'_0(k) \) is the image intensity at point \( (l_{0,x}(k), l_{0,y}(k)) \) obtained by bilinear interpolation. The corresponding coordinate, denoted \((e_{0,x}, e_{0,y})\), with the maximum \( e_0(k) \) is the detected edge point for the orientation \( \theta \). The value of \( r_2 \) records the distance between \( (x, y) \) and \((e_{0,x}, e_{0,y})\).

Fig. 2a shows an example of radial edge detection \( (N_1 = 8) \).

The leukocyte shape characteristic is represented by \( \bar{r} \) and \( \sigma_r \), the mean and the variance of \( r_0 \), respectively. The leukocyte image intensity characteristic \( \sigma_I \) is defined as the image intensity standard deviation inside the leukocyte. In our work, we obtain \( \sigma_I \) by computing the image intensity standard deviation in the circular area centered at \( (x, y) \) with radius \( \bar{r} - \delta \), where \( \delta \) is a positive constant. Here, the use of the circular area is to compute \( \sigma_I \) in an area as large as possible and the use of \( \bar{r} - \delta \) is to lessen the effect of the leukocyte boundary. The local image intensity measurement for the position \( (x, y) \) is defined as the following two-component vector:

\[
Z = [r_0; \sigma_I]^T.
\]

(9)

According to leukocyte image intensity properties, if \( (x, y) \) is near the leukocyte center, the elements of \( r_0 \) should not differ much with each other and \( \sigma_I \) should not be large.

Radial edge detection requires choosing \( r_1 \) and \( r_2 \) appropriately according to the leukocyte size as observed in microscopic images. In Fig. 2b, the edge detection detects the leukocyte boundary where \( r_1 \) and \( r_2 \) are chosen appropriately. With the choice of a large \( r_2 \), the edge detection detects the image clutter in Fig. 2c. In Fig. 2d, the edge detection is unable to detect the boundary because \( r_2 \) is chosen to be too small. We tested the radial edge detection on 100 leukocytes in different microscopic images. If the radial edge detection can detect the leukocyte boundary on all orientations \( (N_1 = 8) \), we regard the edge detection as successful.

By choosing \( r_1 = 0.8 \) \( \mu m \) (2 pixels) and \( r_2 = 3.2 \) \( \mu m \) (8 pixels), the successful edge detection rate is 80%. The unsuccessful edge detections are due to weak image intensity features, partial occlusion of the leukocyte by other leukocytes or structures, and broken leukocyte boundaries.

### 3.3. Sample weighting

Given a set of generated samples and the associated image measurements, the sample weighting is defined by comparing the local image intensity measurement of the sample with that of the target leukocyte center. Assume that the target leukocyte center in the first frame \( (x_1, y_1) \) is known to us. The local image intensity measurement of \( (x_1, y_1) \) is \( Z_1 = [r_0; \sigma_I]^T \) and that of \( Z^{(m)}_{i+1} \) is \( Z^{(m)}_{i+1} = [r^{(m)}_{i+1}; \sigma^{(m)}_{i+1}]^T \). We define \( d^{(m)}_{1,i+1} \) to measure the difference between \( r^{(m)}_{0,i+1} \) and \( r_{0,i+1} \), and \( d^{(m)}_{2,i+1} \) to measure the difference between \( \sigma^{(m)}_{1,i+1} \) and \( \sigma_{1,i+1} \):

\[
d^{(m)}_{1,i+1} = \frac{1}{N_1} \sum_{\theta} (r^{(m)}_{0,i+1} - \bar{r})^2 - N_1 \sigma^2_r,
\]

(10)

\[
d^{(m)}_{2,i+1} = |\sigma^{(m)}_{1,i+1} - \sigma_{1,i+1}|.
\]

(11)
where \( \bar{r} \) and \( \sigma_r \) are, respectively, the mean and the variance of \( r_{0,1} \), and \( N_1 \) is the number of detected boundary points \((N_1 = 8)\).

The sampling weighting should be defined to force the maxima in \( p(Z_{i+1} | X_{i+1}) \) correspond to the target leukocyte image intensity features. If \( s_{i+1}^{m} \) is near the target leukocyte center, the distribution of \( r_{i+1}^{m} \) will not differ much with that of \( r_{0,1} \) and \( d_{i+1}^{m} \) will be small. The values of \( d_{i,1}^{m} \) and \( s_{i+1}^{m} \) will not be large since the image intensity is relatively constant inside the leukocyte and \( d_{2,1}^{m} \) will not be large. These imply \( s_{i+1}^{m} \) should be large when \( d_{i,1}^{m} \) and \( d_{2,1}^{m} \) are small.

The Gaussian function

\[
 u = \frac{1}{2 \pi \sigma_r} e^{-\left( \frac{r^2}{2 \sigma_r^2} \right)}
\]

(12)

is an appropriate choice for the weight criterion, in which \( u \) is maximal if \( v \) equals to zero and \( u \) decreases as \( v \) moves away from zero. The conditions that the sample \( s_{i+1}^{m} \) is near the target leukocyte center require both \( d_{i,1}^{m} \) and \( d_{2,1}^{m} \) be small and they are two independent measurements. Hence, the weight criterion is defined as:

\[
 s_{i+1}^{m} = \frac{s_{i+1}^{m}}{1 + \sigma_{z_1}^{2} + \sigma_{z_2}^{2}},
\]

(13)

where

\[
 s_{i+1}^{m} = -\frac{d_{i+1}^{m} \sigma_{z_1}^{2}}{\sigma_r^2},
\]

(14)

\[
 s_{i+1}^{m} = -\frac{d_{2,1}^{m} \sigma_{z_2}^{2}}{\sigma_r^2},
\]

(15)

and \( \sigma_1 \) and \( \sigma_2 \) are constants. We disregard the constant coefficients in the Eqs. (14) and (15) because \( z_{1,1}^{m} \) will be normalized. After normalization, the weight we assign to the sample \( s_{i+1}^{m} \) is

\[
 s_{i+1}^{m} = \frac{s_{i+1}^{m}}{\sum_{j=1}^{N_1} s_{i+1}^{m}}.
\]

(16)

4. Discussion

The sample generation is crucial to the efficiency of the particle filter. If the sample generation is not well designed, a large number of samples will be needed to achieve a specified performance level. In the Monte Carlo tracker, the weight criterion is based on the local image intensity measurement method, which performs radial edge detection to detect the leukocyte boundary. It is thus desirable that most of the samples be inside the target leukocyte and cover its central region. This requires the predicted position \((\bar{x}_{i+1}, \bar{y}_{i+1})\) not be far away from the target leukocyte center. In Eq. (5), the prediction of \( \bar{x}_{i+1} \) was obtained based on the previous horizontal movements of the target leukocyte. The predictions based on the movements of the previous zero step (simply let \( \bar{x}_{i+1} \) equal to \( \bar{x}_i \)), one step, two steps and three steps were also examined. Prediction based on the two-step movement leads to better tracking results than that of zero step and one step movement prediction but similar tracking results as that of three-step movement prediction. Eq. (6) implies that the samples cover a circular area centering at \((\bar{x}_{i+1}, \bar{y}_{i+1})\) and the size of the area is proportional to \( \sigma_r \), which is properly chosen according to the target leukocyte size as appeared in microscopic images.

To compare the sample generation in the Monte Carlo tracker with that in the CONDENSATION algorithm, we transform Eqs. (5) and (6) into a Markov state transition model

\[
 X_{i+1} = AX_i + W,
\]

(17)

where

\[
 X_i = \begin{bmatrix} x_{i-2} \\ x_{i-1} \\ x_i \\ y_i \end{bmatrix}, \quad A = \begin{bmatrix} 0 & 1 & 0 & 0 \\ 0 & 0 & 1 & 0 \\ \alpha - 1 & -2 \alpha & 1 + \alpha & 0 \\ 0 & 0 & 0 & 1 \end{bmatrix}, \quad W = \begin{bmatrix} 0 \\ 0 \\ \bar{R} \cos \bar{\alpha} \\ \bar{R} \sin \bar{\alpha} \end{bmatrix},
\]

In the CONDENSATION algorithm, the sample \( s_{i+1}^{(m)} \) is generated by

\[
 s_{i+1}^{(m)} = A s_{i+1}^{(m)} + W,
\]

(18)

where \( s_{i+1}^{(m)} \) is resampled from the sample set \( \{ s_{i+1}^{(m)}, \bar{x}_{i+1}^{(m)} \} \). In time, the samples generated in this way tend to disperse and some of them are positioned outside of the target leukocyte boundary. If only several hundred samples are used (more samples lead to a linear increase in computation cost), the tracker will lose the target. This phenomenon is illustrated in Fig. 3a (for the sample size \( M = 100 \)). In the Monte Carlo tracker, the sample \( s_{i+1}^{(m)} \) is generated according to

\[
 s_{i+1}^{(m)} = A \bar{z}_i + W,
\]

(19)

where \( \bar{z}_i \) is the mean of the sample set \( \{ s_{i}^{(m)}, \bar{x}_i^{(m)} \} \). This method is able to locate the majority of the samples inside the target leukocyte and cover its center, as shown in Fig. 3b, and the tracker is able to track the target with an efficient sample generation method.

The weight criterion (Eq. (16)) is based on the fact that the difference between the local image intensity measurement of the sample and that of the target leukocyte center increases as the distance between the sample and the target leukocyte center increases. This implies that \( d_{1}^{(m)} \) (Eq. (10)) and \( d_{2}^{(m)} \) (Eq. (11)) are proportional to \( d^{(m)} \), the distance between the sample \( s^{(m)} \) and target leukocyte center.

This point can be illustrated by the following example. To simplify the notation, we drop the index for the frame number in each notation since we only show how the weight criterion works in one frame. Fig. 4a shows the
target leukocyte in frame 1 of one microscopic video sequence. Fig. 4b shows the target leukocyte and the sample position \( s^{(m)} \) (\( m = 1, 2, \ldots, 11 \)) in frame 8. The distance \( d^{(m)} \) increases with \( m \). The local image intensity measurements of the target leukocyte center in frame 1 and that of \( s^{(m)} \) in frame 8 are recorded by \( Z = [r_0, r_1]^{T} \) and \( Z^{(m)} = [r_0^{(m)}, r_1^{(m)}]^{T} \), respectively.

The distributions of \( r_0 \) and \( r_0^{(m)} \) (where \( \theta = 0, \frac{\pi}{4}, \ldots, \frac{7\pi}{4} \)) are plotted in Fig. 5. The distributions of \( r_0^{(1)} \) and \( r_0^{(2)} \) accumulate around 5 pixels, which are similar to that of \( r_0 \), and
the distribution of $r_{\theta}^{(m)}$ changes as $d^{(m)}$ moves away from the leukocyte center. Fig. 6 shows the relationship of $d_{1}^{(m)}$, $z_{1}^{(m)}$, $d_{2}^{(m)}$, $z_{2}^{(m)}$, and $\pi^{(m)}$ to $d^{(m)}$. We can see that $d_{1}^{(m)}$ and $d_{2}^{(m)}$ are almost proportional to $d^{(m)}$, and $\pi^{(m)}$ is high for the samples near the target leukocyte center and almost zero for those samples farther away from the center.

The weight criterion is defined on the basis of the target leukocyte shape characteristic. While rolling, leukocytes deform from a spherical shape into a teardrop shape. The deformation can be characterized by a deformation index $w/h$, where $h$ is the maximum height of the leukocyte and $w$ is the length of the leukocyte measured at a height $h/2$.

Fig. 5. The histograms of local image intensity measurements $r_{\theta}$ and $r_{\theta}^{(m)}$ ($m = 1, 2, \ldots, 11$).

Fig. 6. The relationship of $d_{1}^{(m)}$, $z_{1}^{(m)}$, $d_{2}^{(m)}$, $z_{2}^{(m)}$ and $\pi^{(m)}$ to $d^{(m)}$ ($\sigma_{1} = 6$ and $\sigma_{2} = 5$).
5. Tracking results

In this section, we demonstrate the performance of Monte Carlo tracker. The parameters were set as \( r_1 = 2 \), \( r_2 = 8 \), \( \alpha = 0.6 \), \( \sigma_1 = 6 \), and \( \sigma_2 = 5 \) (fixed for all experiments). The sample size \( M \) was 300. If \( \bar{r} \) was less than 2.4 \( \mu m \) (6 pixels), we chose \( \sigma_{\bar{r}} = 1.5 \). Otherwise, we chose \( \sigma_{\bar{r}} = 2 \). Before tracking leukocytes in intravital microscopic video sequences, for each sequence, we used template matching to register each frame with the first frame to suppress the jitter caused by the breathing movement of the living animal. The interface translation was removed when we computed the displacement of the leukocyte between frames. Since the two-step movement prediction was involved, we tracked the target leukocyte position in first three frames manually to initialize the process. The flow chart in Fig. 7 illustrates the tracking process.

5.1. Robustness to image clutter

In the Monte Carlo tracker, the image clutter near the target leukocyte may affect the radial edge detection and the observation density peak may correspond to the image clutter. However, as the target leukocyte moves away from the clutter, the peak lacks continuous confirmatory observation and will collapse rapidly. Fig. 8 plots the relationship of \( d_{11}^{(m)} \), \( z_{11}^{(m)} \), \( d_{12}^{(m)} \), \( z_{12}^{(m)} \), and \( \pi_m^{(m)} \) to \( d_0^{(m)} \) in three frames of one microscopic video sequence. In frame 1, \( \pi_m^{(m)} \) is high for the samples near the target leukocyte center. In frame 12, the image clutter caused by the muscle striation attracts the peak of \( \pi_m^{(m)} \) away from the leukocyte center. In frame 23, as the leukocyte moves away from the image clutter, the samples near the target leukocyte center have high \( \pi_m^{(m)} \) again. Fig. 9 displays several frames of the tracking result in this video sequence. The target leukocyte moves through the image clutter caused by the muscle striation from frame 7 to frame 24, and experiences deformation and contrast change, but the Monte Carlo tracker is able to accommodate these hurdles.

5.2. Comparison with the snake tracker

In the snake tracker developed by Ray et al. (2002), the target is located via minimizing an energy function, defined on the basis of internal energy, external energy, shape, size, position, and sampling of the contour. The effects of these constraints on the snake tracker are determined by their individual coefficients. However, it is very difficult to find a balance between these constraints that is able to work well in all scenarios. For example, an edge due to the clutter near the target leukocyte, such as the vessel wall edge, may be so strong that it makes the external energy dominant and the snake tracker may converge to the clutter if we do not increase the coefficients of other constraints. However, if we make the coefficients associated with other constraints higher, the snake tracker may overlook the external energy constraint and generate a boundary that is not collocated with image edges. In the Monte Carlo
tracker, the possible target leukocyte positions are explored by the weight criterion which integrates the edge constraint as well as the shape constraint and the size constraint together. In comparison with the snake tracker, the Monte Carlo tracker is less sensitive to noise and is less affected by the image clutter caused by the vessel wall.

In Fig. 10a, both the Monte Carlo tracker and the snake tracker are able to locate the target leukocyte. Figs. 10b and c are Fig. 10a corrupted, respectively, with the additive Gaussian noise and with the additive salt and pepper noise. To test the sensitivity to noise, we change the Gaussian noise variance and the pepper and salt noise density (larger noise density means that more pixels in the image are affected by noise) and define that, if the difference between the estimated position and the ground truth position is less than a threshold (2 pixels), the tracking is regarded as successful. For each kind of noise and each noise intensity level, we ran the tracker 100 times and recorded the percentage of successful tracking. Fig. 10d and e show the results. The Monte Carlo tracker yields a higher or equal successful tracking rate in 80% of these noisy images.

Fig. 8. The relationship of $d_1, z_1, d_2, z_2,$ and $\pi$ to $d$ in frame 1, frame 12, and frame 23. The notation "." denotes the estimated target leukocyte position.
Fig. 11 shows several consecutive frames in an intravital microscopic video sequence, where the target leukocyte rolls near the vessel wall. The strong vessel wall edge distacts the snake tracker while the Monte Carlo tracker is not affected by the vessel wall edge.

5.3. Tracking rolling leukocytes in vivo

We compare the performance of the Monte Carlo tracker with the centroid tracker, the correlation tracker and the GVF snake tracker in 99 intravital microscopic video sequences. The notation "." marks the estimated target leukocyte position.
video sequences. Each tracker is evaluated by following four aspects (Ray, 2003):

1) **Percentage of frames tracked**
   The leukocyte in each frame is considered as tracked if the distance between the estimated position and the ground truth position (manually recorded by a technician) is less than a threshold (2 \( \mu \)m). Dividing the number of frames tracked by the total number of frames in the video sequence, we obtain the percentage of frames tracked.

2) **RMSE (root mean square error)**
   The RMSE is computed by:
   \[
   \text{RMSE} = \sqrt{\frac{\sum_{i=1}^{N} (\hat{x}_i - x_i)^2 + (\hat{y}_i - y_i)^2}{N}},
   \]
   where \((\hat{x}_i, \hat{y}_i)\) and \((x_i, y_i)\) are, respectively, the estimated target position and the ground truth position in frame \(i\), and \(N\) is the total number of frames in the video sequence. To better compare the accuracy of the tracker, in each sequence, we compute the RMSE only for the frames tracked according to the definition in criterion (1).

3) **Last frame tracked**
   If the last frame in the video sequence is tracked, we regard the sequence as “last frame tracked”.

4) **100% Frames tracked**
   If all the frames in the video sequence are tracked, we consider the sequence as one with “100% frames tracked”.

Fig. 12a shows the tracking results. The Monte Carlo tracker displays better performance than other trackers in terms of a lower RMSE, a higher percentage of frames tracked, and more sequences with last frame tracked and with 100% frames tracked. Among the 99 video sequences, there are 37 sequences in which the target leukocyte rolls near the vessel wall. Fig. 12b shows the number of frames tracked by the Monte Carlo tracker and that by the snake tracker for each of these sequences. Among these 37 sequences, the Monte Carlo tracker outperforms the snake tracker with the same number of frames tracked as the snake tracker in 12 sequences and a greater number of frames tracked in 17 sequences. In some sequences, the target leukocyte image intensity features are very weak, or the image clutter caused by the muscle striation is very severe and its effect on the target leukocyte image intensity features persists. Both trackers fail or the snake tracker is able to track more frames than the Monte Carlo tracker. However, it does not seem that there is a clear characterization of situations in which the snake tracker performs better than the Monte Carlo tracker.

We compared the computational cost for our implementation of the tracking methods. The algorithms were executed with Matlab 6.5 on a PC with a Pentium 4 (3.6 GHz) CPU and 2 GB of RAM. The time needed to process each frame is about 0.174 s for the Monte Carlo tracker, 0.340 s for the snake tracker, 0.025 s for correlation tracker, and 0.028 s for the centroid tracker. If implemented in a compiled language, such as C/C++, the Monte Carlo tracker could achieve real time tracking (at video frame rates), as demonstrated in previous studies (Isard and Blake, 1998).

6. Conclusions

In this paper, we have presented a Monte Carlo tracker to track rolling leukocytes in vivo. Based on the leukocyte movement information and the image intensity features, a specialized sample-weighting criterion is tailored to leukocytes observed in vivo. In tracking 99 intravital
microscopic video sequences, the Monte Carlo tracker shows robust performance and the capability to track the target cell in all the frames in 53 sequences, while a snake-based tracker can only successfully track all the frames in 35 sequences. The Monte Carlo tracker also illustrates more accurate tracking ability with a 0.47 \mu m RMSE, where the snake-based tracker yields an RMSE of 0.52 \mu m. In tracking 37 sequences in which the target leukocyte rolls near the vessel and the image clutter due to the strong vessel edge may affect the performance of the tracker, the Monte Carlo tracker outperforms the snake-based tracker in terms of the ability to track more frames in 19 sequences. In future work, we will focus on extending this method to (simultaneous) multiple target tracking.

The accurate, robust tracker allows bias-free evaluation of anti-inflammatory drugs for which rolling leukocyte velocity distribution is a critical indicator of inflammatory response. The tracker is also an efficacious tool for basic inflammatory disease research in small animals. With a near real-time tracker, velocity distributions can be collected and displayed during a small animal experiment, allowing experimental parameters to be adjusted at the bench top. Further, the automated tracking eliminates tedious and

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**Fig. 12.** (a) Comparison with other trackers for tracking 99 video sequences. (b) Comparison with the snake tracker for tracking 37 sequences in which the target leukocyte rolls near the vessel wall.
error-prone manual post processing, increasing both the accuracy of results and the laboratory throughput.

References


