INTELLIGENT ALGORITHMS FOR CELL TRACKING AND IMAGE SEGMENTATION

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ABSTRACT

Sensitive and accurate cell tracking system is important to cell motility studies. Recently, researchers have developed several methods for detecting and tracking the living cells. To improve the living cells tracking systems performance and accuracy, we focused on developing a novel technique for image processing. The algorithm we propose presents novel image segmentation and tracking system technique to incorporate the advantages of both Topological Alignments and snakes for more accurate tracking approach. The results demonstrate that the proposed algorithm achieves accurate tracking for detecting and analyzing the mobility of the living cells. The RMSE between the manual and the computed displacement was less than 12% on average. Where the Active Contour method gave a velocity RMSE of less than 11%, improves to less than 8% by using the novel Algorithm. We have achieved better tracking and detecting for the cells, also the ability of the system to improve the low contrast, under and over segmentation which is the most cell tracking challenge problems and responsible for lacking accuracy in cell tracking techniques.

KEYWORDS

Cell tracking, segmentation enhancement, Active Contour, Topological Alignments.

1. INTRODUCTION

Tracking cell mobility is an important part of many biological processes. Tissue cells of multi cellular organisms mobilize during embryologic development, generation of new blood vessels, cancer metastasis, and immune response. Understanding the mechanisms of cell motility is essential part for curative and preventative treatments to many diseases. Cell tracking and segmentation with high accuracy is important step in the cell motility research. For instance, tracking the number and velocity of rolling leukocytes is essential to understand and successfully treat inflammatory diseases [15]. Sensitive tracking for moving cells is important to do mathematical modeling to cell locomotion. Moreover, Zimmer [17] modified the snake model to track the movement of the cells and segment the first frame. Another research by Mukherjee [19] he developed a technique to handle segmentation process and tracking problem simultaneously. Li [20] used a technique with two stages; the first one is a tracker and a filter to detect the cell and also the cells which move in and out of the image area. Coskun [12] used imaging data to solve the inverse modeling problem to determine the mobility analysis of the cells. Recently, a number of researchers have been created automated techniques to track and detect the cells mobility. Segmentation is an essential part in many signal processing techniques and its applications. Texture analysis is important in many areas such as image processing, determination of the object shape, scene analysis. The process of segmentation depends on the determination of the best positions of the points which represent the image. The purpose of image segmentation is to partition an image into meaningful regions based on measurements taken from the image and might be grey level, colour, texture, depth or motion. Usually the process to determine the image

starts with image segmentation as initial step. The goal of image segmentation is to cluster pixels into salient image regions, i.e., regions corresponding to individual objects, surfaces, or natural parts of objects. In this paper we managed to introduce a novel technique for image segmentation and cell tracking.

2. RELATED WORK

In recent years, there has been significant research efforts toward the development of automated methods for segmentation and cell tracking for living cells as in [1][2][3][4][5]. Most of the time, images from microscopic studies are corrupted during the recording process and due to the noise from the electronics devices, which affect the quality of the image.

Cell tracking with good accuracy is important in microscopic imaging studies. For instance, Image analysis of leukocytes cells is essential part for curative and preventative treatments to many diseases and also important to understand and successfully treat inflammatory diseases as in Ray et al. [23]. Sensitive tracking for moving cells is important to do mathematical modelling to cell locomotion. Zimmer [17] modified the Active Contour model to detect the mobility of the moving cells and also handle the cell division by providing an initial segmentation for the first frame. Mukherjee et al. [19] developed an algorithm by using threshold decomposition computed via image level sets to handle tracking problem and segmentation simultaneously. Li [20] developed an algorithm with two levels, a motion filter and a level set tracker to handle the cell detection and the cells that move in and out of the image. Coskun et al. [12] used imaging data to solve the inverse modelling problem to determine the mobility analysis of the cells. Recently there have been a number of researchers attempt to create automated algorithms to detect and track the cells from microscopic images as in Mélange [6]; and Mignotte [10].

This paper discusses the tracking cell accuracy as important task in many biological studies to understand the cell behaviour and the way in which cells interact with the world around them. One of the major goals of tracking the mobility of living cells is to find the best way to increase segmentation and tracking accuracy under weak image boundaries, over and under segmentation, which the most cell tracking challenge problems and responsible for lacking accuracy in cell tracking techniques.

3. ALGORITHM

Active Contour and Topological Alignments are used in image processing, particularly in locating object boundaries. Each method has its own advantages and also limitations. Active Contour (snakes), can locate the object boundaries dynamically and automatically from an initial contour. The advantage of Snakes model is the ability of the model to give a linear determination of the object shape at the convergence time, and no extra processing is needed. But Snakes model require detecting strong image gradients to detect the contour. This actually limits the use of Active Contour, because weak boundaries of the image frames and also frames with low contrast will cause over and under segmentation which responsible for decreasing the accuracy of the analysis. To mitigate the effect of this problem with the Active Contour model, and to improve the performance of segmentation and cell tracking, we apply the Topological Alignments method to increase the accuracy of cell tracking and detecting analysis. The Topological Alignments method links segments between every frame and the next one; that will decrease the number of false detections and also the false trajectories. In this paper, we introduce a novel technique based on Active Contour in conjunction with Topological Alignments. We present tracking system and image segmentation algorithm to incorporate the advantages of both Active Contour and Topological Alignments to get a tracking system with high accuracy to detect and analyze the

mobility of the living cells. The novel technique proceeds in two steps: First we do initial segmentation by applying the Topological Alignments and then transformed the output into the input of the Active Contour model to begin the analysis to detect the cells boundaries and determine the mobility of the cells.

Active Contour models (snakes) goal is to apply segmentation process to an image by doing deformation to the initial contour towards the boundary of the object of interest. We do that by deforming an initial contour to minimizing the energy function which defined on contours, as in [13] [15]. We have here two components which represents the energy function; the first part is the potential energy component, and the potential energy component is small when the contour is aligned to the image edge, and the second part is the internal deformation energy component, and the component is small when the contour is smooth. Both components are contour integrals with respect to a parameter of the contour.

Active Contour can be represented by two models depends on the characteristics of the image; edge-based models and region based models. The advantage of Active Contour model is the ability of the model to give a linear determination of the object shape at the convergence time, and no extra processing is needed. But Snakes model require detecting strong image gradients to detect the contour. This actually limits the use of Active Contour, because weak boundaries of the image frames and also frames with low contrast will cause over and under segmentation which responsible for decreasing the accuracy of the analysis.

The Topological Alignments method based on linking the segmentation of two frames in the video sequence as in [17] [25]. From the output of the segmentation procedure, the method finds the maximum weighted solutions between two pairs of frames and then match the segments. The method can deal with low contrast images and shape cells and improves the filtration efficiency.

Figure (1), represents the proposed framework of this research. Starts by loading the cell sequence, and pre–processing stage starts to smooth the image and remove noise, segmentation stage starts to detect the WBC outline boundaries, and segments the image boundary and then, extracts the WBC mobility data. The following algorithm describes the general cell tracking main steps used in this research for automated enhancement technique.

Algorithm

Step 1) Cell image pre-processing and enhancement to reduce the cell image noise.
Step 2) Cell detection; using level set Active Contour segmentation.
Step 3) Cell tracking; using Topological Alignments method, and transforms the raw images into a file that encodes the boundaries of every cell at every time point, and then compute the motion, and shape parameters. We implemented the algorithm in C++ under Windows XP operating system.

The datasets in this research consists of three video sequences protocols of WBC as test data sets for the tracking experiments. Measuring the quality of an automated cell tracking procedure requires a ground truth annotation to compare the output of the computationally produced tracking with the ground truth annotation.

The first test data set consists of 32 microscopic video sequences and the temporal resolution is 30 frames /s (3-sec duration) previously used in Jung *et al*. (1998). These sequences are captured from experiments, and recorded via a CCD camera system (model VE-1000CD; Dage-MTI) on a Panasonic S-VHS recorder. The video frames is recorded at a spatial resolution of 320 x 240 pixels, where the pixel-to-micron ratio is 2.47 pixels /micron horizontally and 2.34 pixels /micron

vertically, the leukocyte motion direction is known a priori and is from left to right hand direction.

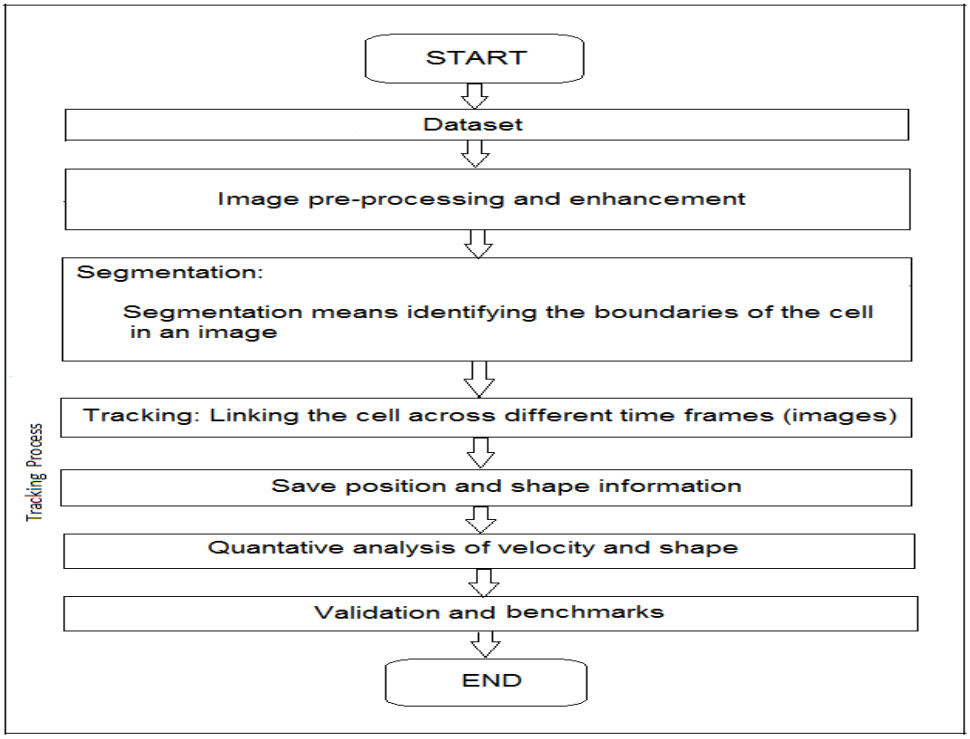


Figure 1. Proposed framework for cell tracking system: segmentation and tracking transforms the images of a cell into a file that extracts the boundaries of every cell at every time point to compute the shape and motion parameters.

The second data set consists of 16 leukocyte sequences previously used in Scott *et al*. (2001). The video sequence is recorded at a spatial resolution of 320 x 240 pixels (where the pixel-to-micrometer ratio is 2.47 pixels /mm in the horizontal direction and 2.34 pixels /mm in the vertical direction) and a temporal resolution of 30 frames/s. All cells were tracked for at least 90 frames (a period of 3 sec). The leukocyte motion direction is from right to left hand direction for the 16 microscopic video sequences.

The third data set (self-created detect) consists of a set of video sequences of 40 live cells. The video recordings are made by attach a charge-coupled device (CCD) camera to a microscope. The video frames were recorded at a spatial resolution of 640×480 pixels, where pixel-to-µm ratio was 4.94 pixels/µm horizontally, and 4.68 pixels/µm vertically. The leukocyte motion direction is from left to right hand direction. The temporal resolution was 30 frames /sec. Each video sequence included 91 frames, more details about the (self-created detect) lab experiment to get mobility analysis data for live cells. Standard operating procedure (S.O.P) is used for the ground truth annotation.

3.1 Cell Image Pre-Processing and Enhancement

Image pre-processing is required to remove the image noise. Median filtering by Kasturi *et al*. (1995) is used to reduce the existing high noise level in the input frames. The median filter is an effective method that can suppress isolated noise without blurring sharp edges. Specifically, the median filter replaces a pixel by the median of all pixels in the neighborhood.

3.2 Cell Segmentation and Tracking Using the Proposed Technique

The aim of cell segmentation algorithms is to partition the image into perceptually similar regions. The goal of segmentation is to simplify and/or change the representation of an image into something that is more meaningful easier to analyze, and to produce a binary image from the grey scale image. In addition, segmentation process also helps to remove noise from the image. There are a few methods to produce segmentation, such as edge and region based segmentation, statistical classification, and thresholding.

One of the famous methods in cell segmentation is Active Contour based segmentation (Such as level set Active Contour, Gradient Vector Flow (GVF) Active Contours), and also thresholding methods is one of the simplest and famous method in image segmentation.

The enhanced technique is using the level set Active Contour by Lee *et al*. (2004), in conjunction with Topological Alignments method by Palaniappan *et al*. (2009), to segment and track the cells, and to track the cell motion and shape evolution. Quantifying cell morphology and motility generally requires segmenting and tracking individual cells from large image sequences. Segmentation means identifying the boundaries of meaningful objects (here: cells) in an image; tracking means linking these objects across different time frames. In practice, a segmentation and tracking algorithm typically produces a data file that contains a description of the boundary of each cell at each time point, for example of the (x,y) coordinates of a finite list of points along that boundary as shown in Figure 2.

These extracted data can be used to quantify parameters characterizing cell shape and motion, generally without the need to go back to the original images. Most of the process is concerned with the segmentation and tracking stage, which is very often the most difficult part of the workflow that begins with image acquisition and ends with the analysis of quantitative results, and get computation of shape and motion parameters (known width of the leukocyte(~ 4 μm)).

Because the shape size and position information about cells is known a priori, it can incorporate these as parameters in our energy function to provide a more robust algorithm. Active Contour shape can be constrained to known elliptical and circular cell shapes. Active Contour size is known because the approximate cell size from the scale of the video is known.

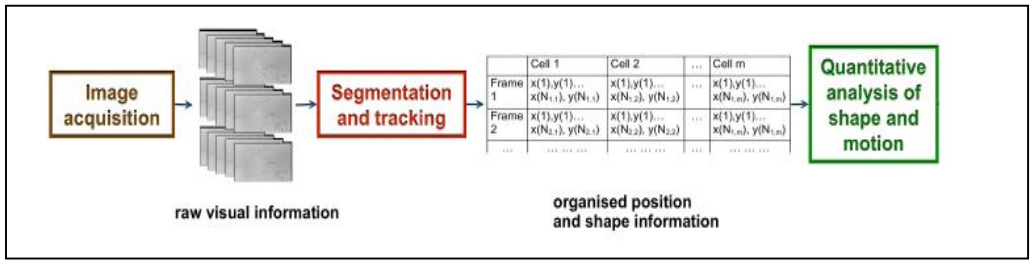


Figure 2. Cell image analysis processes: segmentation and tracking transforms the raw images into a file that encodes the boundaries of every cell at every time point, and allowing computation of shape and motion parameters (Zimmer *et al.*, 2005).

It is importance to combine the shape and size constraints in capturing a leukocyte for tracking. The position constraint incorporates future cell positions and provide updates for the (x, y) position. In addition, the Active Contour re-parameterizes itself (keeping uniform sampling) according to the sampling energy term. The assumed initial radius of the snake tracker is integral

to effective tracking. This parameter allows the Active Contour to formulate the size constraint, which governs the relative size of the circle or ellipse shape constraint. An accurate value of the initial radius as compared to the actual radius of the target cell realizes a more effective Active Contour tracker.

Level set Active Contour segmentation: Level set theory, a formulation to implement Active Contours by Lee *et al*. (2004), was proposed by Osher *et al*. (1988). Redefine the entire domain of an image *I(x, y)* as a disjoint set of subsets. They represented a contour implicitly via a two dimension continuous function $\phi(x, y) : \Omega \rightarrow \Re$ defined on the image plane. The function $\phi(x, y)$ is called level set function, and a particular level, usually the zero level, of $\phi(x, y)$ is defined as the contour

Topological Alignments Tracking Method: level set Active Contour is used to do segmentation to the cell frames, and Topological Alignments method is used to track the cells. The study addresses the problem of linking segmentations of two consecutive frames in the video sequence. Starting from the output of a conventional segmentation procedure, the study aligns pairs of consecutive frames through assigning sets of segments in one frame to sets of segments in the next frame. The Topological Alignments method represent the segmentation of two images from the video sequence as m and n, index set $P = \{1,..., m\}$, and an index set $Q = \{1,..., n\}$. The method assumes that cells move moderately between two consecutive frames.

Topological Alignments Method by Palaniappan *et al*. (2009) has two stages approach: The first stage, the method apply a segmentation procedure on every frame (here, level set Active Contour is used for cell segmentation). The second stage is the linking stage, where the topological alignment links segments between each frame i and the next frame $i + 1$. Now, in order to track one cell, the method is matching a set of segments in frame i with another set of segments in frame $i + 1$. Being based on overlap of segment groups in the two frames, the method can be considered as a topological alignment between two images.

The method links segments between each frame and the next frame, reducing the number of false detections and false trajectories. The Topological Alignments technique achieve this through finding maximum weighted solutions to a generalized "bipartite matching" between two hierarchies of segments, where they derive weights from relative overlap scores of convex hulls of sets of segments.

3.3 The Main Steps of the Proposed Enhancement Tracking Algorithm:

1. Level set Active Contour is used for cell segmentation to identify individual cells in every single frame, then the segmentation data for each cell are stored in a file. The first step toward extracting leukocyte shape is to find a closed contour that satisfies a leukocyte shape prior and minimizes energy functional for image segmentation.
2. Topological Alignments method is applied to track the cells, where the data file contains a description of the boundary of each cell at each time point, consisting for example of the (x, y) coordinates of a finite list of points along that boundary.

a. The main point in this procedure is to assign a weight *w(p, q)* to matching segments sets $p \subseteq P$ and $q \subseteq Q$.

where segmentation of the first image into *m* segments with an index set $P = \{1,..., m\}$, and the segmentation of the second image into *n* segments with an index set $Q = \{1,..., n\}$.

b. Measure weights based on the "relative overlap" of the convex hulls of *p*, *q*, and identify $p` \subseteq P$ with the convex hull of the area covered by all segments in *P*, i.e. $A(p) := \overline{\cup_{x \in p} a(x)}$, where $a(x)$ denotes the area covered by segment *x* and $\overline{X}$ denotes the convex hull of a set *X* of points in the plain.

c. Assign the relative overlap of *p* and *q* as their weight, formally defined as:

$w(p,q) := \left|\overline{A(p)} \cup \overline{A(q)}\right| / \left|\overline{A(p) \cup A(q)}\right|$ and assuming that cells move moderately between two consecutive frames. Sets of segments that achieve a relative overlap close to 1 should be considered as one cell.

d. For each $p \subseteq P$ and $q \subseteq Q$, we introduce a binary variable Xp, q, where $Xp, q = 1$ if and only if $p = S_i$ and $q = T_i$.

e. Maximize over valid partitioning only, where $max \sum_{p \subseteq P, q \subseteq Q} w(p,q) X_{p,q}$ and, correspondingly, overlapping subsets from *Q* by introducing constraints as $X_{p,q} + X_{p',q'} \leq 1$.

f. The constraint matrix *C* resulting from equation (3.12), *C* is the incidence matrix of the bipartite graph $B = (L \cup R, E)$, where $L = \{pp' | p, p' \subseteq P\}$ and $R = \{qq' \mid q, q' \subseteq Q\}$, and *E* introduces one edge for each constraint.

3. Repeat steps (a-f) until all frames of the image sequence are processed. The result as a function of time enable estimation of the position and deformation of the corresponding cells for each frame in the sequence and update shape and position.

4. Segmentation and tracking transforms the raw images into a file that encodes the boundaries of every cell at every time point, directly allowing computation of shape and motion parameters.

By using Topological Alignments to track the cells, the enhanced technique addresses the problem of linking segmentations of two consecutive frames in the video sequence. The output of a conventional segmentation procedure is used to align pairs of consecutive frames through assigning sets of segments in one frame to sets of segments in the next frame. The procedure achieve this through finding maximum weighted solutions to a generalized bipartite matching between two hierarchies of segments, and derive weights from relative overlap scores of convex hulls of sets of segments.

These extracted data for shape and position can be used to quantify parameters characterizing cell shape and motion. Segmentation and tracking transforms the raw images into a file that encodes the boundaries of every cell at every time point, and allow the computation of shape and motion of the cells.

3.4 Manual Method-Ground Truth (Self-Created Detect)

The manual method (ground truth) used to track the mobility of the cells and manually collecting data from the recordings. Based on the Stripe Source Diffusion Technique, which developed by *G*. Grimes and *F*. Barnes (Grimes *et al*., 1973), to track the cells using microscopic technique.

Manual tracking measurements were obtained by allowing an operator to observe the cells movement; its movements were tracked on the computer monitor with the frames.

This manual method investigate the human leukocyte cells mobility changes under the effects of radio frequency fields on the ability of human leukocyte to follow concentration gradients of cyclic-AMP are reported. Blood from healthy adult donors is exposed in vitro to 990 MHz a signal generator at levels of 100 *V/m* and magnetic flux densities of 0.26 μT, and to fields from a NOKIA cellular phone for approximately 3 seconds intervals. Once the data are collected, and usually calculate the chemotactic index, the initial concentration, the concentration gradient, and the diffusion constant.

Squared grid sheet is placed on the screen so that the mobility of the cells could be tracked on the screen as shown in Figure 3. The screen is calibrated so that the diameter of one white blood cell (4 µm) is equal to one square on the screen. The collected data is used to calculate the mobility of the cells, and allow the computation of shape and motion of the cells.

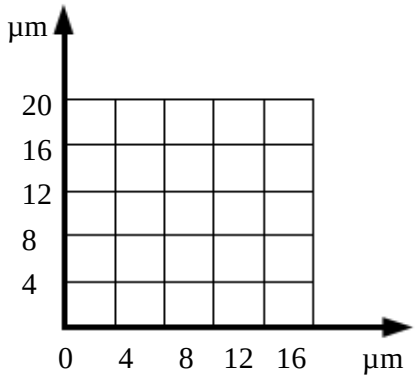


Figure 3. Squared grid sheet is placed on the monitor.

Shape Index: Shape index is an indicator for the shape change. (Koenderink and van Doorn, 1992) developed a single-value, angular measure to describe local surface topology in terms of the principal curvatures. It is a quantitative measure of the surface shape. Where *n* is the surface normal at position *x, y*. This shape index is defined as:

$$S = \frac{2}{\pi}\arctan\frac{k_2 + k_1}{k_2 - k_1} \qquad k_1 \geq k_2 \tag{1}$$

$$H = \begin{pmatrix} -(\frac{\partial n}{\partial x})_x & -(\frac{\partial n}{\partial x})_y \\ -(\frac{\partial n}{\partial y})_x & -(\frac{\partial n}{\partial y})_y \end{pmatrix} \tag{2}$$

where shape index (*S*) defines the shape, and the principal curvatures are the maximum curvature k_1 and minimal curvature k_2. k_1 and k_2 can be found by solving $|H - kI| = 0$ for *k*, where I is the identity matrix. Where *H* matrix is the eigenvalues of the shape and $(\frac{\partial n}{\partial x})_x$, $(\frac{\partial n}{\partial x})_y$, $(\frac{\partial n}{\partial y})_x$, and $(\frac{\partial n}{\partial y})_y$ denote the *x* and *y* components of the parenthesized vector respectively. The collected data (video sequences) is used as a protocol to test the automated methods, and to compare the results from the manual method (ground truth) and the automated methods.

3.5 Accuracy Performance Measure

The performance of the enhanced cell tracking technique is measured by how well the system can track the WBC. Two methods are used to evaluate the enhanced technique.

- Percentage of frames tracked. If a computed cell center is within one cell radius of the manually observed cell center, then we consider that frame as tracked. The percentage is computed as the ratio of number of frames tracked to the total number of frames in the sequence.

$$f_P = \frac{N}{N_{total}} \tag{3}$$

where f_P is the percentage of frames tracked, N is the number of frames tracked, and N_{total} is the total number of frames in the image sequence.

- The second method is used to measure the performance of our enhanced technique is calculating the Root Mean Squared Error (RMSE) between the manual (ground truth) and the computed displacement. In addition, compare the RMSE achieved with the RMSE for the other methods (Jung *et al*., (1998), and also earlier observation of Ley et al., (1996)).

The RMSE (in microns) describes how accurately the tracker tracks the cell as compared predicted (computed) to the actual (ground truth or manual) data. RMSE gives the standard deviation of the model prediction error. A smaller value indicates better model performance. The root mean square error (RMSE) is giving a sense of the predicted values error. Also how close the predicted values are to the actual values. The RMSE mathematical formula is giving by:

$$RMSE = \sqrt{\frac{\sum_{i=1}^{n} (X_{actual,i} - X_{predicted,i})^2}{n}} \tag{4}$$

where X_{actual} is actual values and $X_{predicted}$ is predicted values, and *i* represent the current predictor, and *n* represents the number of predictors. The combination of the percentage of frames tracked and the RMSE yields the qualitative performance Ratings.

3.6 Validation and Benchmarks

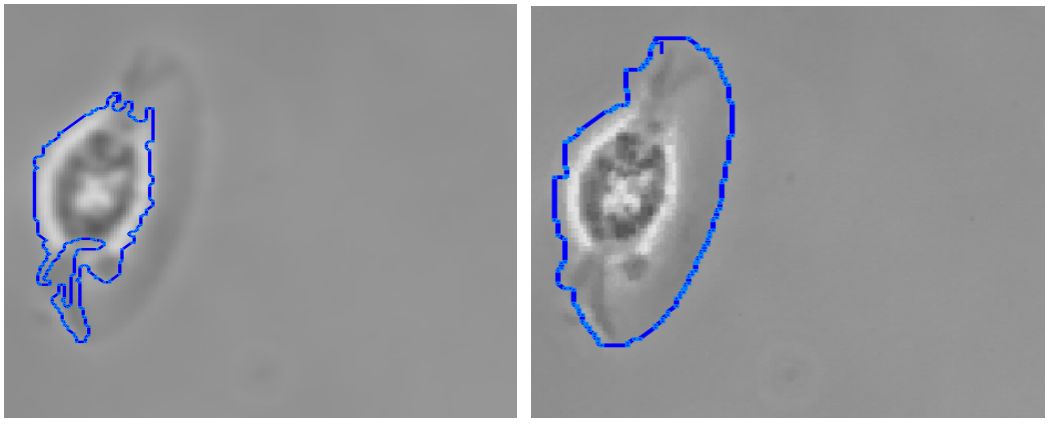
To validation and evaluate the quality of an automated cell tracking procedure, a comparison have been made between the manually marked data, also known as ground-truth, of three video sequences protocols and the automated extracted data, to compare a computationally produced

tracking with the ground truth annotation. In addition, compare the experiment results with the other research methods results such as Jung *et al*., (1998), Scott *et al*. (2001), and earlier observation of Ley *et al*. (1996).

4. RESULTS AND DISCUSSION

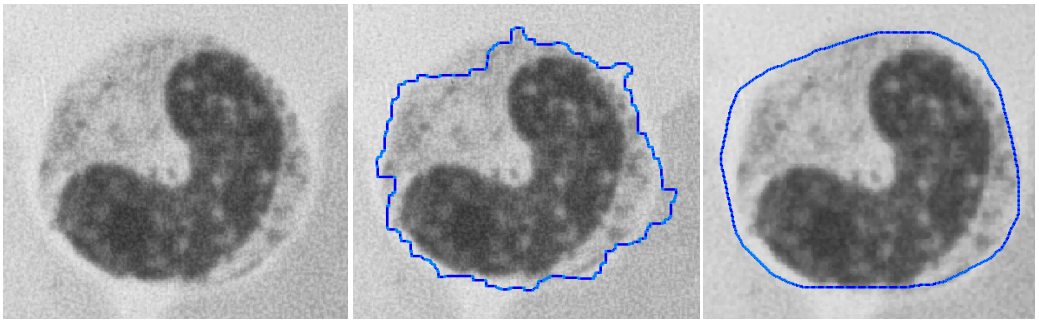
In order to obtain validation of our approach, we have tested the algorithms in by using three different protocols. Some results for both manual and automated tracking of the leukocytes are given in help of a tracking system as function of time and position. The cell velocity is calculated from the mass center across the frames. Some results for both manual and automated tracking of the leukocytes are given in Table 1. Average movement velocity was $\upsilon = 4.2 \pm 0.4$ μm/min (manual) and 4.8 ± 0.5 μm/min (automatic), consistent with earlier observations in [23]. The RMSE between the manual and the computed displacement was less than 12% on average. The Active Contour method gave a velocity and change shape RMSE of less than 11%, improves to less than 7% by using the novel algorithm presented here. Our results indicate better segmentation and more accurate tracking for detecting and analyzing the mobility of the living cells. We have achieved better tracking and detecting the cells, also the ability of the system to improve the low contrast, some results are shown in Figure 4, Figure 5 and Figure 10, Figure 11 show the novel velocity and the shape index for the cells. The novel image processing technique used in the tracking system successfully address the major problems associated with tracking cells, image with low contrast; more accurate tracking for detecting and analyzing the mobility of the living cells.

The results show the advantages of using our novel techniques to enhance the image and the effect on detecting the cell contours with better and accurate segmentation. The results indicate improvement in segmentation performance by using the Topological Alignments, which leads to improve cell detection results. Our results indicate better segmentation and more accurate detecting and analyzing leukocytes cells, also the ability of the system to improve the low contrast, under and over segmentation, some results shown in Figure 6, and Figure 7.



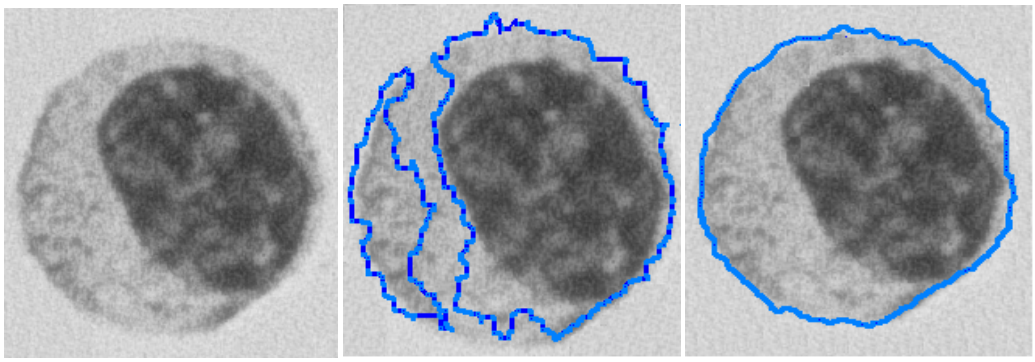
(a) (b)

Figure 4. Better accuracy segmentation by using the novel algorithm.(a) By using Active Contour algorithm (b) By using the novel algorithm we get better contrast and detection.



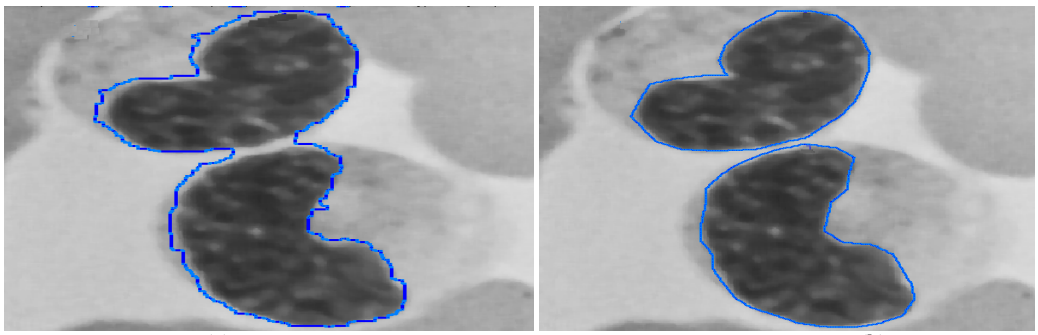
(a) (b) (c)

Figure 5. Example image from grayscale images (a) Original image with noise, (b) Cell detection by using Active Contour, (c) Better detection by using the Novel algorithm.



(a) (b) (c)

Figure 6. Example image from grayscale images (a) Over segmentation problem by using Active Contour (b) Over segmentation problem solved, by using the Novel algorithm.



(a) (b)

Figure 7. Example image from grayscale images (a) Over segmentation problem by using Active Contour (b) Under segmentation problem solved, by using the Novel algorithm.

Table 1. Some mobility results for both manual (ground truth) and enhancement automated tracking of the leukocytes cells.

Cell	Vel_{GT} (μm/s)	Vel_{Exp} (μm/s)	$Shape.Index_{GT}$	$Shape.index_{Exp}$
1	7.0	7.4	0.8	0.83
2	3.5	3.5	0.9	0.83

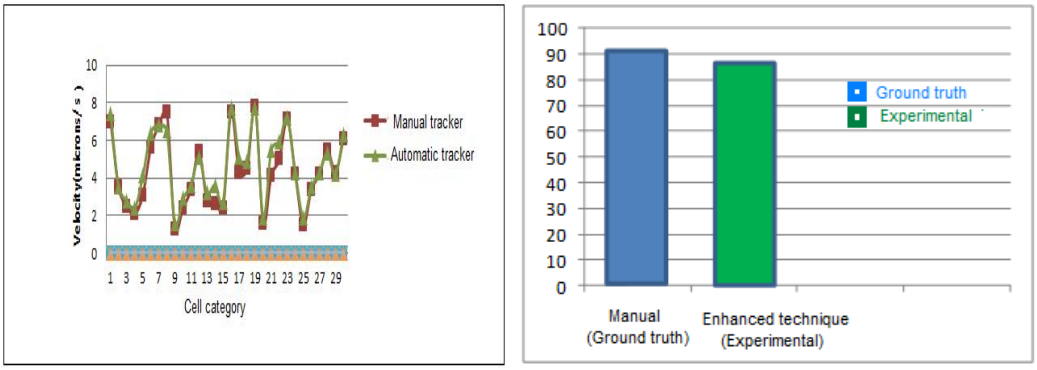
3	2.5	2.8	0.5	0.53
4	2.1	2.4	0.73	0.8
5	3.1	4.1	0.83	0.83
6	5.6	6.4	0.5	0.56
7	6.8	6.8	0.43	0.53
8	7.5	6.5	0.83	0.93
9	1.3	1.6	0.96	1.0
10	2.4	2.9	0.56	0.56

Vel_{GT} :GROUND TRUTH Vel_{Exp} :EXPRIMENTAL. (ENHANCED TECHNIQUE) VELOCITY

$Shape.Index_{GT}$:GROUND TRUTH (CHANGE SHAPE INDICATOR) $Shape.index_{Exp}$:EXPERIMENTAL (ENHANCED TECHNIQUE)

The average movement velocity obtained is $\upsilon = 5.5 \pm 0.4$ μm/s (experimental) and 5.8 ± 0.5 μm/s (ground -truth), consistent with earlier observations of Jung *et al*. (1998), and also earlier observation of Ley *et al*. (1996). The RMSE between the manual (ground-truth) and the computed displacement was less than 8% on average, where the RMSE observed by Jung et al., (1998) was 12%. The average velocity error was less than 12% improves to less than 8% by using the enhanced algorithm (experimental) presented here, where a lower RMSE indicates a higher accuracy. The RMSE calculated by using the values from Table 1 and the formula in Equation 4.

The experimental results indicate improvements in WBC segmentation Figure 6, Figure 7, and more accurate tracking for detecting and analyzing the mobility of the WBC, and achieved better tracking of the cells and solving the segmentation problems, also the ability of the system to reduce the noise as shown in Figure 5, Figure 6, Table 1, and Figure 8(a), shows a comparison of average velocities (per cell) between the manually (ground-truth) recorded measurements and the automated tracker results for the tracked cells. Figure 8(b), shows the percentage of sequences with 100% frames tracked for tracking 32 sequences for the cells.



(a) (b)

Figure 8. (a) Some results from the experiments, velocity for a single WBC. Manually (ground- truth) and automatically computed measurements. (b) Percentage of sequences with 100% frames tracked for tracking 32 sequences.

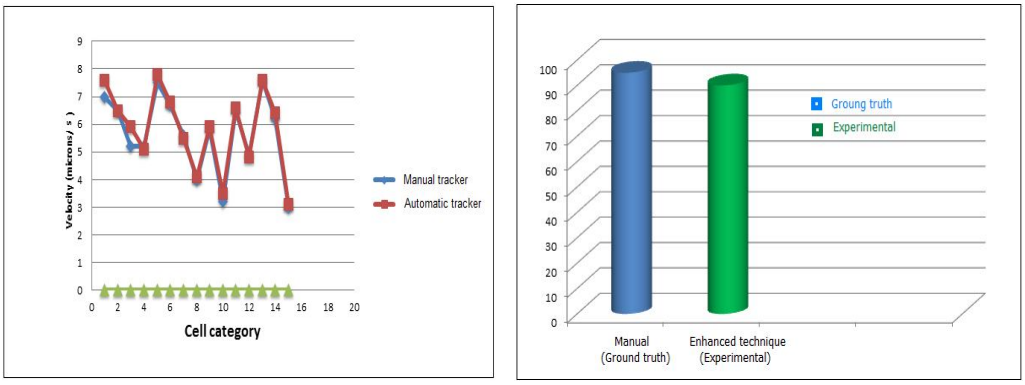
The enhanced image processing technique used in the tracking system successfully address the major associated with tracking cells, image with low contrast; better tracking for detecting and analyzing the mobility of the living cells.

Another validation test for the enhanced technique by using set of video sequences for 16 live cells previously used in Scott *et al.* (2001). In order to obtain another validation of our approach, the experiment carry out a test by using set of video sequences previously used in Scott *et al.* (2001). The experimental results indicate better and accurate tracking analysis by using our enhanced algorithm to track the mobility of WBC from video sequences as shown in Figure 9(a), and Figure 9(b) shows the percentage of sequences with 100% frames tracked. The enhanced algorithm increased the accuracy of the results of the velocity calculations. The average movement velocity result were consistent with the results obtained by Scott *et al.* (2001) and the RMSE according to the enhanced technique results is less than 10%, where the RMSE for Scott *et al*. (2001) was less than 12%, as shown in Table 2.

Table 2. Some mobility results for both manual (ground truth) and automated (enhanced tracking technique) of the living cells.

Cell No	Vel_{GT} (μm/s)	Vel_{Exp} (μm/s)
1	7.0	7.6
2	6.5	6.5
3	5.2	5.9
4	3.5	3.4
5	5.2	5.1
6	7.5	7.8
7	6.7	6.8
8	5.6	5.5
9	4.0	4.1
10	5.8	5.9

Vel_{GT} : GROUND TRUTH Vel_{Exp} :EXPRIMENTAL. (ENHANCED TECHNIQUE) VELOCITY



(a) (b)

Figure 9. (a) Some results from the experiments, Comparison of average velocities (per cell) between the automatic (automatically computed measurements) enhanced technique and manually (ground- truth) tracked cells. (b) Percentage of sequences with 100% frames tracked for tracking 16 sequences.

The third protocol is a set of video sequences (self-created detected) by using a microscopic technique as video sequences for 40 living cells to be used to test and evaluate the experimental tracking system, and used to make comparison between automatic tracking and human controlled

tracking. The 40 living cells as video sequences collected manually to be used to test and evaluate the enhanced tracking system (i.e., experimentally determined in this research.

The automated tracker was used to compute the corresponding 40 cell positions and the cell shape change. Manual tracking measurements were obtained by allowing an operator to observe the cells movement, its movements were tracked on the computer monitor with the help of a tracking system as function of time and position. The speed of a cell is calculated as the displacement of its center of mass across frames. Results for both manual and automated tracking of the leukocytes are given in Table 3.

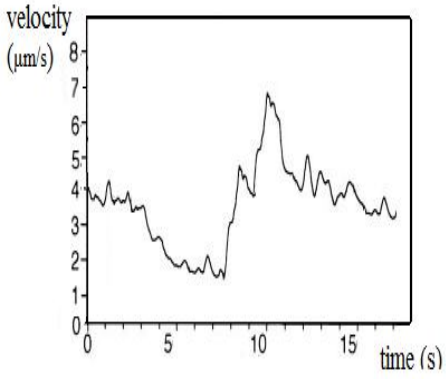


Figure 10. Cells velocity, the WBC velocity-self created detect(Vel_{GT} : ground truth)

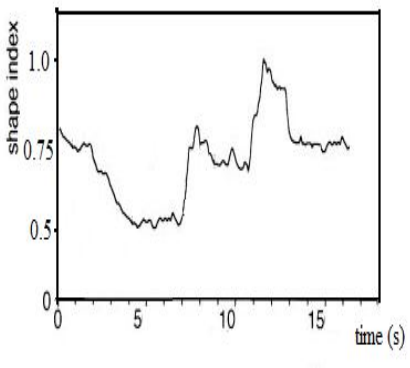


Figure 11. $Shape-index_{GT}$ (ground truth) the WBC change shape indicator, self- created detect.

The root mean squared error (RMSE) between the automated computed displacements and the manually measured displacements was less than 8% on average, where it was 12% of Jung *et al.* (1998). The results indicates better mobility analysis for speed and changing shape due to the improvement in segmentation performance by using the Topological Alignments, which leads to improve cell tracking results as shown in Table 3.

Table 3. Some mobility results for both manual (ground truth) and automated tracking of the leukocytes cells.

Cell	Vel_{GT} *(μm/s*	Vel_{Exp} *(μm/s)*	$Shape.Index_{GT}$	$Shape.index_{Exp}$
1	4.2	4.4	0.46	0.5
2	2.7	2.4	0.9	0.9
3	5.5	5.7	0.93	0.9
4	2.3	2.4	0.4	0.4
6	4.7	4.9	0.53	0.56
7	5.4	5.7	0.76	0.83
8	6.7	6.6	0.93	0.93
9	2.2	2.3	1.0	1.0
10	4.1	4.4	0.86	0.9

Vel_{GT} :GROUND TRUTH

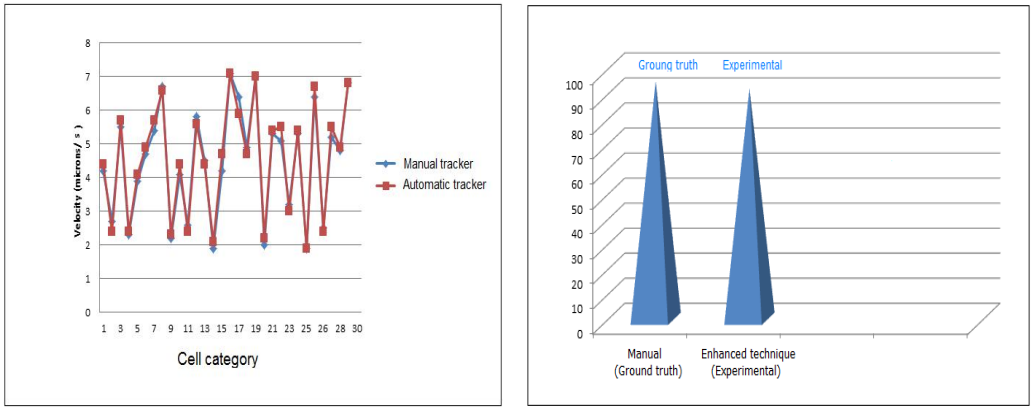
Vel_{Exp} :EXPRIMENTAL. (ENHANCED TECHNIQUE) VELOCITY

$Shape.Index_{GT}$:GROUND TRUTH (CHANGE SHAPE INDICATOR)

$Shape\ .index_{Exp}$:EXPERIMENTAL(ENHANCED TECHNIQUE)

The results from Table 3., Figure 12(a), shows the ability of the enhanced technique to segment and track the WBC with good accuracy, based on the results in Jung *et al.* (1998), and also the

experiment results achieved from the manual tracking. As shown in Figure 12(a) and Figure 12(b). The comparison of average velocities (per cell) between the manual tracking and the automated enhanced technique shows the similarity between the velocity average values for most of the cells as shown in Figure 12(a), which indicate the high accuracy of using the enhanced technique to track the WBC. Figure 12(b) shows the percentage of sequences with 100% frames tracked.



(a) (b)

Figure 12. Some results from the experiments, Comparison of average velocities (per cell) between the manually recorded measurements and the automated tracker results for the tracked cells. (b) Percentage of sequences with 100% frames tracked for tracking 40 sequences.

5. CONCLUSION

Active Contour and Topological Alignments are used in image processing, particularly in locating object boundaries. Each method has its own advantages and also limitations. Active Contour (snakes), can locate the object boundaries dynamically and automatically from an initial contour. The advantage of Snakes model is the ability of the model to give a linear determination of the object shape at the convergence time, and no extra processing is needed. But Snakes model require detecting strong image gradients to detect the contour. This actually limits the use of Snakes, because weak boundaries of the image frames and also frames with low contrast will cause over and under segmentation which responsible for decreasing the accuracy of the analysis. To mitigate the effect of this problem with the Snakes model, and to improve the performance of segmentation and cell tracking, we apply the Topological Alignments method to increase the accuracy of cell tracking and detecting analysis. In our experiments, we compared our algorithm with traditional snake. The results show that the algorithm can demonstrate the segmentation accuracy under weak image boundaries, low contrast, under and over segmentation of living cells, which the most cell tracking challenge problems and responsible for lacking accuracy in cell tracking techniques. Our results indicate better segmentation and more accurate tracking for detecting and analyzing the mobility of the living cells. We have achieved better tracking and detecting for living cells, also the ability of the system to enhance the segmentation for low contrast, under and over segmentation problem. In this paper we focused on solving the under and over segmentation and low contrast problems, however in our future work we will continue our research over the cell tracking by using shape descriptions and other features and consider other problems with image segmentation such as images with high noise. Although the proposed methods produce very robust and promising results, there are still a few aspects could be improved. The required computation is relatively larger than traditional methods increasing the convergence time. The fast advance of computer processor may solve this problem.

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