Automatic Intravital Video Mining of Rolling and Adhering Leukocytes

Xin C. Anders[1], Chengcui Zhang[1], Hong Yuan[2]
[1]*Department of Computer & Information Sciences, the University of Alabama at Birmingham*
[2]*Department of Radiation Oncology, School of Medicine, Duke University*

Abstract

In this paper, we present an automatic spatio-temporal mining system of rolling and adherent leukocytes for intravital videos. The magnitude of leukocyte adhesion and the decrease in rolling velocity are common interests for inflammation response studies. Currently, there is no existing system which is perfect for such purposes. Our approach starts with locating moving leukocytes by probabilistic learning of temporal features. It then removes noises through median and location-based filtering, and finally performs motion correspondence through centroid trackers. By extracting the information about moving leukocytes first, we are able to extract adherent leukocytes in a more robust way with an adaptive threshold method. The effectiveness and the efficiency of the proposed method are demonstrated by the experimental results.

1. Introduction

1.1. Background

Biological video mining is very different from conventional video mining such as in a video surveillance system. Automatic intravital video mining of moving cells are particularly difficult. For example, in contrast to the steady appearances of pedestrians and cars in a traffic video surveillance system, moving cells are very irregular in shapes and sizes. Furthermore, subject movements due to animals' respiration, the strong dependency on biologists' microscopy skills, contrast changes, and noise all add more road-locks to automatic video mining.

In this paper, our focus will be on automatic intravital video mining of rolling and adherent leukocytes during inflammation responses. It is well known in biologists' communities that leukocytes roll along vascular beds, arrest, and transmigrate before they are recruited to inflammatory sites and secondary lymphoid tissues during an inflammation response [1]. The magnitude of leukocyte adhesion and the decrease in rolling velocity are the main predictors of the inflammatory response. In [2] and [3], the measurement of leukocyte rolling and adhesion is done manually with a frame-to-frame video analysis. This type of manual data collection is time and labor consuming and subject to bias from observers. Automatic spatio-temporal mining of rolling and adherent leukocytes from intravital videos can significantly increase the accuracy of the data collection and liberate biologists from the unnecessarily tedious analyses.

Currently, there are several approaches to tracking moving leukocytes from *in vivo* microscopy video sequences. In [4], local features such as color and temporal features are combined to develop a tracking system. It is reported to be capable of automatically tracking moving leukocytes. In order to perform motion correspondence between frames, they assume that all leukocytes roll along the vessel centerline. However, this assumption is not appropriate for all leukocytes activated during an inflammation response. Our video clips show that a significant amount of activated leukocytes indeed roll along the vessels' boundaries. In another method of tracking moving leukocytes [5], after background removal, morphological filters are used to remove further noises. The problem of this method is that the shape/size changes of leukocytes can pose a big challenge for selecting a fixed structure element for morphological operations. Furthermore, both papers do not deal with detecting adherent leukocytes at all.

1.2. General descriptions of our approach

Figure 1 shows typical frames of rolling and adherent leukocytes from *in vivo* grayscale video sequences. From the figure, it is immediately apparent that both moving and adherent leukocytes have relatively higher intensities compared with their surrounding pixels in each frame. This observation prompts us to consider that using the intensity threshold method is probably able to extract leukocytes in each frame. However, a simple global threshold method cannot extract all moving leucocytes because some of them tend to appear blurred in some frames as shown in Figure 1(c)-(d). Furthermore, as the separation of adherent and moving leukocytes is desired in most inflammation response studies, the presence of adherent leukocytes frequently introduces false correspondence in the temporal tracking of moving leukocytes. From previous studies in [4] and [5], the spatio-temporal feature between frames seems to be a good target for detecting moving leukocytes. Therefore, in our approach, we detect moving leukocytes first from spatio-temporal features and then use this information to facilitate the detection of adherent leukocytes with the intensity threshold method. By the end, we have two

Proceedings of the 5th International Conference on Machine Learning and Applications (ICMLA'06)
0-7695-2735-3/06 $20.00 © 2006 IEEE

separate data sets on hands: moving and adherent leukocytes.

Our video mining approach includes two main parts.

1) Locating moving leukocytes and computing the average velocity:
   - Probabilistic learning from spatio-temporal data between frames is used to extract moving leukocytes for each frame (Section 3.1);
   - Further improvements on the above data with median filtering and location-based filtering with a binary image of vessel segmentation (Section 3.2);
   - Centroid trackers and minimal distance matching is used to correspond motion between frames, based on which the velocity can be calculated (Section 3.3).
2) Extracting adherent leukocytes:
   - Extract both moving and adherent leukocytes from each frame with an adaptive global intensity threshold method (Section 4);
   - Remove the moving leukocytes obtained from Section 3 and consider leukocytes firmly adherent if they stay in the same location for a sufficiently long period (Section 4).

The contributions of this paper are as follows:

- A new algorithm of using probabilistic learning, filtering, and centroid trackers for automatic spatio-temporal mining of moving leukocytes from in vivo grayscale video sequences is proposed. In this algorithm, spatio-temporal intensity-based features from all frames are used to predict the current frame. Later, median and location-based filtering is used to remove noise from the prediction result. Then centroids of moving leucocytes are automatically extracted. The correspondence of moving leukocytes between frames is achieved by minimal distance matching.
- By detecting moving leukocytes in frames first, we are able to extract adherent leukocytes in a more robust way with an adaptive global intensity threshold method. The adaptive method is designed to adjust the global threshold for each frame respectively since the intensity level does change among frames in *in vivo* video microscopy.

2. Intravital video microscopy

In this paper, the closed cranial window model on rats was used to study the brain microcirculation. The scalp and the tissue from a 1.5x1.5 cm area bilaterally over the parietal cortex of rats were removed and a glass plate was glued to the surrounding bone to create a window. After recovery, animals were given 20 Gy radiation locally delivered to the brain. Ionizing radiation has been known to induce inflammatory responses in normal tissues including the central nervous system [2] [6]. Prior to microscopy video recording, rhodamine 6G was injected through the tail vein to fluorescently label leukocytes in order to visualize the blood vessels. Leukocytes rolling and adhesion are thus visible in grayscale videos (see Figure 1).

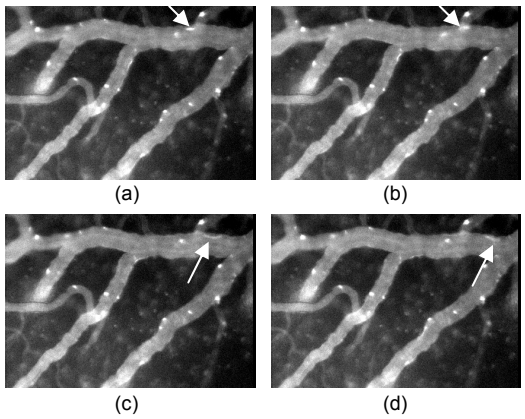

Figure 1. Four consecutive frames (a)-(d) from an in vivo microscopy grayscale video sequence. The bright white dots are mostly leukocytes and the tree-like structures are the blood vessels of study. The arrows mark one moving leukocyte over the four consecutive frames.

3. Video mining of moving leukocytes

The optical flow techniques and background subtraction are popular approaches for tracking moving objects. The basis of optical flow is to estimate the optical flow at all points of a frame and significant points are grouped based on principles of motion coherence [7]. However, since there are also erythrocytes and other blood cells circulating inside and outside the targeted vessels, the optical flow technique can lead to a high false positive rate and make it inappropriate for tracking moving leukocytes from microcirculation video. Another drawback of the optical flow is its sensitivity to contrast changes.

Background subtraction comes to everyone's mind naturally since ideally we can consider that all have steady backgrounds and the rolling leukocytes are the only moving parts. The simplest background subtraction is to calculate an average image of all frames and then subtract each frame from this average, and finally threshold the result. Yet, the performance of tracking cells from *in vivo* videos is affected by many factors due to poor video quality such as camera/subject movement, noise and clutter, cell deformation, and contrast changes. Hence, the simplest form of background subtraction is not good enough and needs further improvement. In our approach, we use probabilistic learning in background subtraction and then apply median and location-based filtering. After locating moving leukocytes for each frame, we perform motion correspondence with centroid trackers and compute the average rolling velocity.

Proceedings of the 5th International Conference on Machine Learning and Applications (ICMLA'06)
0-7695-2735-3/06 $20.00 © 2006 IEEE

3.1. Probabilistic learning

Given x_{1j}, x_{2j}, … x_{Nj} be the grayscale intensity values (0 to 255) of a pixel at the location j ($1 \le j \le$ *Total number of pixels in a frame*) over N consecutive frames. The probability density function that this pixel will have intensity value x_{tj} in the frame t can be non-parametrically estimated with the kernel estimator K [8]. We choose the kernel estimator function K to be a normal distribution, which means that the pixels at the same location of the N frames are considered to follow a Gaussian distribution by themselves. Therefore, the probabilistic density can be estimated with

$$P(x_{tj}) = 1/N \times \sum_{i=1}^{N} (1/\sqrt{2\pi\sigma_j^{\,2}}) \times e^{-0.5*(x_{tj}-x_{ij})^2/\sigma_j^{\,2}} \quad (1)$$

σ_j is the temporal invariance of intensity I for a pixel at the location j over N frames and is calculated as

$$\sigma_j = 1/(N-1) \times \sum_{i=1}^{N} (I_i - \bar{I})^2 \quad (2)$$

Using this probability estimate, a pixel at the location j in the frame t is considered to be a foreground pixel if $P(x_{tj}) < th$ where th is a global threshold over all frames that can be adjusted to achieve a desired percentage of false positives [8]. Our studies show that a threshold of 0.003 can achieve optimal results for this problem domain. With this model, a foreground pixel is a part of moving leukocytes in each frame since adherent leukocytes and blood vessels will be identified as background by this model. Based on our experiments, the probabilistic learning from temporal features is able to extract all moving leukocytes from each frame. An example of extracted moving elements is shown in Figure 2. The arrows in Figures 2(a) and (b) point to the appearance of a moving leukocyte in two consecutive frames. In Figure 2(c), the extracted moving elements are depicted as white dots. As expected, the extracted moving elements include not only moving leukocytes, but also noises.

3.2. Noise removal

As shown in Figure 2, the data obtained from probability learning is not perfect and we can see a lot of noise signals in Figure 2(c). Further enhancement is necessary to remove noise from real signals. As mentioned earlier, one of the problems faced in vivo microcirculation videos is fluids flowing outside the vessels. Leukocytes moving outside the target vessels are certainly not of our interests. We can remove moving objects outside vessels with a location-based filtering. This can be achieved with a binary image of vessel segmentation. A simple way of vessel segmentation is to create a binary image of each frame using a global threshold method based on Otsu's thresholding algorithm [12]. Figure 3 shows such a binary image example obtained from the frame in Figure 2(a). It is worth mentioning that each frame is associated with its own vessel binary image which might be slightly different from the others. By doing this, the slight changes in vessel shapes due to animal respiration or other reasons can be accommodated. The accurate location of vessel areas is important because there are many leukocytes moving along vessel boundaries.

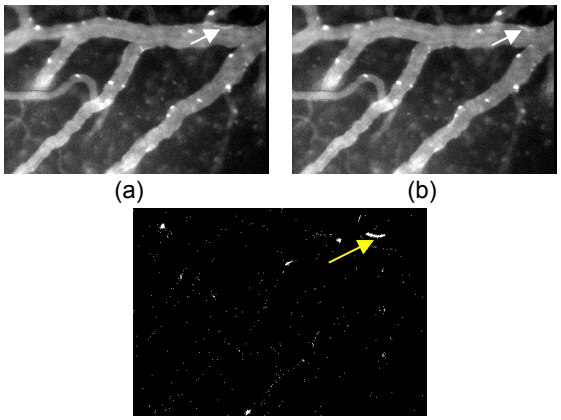

Figure 2. Extract moving leukocytes through probabilistic learning.

Figure 3. An example of the binary image as a result of vessel segmentation obtained from the frame in Figure 2(a) with a global threshold method [12].

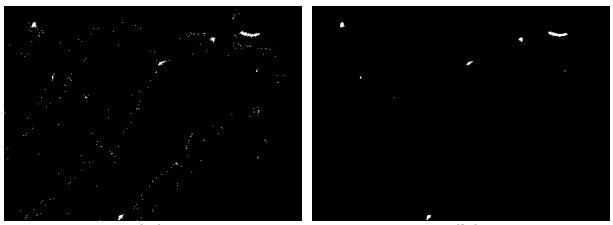

Figure 4. Noise removal through filtering.

Based on our experiments, the vessel segmentation is good enough to filter out moving objects outside the vessels; for an example, see Figure 4(a). However, even after we remove the objects outside the vessels, there is still noise. From Figure 4(a), we can see many tiny white dots (most of them are one pixel in size) spread around. Since they are relatively small and mostly isolated, the median filtering is a good choice to remove them without losing real signals. With the median filtering, the value of an output pixel is determined by the median of the

neighborhood pixels. An example of median filtering is demonstrated in Figure 4(b).

3.3. Motion correspondence and velocity computation

As mentioned in Section 1.1, the velocity of moving leukocytes is a qualitative measurement of inflammation responses. Current methods of tracking cells from *in vivo* videos include correlation trackers [5] [9] and centroid trackers [11]. Correlation trackers use a fixed template for target cells and correlate it with the images to trace the target cells. This type of correlation is inappropriate for tracing moving leukocytes during inflammation responses because activated leukocytes tend to change their shapes a lot. We indeed observe this from the video sequence used in this paper. In contrast, centroid trackers are able to trace deformable cells by following their intensity mass center positions over frames. Therefore, we decide to use centroid trackers to trace moving leukocytes in this paper.

The binary image obtained from Section 3.2 (Figure 4(b) is an example) for each frame actually contains spatial information about moving leukocytes in each frame. Then a seeding and growing approach is used to extract each group of spatially connected leukocytes, and finally the centroid of each of such group can be located. After all centroid positions are located, we apply our matching algorithm to each frame and its previous frame. The matching algorithm is designed as below:

- Start with a centroid position in the current frame, find the centroid position in the previous frame with the smallest distance;
- If the smallest distance does exceed a pre-defined limit, we consider this pair to be a match;
- Remove the matched pairs from the previous and the current frames and repeat the first step until all the centroid positions in the current frame are tried.

In our experiments, the limit is set at 30 pixels, which is determined by the camera calibration parameters and the maximal velocity we can expect. The results from our experiments show a very low false positive rate and a reasonable recall rate when verified manually, which proves that the chosen limit is reasonable for our case.

After acquiring all matched centroid positions between frames, we can compute the mean velocity V_e by averaging the Euclidean distances between matched pairs. We get an average velocity of 6.218 pixels per frame in our experiments. It is easy to convert it to a real-world velocity measurement V_c by the following equation when a calibration of in vivo microscopy c (length per pixel) and the time elapse between frames t are available:

$$V_c = V_e * C / t \tag{3}$$

4. Extracting adherent leukocytes

From Figure 1, it can be seen that leukocytes tend to stand out from their surrounding areas in each frame. This suggests that local features (e.g., local intensity distribution) in each frame might be sufficient for extracting those leukocytes. The local range feature extraction, where each output pixel contains the range value (maximum value – minimum value) of its neighborhood pixels, is tried first. The choice of the neighborhood turns out to be very tricky. A circular shape neighborhood is reported to be a good choice in Acton et al. for tracking leukocytes [5], but we find it very difficult for our case. Even though normal leukocytes have a disk shape, leukocytes in inflammation responses tend to change their shapes a lot and the shapes can be quite irregular. We finally find out that the traditional 3-by-3 rectangle neighborhood is actually the best choice. The local range filtering indeed captures all moving and adherent leukocytes in the frame. However, the resulting local range image also outlines the vessel boundaries.

Since leukocytes tend to adhere to the vessel boundaries during inflammation responses, it is hard to separate those adhering leukocytes from the boundaries. The single global intensity threshold is another method which might be used to extract leukocytes of our interest out of each frame. Yet, the intensity change between frames from *in vivo* microscopy video sequences could not justify a single intensity threshold for all frames. Therefore, we design an adaptive algorithm to select an intensity threshold for each frame, respectively. The algorithm first starts with the median intensity value of each frame as the threshold, and the pixels whose intensity values are greater than this threshold will be tentatively marked as leukocyte pixels. It then gradually increases the value of the threshold as long as the percentage of marked pixels is more than 0.5%. 0.5% is chosen as the cutoff value through a simple sampling and training process. Figure 5 shows an example result of applying this method to an original frame. However, based on our observations from experiments on a real *in vivo* video sequence, there are two major problems in this straightforward use of the adaptive global threshold technique. One problem is that it sometimes also extracts moving leukocytes from each frame. This problem can be easily solved when we have the location information of moving leukocytes (see Section 3) in each frame. Another problem is that adherent leukocytes occasionally disappear and reappear in certain frames due to intensity changes or the poor quality of videos. In order to solve this problem, we use a heuristic rule, where we consider the leukocytes to be firmly adhering if we observe them at the same place for more than 9 frames across the entire video sequence. Such leukocytes will be copied from the frames where they do appear and superimposed onto

those frames where they are apparently missing. By doing so, the overall spatio-temporal consistency of adherent leukocytes is incorporated into the decision process in detecting adherent leukocytes.

Figure 5(a) shows an original video frame which is the same as Figure 2(a). Figure 5(b) shows the binary image after applying the adaptive global threshold, where the white pixels represent the extracted leukocytes and the arrows point to those moving leukocytes. Similarly to how we remove noise from detected moving leukocytes in Section 3.2, we also use the vessel segmentation result to remove the signals outside the vessel area of interest. It is worth mentioning that when we compare Figure 2(c) and Figure 5(b), we can see that the moving leukocyte as pointed out in Figure 2(c) cannot be detected by the global threshold method, which exemplifies the need for a more sophisticated spatio-temporal reasoning method such as the one we described in Section 3.1. Figure 5(c), the binary image after removing the moving leukocytes and detecting (and recovering) the firmly adhering leukocytes is shown. With a binary image such as the one in Figure 5(c) at hand, it is easy to compute the magnitude of leukocyte adhesion represented by the total areas of pixels or *um* when a calibration for *in vivo* microscopy is available.

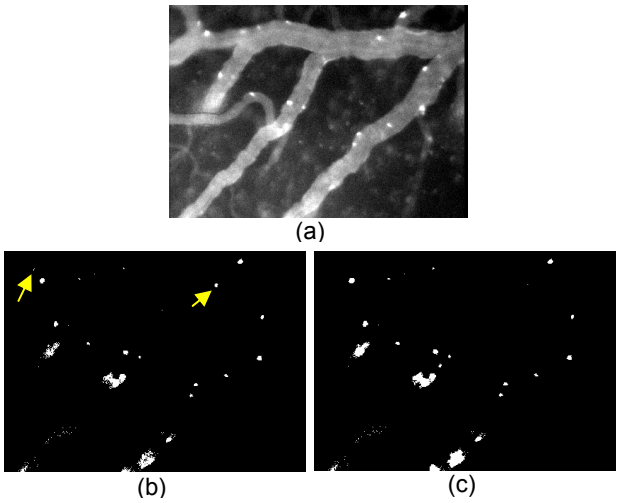

Figure 5. Adaptive global intensity threshold method and its improvements.

5. Experiments and discussions

The grayscale video sequence used in this study comes from an in vivo video microscopy during a typical inflammation response where activated leukocytes roll and adhere to the vessels. Our strategy is to detect moving leukocytes first and then utilizes the information about the moving leukocytes to detect adherent leukocytes.

Our video mining of moving leukocytes include three main steps – locating moving leukocytes through the spatio-temporal probabilistic learning of intensity based features, noise removal with median filtering and location-based filtering, and centroid trackers for tracing moving leukocytes over frames and computing the average rolling velocity. As mentioned earlier, there are several approaches locating moving leukocytes and computing rolling velocity from in vivo microscopy video sequences based on background subtraction with temporal features [4] [5] [10]. In [5], the background subtraction is achieved by subtracting the average of all frames from each frame. In [4] and [10], a temporal invariance image is computed for each frame and a single threshold is then selected to remove the background. We test both methods with our video sequence and both yield poor performances. This is probably due to severe noises, cell deformations and background movements. In order to overcome the problems, we introduce the use of spatio-temporal probabilistic learning to extract moving leukocytes as foreground pixels and the performance is satisfactory. This demonstrates that a probability threshold is more appropriate than a low level intensity threshold, especially for those video sequences full of severe noises and contrast changes. However, the background subtraction alone is likely to suffer from excessive noise. Further enhancements following background subtraction are necessary to overcome this problem. In [4], a location-based filtering is used to remove false positive leukocytes if they are outside a vessel region. Acton et al [5] approach this problem by using morphological filters. However, none of them deal with the detection and separation of adherent leukocytes from moving leukocytes. In addition, the morphological filtering method performs poorly on our video sequence. This is probably due to the irregular shapes of leukocytes and their deformations. After location-based filtering, we also apply median filtering on our data to further remove those isolated random noises. Our approach of locating moving leukocytes is presented in an example as in Figure 4(b). After locating moving leukocytes in each frame, we continue our mining with centroid trackers to obtain motion correspondence and compute the rolling velocity. The rolling velocity is the main interest of moving leukocytes for many inflammation response studies because it offers biologists a qualitative measurement of each inflammation response. In [4], it is assumed that leukocytes roll along the vessel centerline and therefore a vessel centerline extraction from thinning can help in predicting the direction of moving leukocytes in the next frame. However, this assumption is against our observations that under inflammation response, leukocytes rarely roll along the centerline and move along the vessel boundaries instead. Acton et al 2002 use correlation trackers to match a template of the target leukocyte to each frame in order to find the instances of the target. This method is insufficient and very vulnerable to leukocytes' deformations. We decide to apply the

centroid trackers in our motion correspondence because it is less vulnerable to the frequent shape changes of leukocytes. We match leukocytes between frames by finding the pairs with the minimal distance which is below a selected limit. Our experiments on automatically tracing 118 leukocytes show a false positive rate as low as 1.6% when double-checked manually. The rolling velocity is 6.218 pixels per frame, which is verified by our manual calculation. It is easy to convert it to a conventional speed representation by Equation 3. However, we observe a relatively low recall rate (≈50%) for tracking moving leukocytes since they tend to disappear in some frames and reappear afterwards. Since the measurement of the average rolling velocity is the main interest, this is justified as long as enough leukocytes are tracked with a very low false positive rate. More discussion about this issue is presented at the end of this section.

There are currently no studies about automatic detection of adherent leukocytes that can be found in the literature. In this paper, we are able to extract adherent leukocytes by combining an adaptive global intensity threshold method with the information about the moving leukocytes detected earlier. Our experimental results show a recall rate of 93% with a zero false positive rate when double-checked manually.

It is observed from our video sequence that moving leukocytes occasionally disappear in one frame and reappear in the next frame. We are concerned that our motion correspondence algorithm may be vulnerable to the errors caused by this phenomenon and draw a potential criticism. In [11], Ghosh and Webb address the similar concern in their approach to automatically detect cell receptors. They propose including a storage matrix for the unmatched pixels in the previous frame against the current frame. Then when the matching for the next frame starts, a scan through the storage matrix of the previous frame is also performed to find whether there is any reappearance. We test this algorithm on our video sequence. However, our results show the false positive rate of this algorithm is as high as 61% when double-checked manually. By including those false leukocytes into our measurement, we are also introducing more significant errors into our velocity measurement. Therefore, we argue that in this case, precision is more important than recall, and that omitting those disappearing and reappearing leukocytes is okay for the measurement of the average rolling velocity as long as enough leukocytes are traced.

6. Conclusions

In this paper, we present an automatic intravital video mining system of leukocytes rolling and adhesion. Video mining of in vivo microscopy video sequences is very difficult due to severe noises, background movements, leukocytes' deformations, and contrast changes. In our approach, we first locate moving leukocytes by applying a spatio-temporal probabilistic learning. The proposed saptio-temporal probabilistic learning from intensity based features for background subtraction is superior to other background subtraction methods based on temporal average subtraction or temporal invariance threshold. We further remove noises by applying median and location-based filtering. After moving leukocytes are located, centroid trackers are used for motion correspondence. Our motion correspondence results show a very low false positive rate as low as 1.6% when double-checked manually and are demonstrated effective in correctly calculating the rolling velocity. Another contribution from this paper is that we extract the information about moving leukocytes first and therefore are able to extract adherent leukocytes with an adaptive global threshold method. Our result shows a recall rate of 93% with a zero false positive rate when double-checked manually.

References

[1] M.B. Lawrence and T.A. Springer, "Leukocytes roll on a selectin at physiologic flow rates: distinction from and prerequisites for adhesion through integrins," C*ell*, 65, 1991, pp. 859-873.

[2] S.I. Anderson, R. Shiner, M.D. Brown, and O. Hudlicka, "ICAM-1 expression and leukocyte behavior in the microcirculation of chronically ischemic rate skeletal muscles," *Microvascular Research*, 71 (3), 2006, pp. 205-211.

[3] H. Yuan, D.J. Goetz, M.W. Gaber, A.C. Issekutz, T.E. Merchant, and M.F. Kiani, "Radiation-induced up-regulation of adhesion molecules in brain microvasculature and their modulation by dexamethasone," *Radiation Research*, 163, 2005, pp. 544-551.

[4] E. Eden, D. Waisman, M. Rudzsky, H. Bitterman, V. Brod, and E. Rivlin, "An automated method for analysis of flow characteristic of circulating particles from *in vivo* video microscopy," *IEEE Transactions on Medical Imaging*, 24 (8), 2005, pp. 1011-1024.

[5] S.T. Acton, K. Wethmar, and K. Ley, "Automatic tracking of rolling leukocytes *in vivo*," *Microvascular Research*, 63, 2002, pp. 139-148.

[6] K. Narayan, R. Withers, C. Garcia, K. Masoh, and S. Kumar, "Guinea pig spinal cord as a model for the study of late radiation injury and repair," *International Journal of Oncology*, 4, 1994, pp. 809-814.

[7] A. Mclvor, Q. Zang, and R. Klette, "The background subtraction problem for video surveillance systems," *Lecture Notes in Computer Science*, Springer Berlin / Heidelberg, International Workshop RobVis, Auckland, New Zealand, 2001, pp. 176-188.

[8] A. Elgammal, D. Harwood, and L. Davis, "Non-paramtric model for background subtraction," *Lecture Notes in Computer Science*, Springer Berlin / Heidelberg, ECCV 2000: 6th European Conference on Computer Vision, Dublin, Ireland, 2000, pp. 751-767.

[9] G. J. Schutz, H. Schindler, and Th. Schmidt, "Single molecule microscopy on model membranes reveals anomalous diffusion," *Biophysical Journal*, 73, 1997, pp. 1073-1080.

[10] Y. Sato, J. Chen, R.A. Zoroofi, N. Harada, S. Tamura, and T. Shiga, "Automatic extraction and measurement of leukocyte motion in microvessels using spatiotemporal image analysis," *IEEE Transactions on Biomedical Engineering*, 44 (4), 1997, pp. 225-236.

[11] R.N. Ghosh and W.W. Webb, "Automated detection and tracking of individual and clustered cell surface low density lipoprotein receptor molecules," *Biophysical Journal*, 66, 1994, pp. 1301-1318.

[12] N. Otsu, "A threshold selection method from gray-level histogram," IEEE Trans. Syst. Man Cybern., 8, 62–66, 1979.