RESEARCH

Centroid tracking and velocity measurement of white blood cell in video

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Abstract

Automated blood cells tracking system has a vital role as the tracking process reflects the blood cell characteristics and indicates several diseases. Blood cells tracking is challenging due to the non-rigid shapes of the blood cells, and the variability in their videos along with the existence of different moving objects in the blood. To tackle such challenges, we proposed a green star based centroid (GSBC) moving white blood cell (WBC) tracking algorithm to measure its velocity and draw its trajectory. The proposed cell tracking system consists of two stages, namely WBC detection and blob analysis, and fine tuning the tracking process by determine the centroid of the WBC, and mark the centroid for further fine tracking and to exclude the bacteria from the bounding box. Furthermore, the speed and the trajectory of the WBC motion are recorded and plotted. In the experiments, an optical flow technique is compared with the proposed tracking system showing the superiority of the proposed system as the optical flow method failed to track the WBC. The proposed system identified the WBC accurately, while the optical flow identified all other objects lead to its disability to track the WBC.

Keywords: White blood cell, Cell detection, Velocity measurements, Trajectory analysis, Video processing, Blob analysis, Tracking system

Introduction

The blood white/red cells tracking and velocity measurements are active research areas that have an impact to study the blood flow characteristics, cell diseases, lesion vasculature, cerebral micro-vessels, and the mechanism of the white blood cell (WBC) to engulf the bacteria [1–4]. In clinical applications, WBC detection and tracking without the inefficient culture processing facilities the rapid diagnoses and the accurate study of the WBC characteristics and motion. Such tracking system requires the detection of the WBC from the body fluid, which is a complex and challenging process. In cell biology, video microscopy processing becomes indispensable for quantitative analysis of the cell motion trajectory. Using video's frames provides significant information about the working structures and mechanisms in living cells [5].

For computerized detection and quantitative analysis of the objects' trajectories in cell biology video, Sbalzarini and Koumoutsakos [6] implemented an effective feature point tracking technique. This tracking employed the moment scaling spectra technique based on the initial detection of the feature point algorithm, which has been applied to each frame image. Afterwards, a linking algorithm has been used to identify the corresponding points in the object of interest in the subsequent frames to link the positions into trajectories. Synthetic frame sequences of moving point blobs have been used to evaluate the feature point detection algorithm. Reyes-Aldasoro et al. [7] designed a tracking algorithm for measuring the red blood cells velocity during their travel via the tumours' microvessels. A pre-processing for artefacts and noise removal has been applied before the cells segmentation from the background. Between the subsequent frames in the video sequence, a keyhole model has been used in the tracking system to describe the possible movement of the segmented cell. The shape of a keyhole has been defined using the history of cell movement, including

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the past, present and predicted arrival position of the cells. This keyhole model has been used to determine if the cells were in adjoining frames to be linked to form paths. In addition, a circular region around the parent cell's centroid was used as a probability region, if there was no history.

The white-/red- blood cells, platelets, and the circulating particles have been tracked by Eden et al. [8], designed an automated CellTrack system to analyse the cells circulatory patterns and their behavioural characteristics in vivo video using the artificial neural network to classify the specific blood cells type. This system entailed two stages, namely (i) a global processing stage to extract the center lines and the vessel borders based on their temporal patterns and colored, and (ii) a local processing stage to extract the positions and velocities of the circulating cells.

Generally, several biological applications include the tracking of objects, such as the WBCs that have unknown motion type and velocity. The tracking process in such applications is hindered by the stochastic characteristics of the motion, or by trajectories involving several motion modes, such as uniform and random, or by the existence of several objects in the blood. In addition, the motion correspondence is considered an essential process to connect the target (region of interest 'ROI') in a frame to the same ROI in the previous and subsequent frames. This process is complex in the video-microscopy due to the existence of multiple objects with changing appearance and overlapping paths.

Consequently, the current work proposed a two-stage tracking system, named Green star based centroid (GSBC) to track, measure the velocity and trajectory of a moving WBC while its attacking to a harmful small bacterium. The proposed tracking system includes pre-processing, segmentation, blob analysis, detection of the WBC followed by fine tuning of the tracking process, determining the WBC trajectory, and measuring its velocity.

The structure of the remaining sections is as follows. Section 2 includes the methodology and a detailed explanation of the two main stages in the proposed WBC tracking system. Section 3 discusses the obtained results with a comparative study with the failed tracking using the optical flow technique. Finally, the current work is concluded in Sect. 4.

Methodology

To detect and track the white blood cell in a video, the coloured test video is converted into grey scaled successive frames. Then, an improved canny edge detector is applied according to the following steps:

(i) Smooth each frame with a Gaussian filter to reduce noise, unwanted details, and textures, using the following expression [9]:

$$g(m, n) = G_{\sigma}(i, j) * f(m, n) \quad (1)$$

$$G_{\sigma}(i, j) = \frac{1}{\sqrt{2\pi}\sigma} \exp\left(-\frac{i^2 + j^2}{2\sigma^2}\right) \quad (2)$$

where $G_{\sigma}(i, j)$ is a filter kernel function.

(ii) Compute the appropriate gradient of $g(m, n)$ by using the Sobel gradient [10] to obtain:

$$M(m, n) = \sqrt{g_m^2(m, n) + g_n^2(m, n)} \quad (3)$$

(iii) Apply the thresholding method on M to exclude the background regions [11] as follow:

$$M_T(m, n) = \begin{cases} M(m, n) & \text{if } M(m, n) > T \\ 0 & \text{otherwise} \end{cases} \quad (4)$$

(iv) Suppress non-maxima pixels in the edges in M_T obtained above to thin the edge ridges (as the edges might have been broadened in step (i)). To perform this process, it is essential to ensure that the non-zero $M_T(m, n)$ is greater than its two neighbours along the gradient direction to keep $M_T(m, n)$ unchanged; otherwise, it will be set to 0 value.

(V) Threshold the previous result by two different thresholds T_1 and T_2 (where $T_1 < T_2$) to obtain two binary images BI_1 and BI_2 , where the results with large values of T_2 has less noise and fewer false edges, but greater gaps between edge segments. Link edge segments in BI_2 to form continuous edges by tracing each segment in BI_2 to its end and then search its neighbors in BI_1 to find any edge segment in BI_1 to bridge the gap until reaching another edge segment in BI_2 .

Afterward, a dilation of the output of the canny edge step is performed. Assume A be the canny edge result image and B the structuring element [12], and the dilation would be performed as follow:

$$A \oplus B = \left\{ Z \mid (\hat{B})_z \cap A \neq \emptyset \right\} \quad (5)$$

To fill the hole in the dilated image, the following expression is used:

$$X_k = (X_{k-1} \oplus B) \cap A^c \quad (6)$$

The final stage is to isolate the required region that contains the WBC by removing the small objects that

have fewer than P pixels, from binary frame. The purpose of blob extraction is to blob identify the WBC in the successive video frames blob. The 4-connectivity is applied since it requires fewer computations to process the successive video frames faster.

The preceding steps are considered the first stage of the proposed WBC tracking system for extracting the blobs. This is followed by the second stage to identify/classify the different blobs as a WBC or not. This identification/classification process consists of two steps, namely (i) present each blob by a number of characteristics (features), and (ii) apply a matching method to compare the features of each blob. In the present work, the recursive grass-fire algorithm is used as a matching method to compare the features of each blob with the WBC features.

During the feature extraction process, each blob is converted into a few representative features. However, there are still some regions inside the frame when it is connected with border. Thus, before calculating the features, any blob, which is connected to the frame's border, should be excluded, where there is no information about the WBC part that exist outside the frame. Consequently, the main extracted feature is the area of a blob, which is measured using the number of pixels that the blob consists of. This feature is often used to remove the too small or too big blobs from the images in the frame. Generally, the bounding box of a blob, which is the minimum rectangle that contains the blob, is defined by going through all pixels for a blob and finding the four pixels. From these values the width and height of the bounding box are given. A bounding box can be used to identify the WBC (ROI) based on the degree of the pixels connectivity.

The state of the art of blob detector is based on normalized Laplacian of Gaussian (LOG)_{norm}, [13] which is given by:

$$L(x, y, t) = g(x, y, t) * f(x, y, t) \quad (7)$$

where $g(x, y, t) = \frac{1}{2\pi t^2} e^{-\frac{x^2+y^2}{2t^2}}$ is the Gaussian kernel and $f(x, y, t)$ is the video frame. The (LOG)_{norm} can be estimated as follow:

$$\nabla_{norm}^2 L = t(L_{xx} + L_{yy}) \quad (8)$$

Typically, the basic scenario of the blob analysis solution consists of the following steps: (i) extraction of the region corresponding to the objects (or single object) using the thresholding techniques, (ii) refinement the extracted ROI using region transformation techniques, where the extracted region is often flawed by noise of various kind (e.g. due to inconsistent lightning or poor image quality), and (iii) analysis of the refined region to compute the analysis measurements and to split the multiple objects into individual blobs each of which is inspected separately.

In addition, in order to guarantee the keep tracking process of the WBC in the whole video, a simple predictor-based system that consists of two main parameters, namely the age, and the visibility count is proposed [14, 15]. Since the object (ROI) appears initially, so it has age, which means it has been appeared. However, it may be not be visible in some frames and may be visible, thus, a visibility counting is required. Consequently, in this predictor, the 'age' expresses the number of frames that includes the white cell (ROI) during the tracking from the first time it was detected, and the 'visibility count', which represent the total number of frames in which the track was, detected visible [16]. These two parameters are used to predict the new locations of the WBC according to the following algorithm.

Algorithm: Proposed predictor- based system

Start

Set initial object age=1

Set initial object visibility count=1

If detection=prediction

Increment the age and visibility count by one (Age +=1) and Visibility count +=1

End

Stop

Fig. 1 The main parts in the video frames and their relative size that affects the tracking process before modifications, where '1' is the bacteria, '2' is the centroid of the white blood cell, and '3' is the bounding box

However, it is noticed that when the WBC start to attack the bacteria and they are touched together, the bacteria have been included within the bounding box with the region of the detected and tracked also as a WBC due to the small relative velocity between the WBC and the bacteria. This is due to the fact that the difference in the distance between them was very close taking in considerations the obvious difference between their sizes of interest as illustrated in Fig. 1. This mechanism affected the morphological operations that needed to have a good tracking accuracy. Figure 1, shows the drawbacks on the traditional tracking system. Accordingly, we have developed a fine tuned tracking mechanism to guarantee good tracking quality.

To overcome these limitations, a proposed modification by cascading another second stage on the previously

discussed tracking system is included. The main concept is to replace the bounding box (that may include both the WBC and the bacteria when they are too close) by overwriting a green star (*) notation at the centroid of the correct tracked WBC (as shown in Fig. 1) to produce a green masked video for the next stage. Accordingly, compromising algorithm is proposed for fine-tuning of the tracking system based on **Green star based centroid (GSBC)** moving WBC tracking. Thus, in order to identify the green centroid, the HSV is employed, where the HSV color space is quite similar to the way in which humans perceive color. In the present work, the HSV thresholds are used for the green masked WBC according to estimating the histogram of HSV masks [17]. The last step is to filter out small blobs that less than a predefined threshold [18]. Now we get a fine tuned WBC tracking. The block diagram for the whole proposed system is represented as follows in Fig. 2.

Furthermore, the speed of the WBC is calculated using the following procedure. In addition, we locate the centroid in two successive frames in both X- and Y- directions, where the Euclidean distance can be calculated using the following expression:

$$distance = \sqrt{[x(i) - x(i - 1)]^2 + [y(i) - y(i - 1)]^2} \quad (9)$$

Then we store all these centroids in matrix $z(i)$ to calculate the median (ME) of this matrix. The WBC speed is calculated as follow:

$$speed = ME * \frac{number\ of\ frames}{video\ duration} \quad (10)$$

The following algorithm despite the main steps for estimating speed of the detected moving WBC.

Algorithm: Proposed speed estimator based moving white cell

start

Create square structuring element (SE)

Create blob analysis object

Obtain the bounding box_

Read the frames ***Measure*** the properties of the frame's connected regions

Measure the Euclidean distance

Calculate the median (ME)

Calculate the speed

end

Fig. 2 Block diagram of the proposed whole fine-tuned WBC tracking system

Experimental results

A video of a white blood cell chases bacteria [19] is used in the following work. The results of each step in the first stage of the proposed detection procedure are illustrated in Fig. 3.

Figure 3 illustrates a correct detection for the WBC. In addition, the HSV thresholds are used for the green masked WBC according to estimating the histogram of HSV masks in the second stage, using Hue thresholds = [0.24, 0.44], Saturation thresholds = [0.8, 1.0], and value thresholds = [20, 125] based on the histograms for Hue (H), Saturation (S), and Value (V) channels as shown in Figs. 4 and 5. The threshold values selection was

performed according to the concluded histograms in the following figures.

The HSV thresholds are used for the green masked WBC according to estimating the histogram of HSV masks as shown in Figs. 4 and 5 for frame number 53 of 426 and frame number 103 of 426. Furthermore, the tracking trajectory of the WBC is demonstrated in Fig. 6, where also Table 1 reported sample of the centroid coordinates indicating the location of the WBC within the frame.

Figure 6 illustrated the trajectory of the WBC during attacking the bacterium, where the x-axis represents the direction of the WBC's centroid location (right or left). For example, if the WBC moves from left to right negative direction is indicated on the x-axis, while positive values on the x-axis indicated motion from left to right. Moreover, the y-axis represents the motion direction of the WBC's centroid location (up or down). For example, the positive values on the y-axis indicates the motion of the WBC from down to up (upwards) relative to the reference starting point (red point), while the negative values indicated motion from up to down (downwards). In the video under consideration, the WBC starts to move from left to right for short time, then changed its direction to move toward left to attack the bacterium. The visual inspection is used to evaluate the accuracy the proposed complete tuned tracking system for the WBC and harmful bacterium without any means of overlapping. Figure 6 represents a good indication for the system accuracy showing the white blood cell trajectory. Additionally, Table 1 reported samples from the 426 frames, of the coordinates/centroid directions in degrees of the moving WBC, where the absolute values are reported.

For each frame, the centroid direction in degrees is illustrated in Table 1. Furthermore, Fig. 7 demonstrated the speed of the WBC while attacking the small bacterium.

Figure 7 represented the speed of the WBC motion which is estimated according to both time and Euclidean distance. Horizontal axis (x-axis) represents successive video frames number, while the vertical axis (y-axis) represents the motion speed per frame. Generally, Figs. 6 and 7 established the random behaviour and speed of the WBC speed while tracking the bacterium due to the random behaviour of harmful

Fig. 4 Frame #53 of 426: **a** Original frame, **b–d** Hue, saturation, and value; respectively, **e–g** Histogram of H, S, V; respectively, and **h–j** Hue-, Saturation-, Value- masks; respectively

bacterium. In addition, a comparative study is conducted to compare the proposed method with the use of the optical flow tracking method. Accordingly, Fig. 8 illustrated the performance of the optical flow while tracking process.

Figure 8 established that the optical flow suffers from a complete tracking error due to several reasons, namely its sensitivity to noise; it requires large number of calculations that affected its performance to track

the high speed moving WBC, it has poor anti-noise performance which makes it not suitable for real-time demanding occasions. By comparing the optical flow results in Fig. 8 with the results of our proposed system (Figs. 5 through 7), this shows the ability of the proposed method to localize and track the WBC while attacking the bacterium.

Typically, the blood cells' tracking is a challenging process due to the blood cells' non-rigid shapes. Thus, the

Fig. 5 Frame #103 of 426: **a** Original frame, **b–d** Hue, saturation, and value; respectively, **e–g** Histogram of H, S, V; respectively, and **h–j** Hue-, Saturation-, Value- masks; respectively

main motivation of the proposed system was to identify accurately and track the rapidly moving WBC during its attacking to a very small bacterium, where there is a large difference between the size of the WBC and the bacterium. Accordingly, a green star based centroid (GSBC) moving WBC tracking algorithm is proposed in the present work to measure the velocity of the WBC and to draw its trajectory. This green centroid was proposed

to guarantee the accurate localization of the WBC. In addition, a fine tuned tracking system is claimed based on blob analysis to track the WBC in a video. An automatic ROI detection is achieved by identifying the appropriate number of connected component which satisfied a complete isolation between the WBC and the harmful bacteria. This isolation is performed by removing the small objects having number of pixels which is

Table 1 Absolute value of the WBC's centroid directions in degree

Frame number	Centroid location coordinate in horizontal x-axis direction in degree	Centroid location coordinate in vertical y-axis direction in degree
1	146.54	94.36
2	143.99	92.69
3	139.97	90.99
4	137.055	89.62
5	135.12	88.41
50	88.64	145.39
51	85.28	145.87
52	86.36	150.28
53	143.87	153.86
54	139.63	153.13
55	136.18	153.08
100	126.41	122.26
101	124.34	123.74
102	121.75	125.18
103	116.86	130.66
104	179.62	124.09
105	176.69	123.87
200	171.39	79.53
201	170.39	79.16
202	169.22	77.99
203	164.98	76.28
204	163.86	74.56
205	162.32	74.31
290	128.98	72.39
291	124.13	72.08
292	122.69	69.74
293	120.07	68.49
294	116.91	68.65
295	114.82	68.58

less than P pixels. In the present work, the blob extraction is employed to identify the WBC in the successive video frames blob, where 4-connectivity is used since it requires fewer computations to process the successive video frames faster. In the present work, it is noticeable that once the WBC starts to attack the bacteria, they are touched together. Accordingly, the bacterium was included within the bounding box in the detected/tracked region due to the small relative velocity between the WBC and the bacterium. This is due to the fact that the difference in the distance between them was very close taking in considerations the obvious difference between their sizes of interest.

Conclusion

Blood cells detection and tracking has a vital role to study the behaviour and the mechanism of the biological cells in different applications. In the present work, a video-microscopy for WBC attacking a small bacterium is considered. The proposed tracking system consists of the WBC detection stage, which was responsible to detect and localize the WBC in a given frame within the video using blob analysis. Afterwards, for fine-tuning of the tracking process, a proposed green star marker is used to mark the centroid of the WBC with excluding the bacteria as they become so close in some frames. In addition, the velocity of the moving WBC and its trajectory were illustrated.

Fig. 7 Speed of the white blood cell per frame

Fig. 8 Optical flow output: **a** thresholding output, **b** motion vector output, and **c** tracking output

The proposed system is compared to the optical flow technique, which established the superiority of the proposed system and the failure of the optical flow to track the WBC due to the poor quality of the video and the existence of multiple objects.

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Received: 31 May 2018 Accepted: 16 October 2018

Published online: 01 November 2018

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