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AUTOMATED CELL MIGRATION TRACKING TECHNIQUE: A REVIEW

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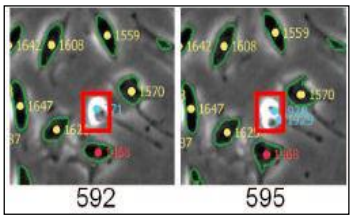
Graphical abstract



Abstract

Automated cell migration tracking is important in detecting the cell movement in order to help in cell status analysis especially when there are a huge numbers of cells in one image frame. Automated cell tracking processes involve detecting, segmentation and labelling the cell. Each step is crucial and will affect the next step. The common problems are cell proliferation, overlapping and clustering. Consequently, this review not only focuses on the overview of current techniques used to complete the cell migration tracking tasks, but also the comparison of these techniques and some suggested future work(s).

Keywords: Cell migration, tracking, segmentation, labelling

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1.0 INTRODUCTION

Over recent years, there has been an explosive growth Cell migration is a translation process of cells from one location to another. It is important for those involve in biomedical field to understand cell behaviour through knowing the migratory movement of cell. Inappropriate migratory movement of failure in migration of cells will lead to severe consequences such as immunosuppression and tumour dissemination. Therefore, cell migration tracking also help in detecting embryonic development and wound healing analysis [1], [2].

However, it is very hard for a doctor or related person to track the cell by manually. There are a huge numbers of cells in the image frames, consequently, an automated cell migration tracking is needed to complete this task [3]. This paper reviewed the challenges and current techniques in cell migration tracking. In session II, the challenges in cell migration tracking are discussed and cell migration steps are reviewed in session III. Apart from that, the strength, weakness and future work of certain techniques are also reviewed in the Table 1 as a comparison of the existing techniques.

2.0 CHALLENGES OF CELL MIGRATION TRACKING

The first attempt of automated cell tracking was implemented in 30 years ago [3]. But, the numbers of researches in cell tracking has increased over the years. From the previous work, the automated cell tracking can be concluded in several steps, which are cell detection, cell segmentation and cell tracking.

The intensity values in the images are the main component to detect the cell [2]. It can be used to identify the cell position by detecting the centre of the nucleus and the centroid of different parts in the cells [1]. This can be challenging if the intensity values of cell itself is similar to the background. On the other hand, the successful of cell detection helps to produce a better segmentation results.

Thresholding is the common used method to implement segmentation of the cell [1]–[5]. Thresholding determines the division region with an intensity value called threshold [6]. If the pixels are greater than the threshold, the pixels are grouped together into one class, and all other pixels are grouped into another class [7].

Since thresholding approach depends on the intensity value of the image, the segmentation of the

cell from background becomes challenging if noisy image is used [2]. Apart from that, photobleaching, autofluorescence, low contrast, halo artifacts and gradients in the images also cause the thresholding approach fail in segmenting the cell [3]. For this reason, suitable filters should be applied to the images before segmentation process in order to remove the artifacts and noise. This enables the segmentation to be done correctly[8].

In addition, the cell migration, cell density and cell proliferation increase the difficulties in segmentation [9], [10]. The position of a cell changed from frame to frame depending on the movement of the cell. The movements of cells make them enter the view or exit the view as shown in Figure 1. The cells will also overlap or cluster together. When the cells overlap together, it is hard to detect the cell separately. Figure 2 illustrates cell proliferation process. The process occurs

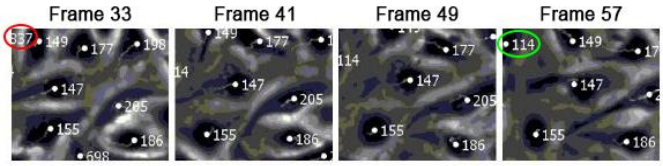

Figure 1 The cell exists the view of field is circled with red color ellipse and the cell enters the view of field is circled with green color ellipse [8]

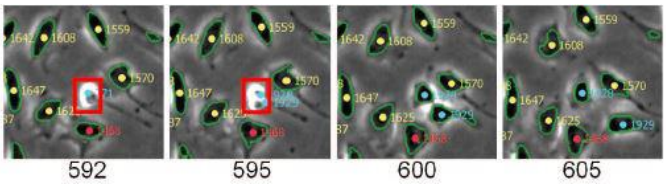

Figure 2 Red color boxes highlight the mitosis event of a cell. The cell is separated into two cells and they are indicated by blue contours [11]

When a single cell enters the mitosis process and divides to two cells. The segmentation results are very important in cell migration tracking process. Therefore, the segmentation of cell also need to consider the changes of cell frame by frame since the tracking process will start from the first frame to the last frame of the cell migration video. Previous research also proved that the frame by frame segmentation gave better results compared to others [9], [11].

The tracking process is done by labelling each cell from frame to frame and converting the image to a video in order to observe the movement direction of a cell. The main challenge of labelling is to connect the cell from frame to frame, which is also called linking of cell [3]. Each cell shall be connected in different frames to show the migration path of the cell. Besides that, the proliferation of the cell causes the divided cell to become mother and children relationship. This raises another problem which is the determination of relationship between the cells in linking step.

3.0 CELL MIGRATION TRACKING PROCESS

There can be hundred of cells in a frame. It is very hard for an expert to track the cell one by one due to the time and energy constraint. Therefore, an automated cell migration tracking is needed to replace manual tracking [3]. Cell migration tracking is divided to two steps, which are cell detection and cell tracking. Cell detection is divided to two sections, based on segmentation or evolution approaches. Cell detection is the main step in migration tracking. If the detection of cell is not accurate, it will lead to tracking error. Cell labelling is the process to label the cell frame by frame in order to track the migration of cell.

3.1 Cell Detection based on Segmentation

Several cell detection based on segmentation approaches are reviewed here. Currently, the commonly used segmentation techniques are edge-based segmentation and thresholding. Frame by frame segmentation is introduced to gain better results [9], [11]. Edge-based segmentation is a fast computation technique because it does not require priori information [12]. As the name given, edge-based technique detects the edge of an image. The boundaries between objects and background are drawn in the segmented image, at which the image brightness will be sharpened, and it may cause discontinuous edges [13]. Number of boundaries is equal to the number of objects in the image. This is because the approach detects the boundary of an object in image [7]. There are three steps in edge detection process: filtering, enhancement and detection [14].

The quality of images is often influenced by random variations in intensity values, called noise. Filtering is performed to reduce noise in an image. However, noise reduction may cause a loss of edge strength. A proper filtering approach shall be determined to prevent loss of edge strength. The changes of intensity in neighbouring pixels in an image facilitate the detection of edges. The enhancement of pixels can be implemented by computing the gradient magnitude. Lastly, edge points are detected by thresholding according the non-zero value for the gradient since all points are edge for particular applications.

Thresholding works with scalar images by creating a binary partitioning of the image intensities. Thresholding determines the division region with an intensity value, called threshold [6]. The thresholding approach can be used with Euclidean distance and watershed transform to solve the problem of cell nuclei clustering. The limitation of these approaches are they cannot solve the cluster of cell problem if the cells overlap on the top of one another [4].

Otsu algorithm is one of the thresholding techniques used in cell segmentation [15], [16]. Otsu algorithm can segment isolated cells. Otsu selects threshold automatically for its simple calculation and good adaption. The algorithm involves separation of pixels into two clusters according to threshold values. After partitioning into two clusters, mean is determined and the algorithm squares the difference between the means. The last step is to multiply the number of pixels in one cluster with the number in the other cluster [17].

The accuracy of segmented image depends on the threshold value. If the threshold value is smaller than the pixel value of desired part image, it leads to under segmentation of the image. The produced image will exclude some of the desired part in image. On the other hand, a greater threshold may include unwanted part in image due to over segmentation. Besides that, failure to determine the threshold value will gives a disconnected result in the edges of segmented region [7].

The combination of H-maxima transform and watershed is another approach used to detect and segment the cell. H-maxima transform is the technique used to detect the cell and watershed transform is used to segment the cell [18]. Isolated regions are extracted through 8-connectivity h-maxima transform. If h is low, then it will produce many seeds and vice versa. In the paper, Suresh and Jayalakshmi expected the intensity of the seed is higher than h. Therefore, the small and faint objects can be eliminated. In watershed segmentation part, distance-transformed image is the input of the algorithm to separate the image to different meaningful regions. The intensity values of each pixel are used to determine the segmentation line. If the magnitude of current pixel is lower than two different minima, then current pixel is treated as segmentation lines to separate the cluster cells.

3.2 Cell Detection based on Model Evolution

In this section, three model evolution approaches are reviewed. These include mean-shift, active-contour and level-set methods.

Mean–shift identifies a local mode of an image. The process is implemented iteratively by moving the kernel position from original position towards darkest or brightest part of the image. Different kernel may attract either dark or bright colour pixels. The sample mean of current position is calculated and then the mean found becomes the current kernel position [19]. In applying mean-shift to cell detection context, each cell should have highest intensity at the centre of the cell[20].

Active-contour approach requires an initial point inside each cell. Therefore, the local maxima is found to identify the location of the cell when the pixel values of neighbour points are smaller than current point[21]. One of the active-contour approaches is sliding band filter (SBF), which is one of the convergence index(CI) filters [16]. By using SBF, the location and shapes of cell nucleus and cytoplasm are detected based on the convex shape of cell. A band of fixed width forms the support region of the filter. The position of the support region may be changed in different direction to maximize the convergence index at each point. SBF approach is proven to give better filtration function even when the shapes of the cells are irregular.

A simple two-phase level set method is divided into two regions, the region inside the cell, and the region outside the cell which is treated as background. This method can separate the cell from background if the cell has distinct intensity value with background colour[22]. The number of phases for level set method depends on the need of the research.

All of these methods function with certain limitations. Cell proliferation cannot be tracked through mean-shift and active-contour while re-initialisation is needed if the moving speed of cell is very fast or the cell is beyond the field of view for level-set based methods[23].

3.3 Cell Labelling

The challenge in cell labelling is to link the cell from previous frame to next frame. This is because the cell is moving from frame to frame, and cell could split due to over segmentation [24]. The simplest form of approach to solve the problem is to link the cell to the nearest cell in the next frame. This method only works well when the cells are well separated in the frame[3].

In contrast, neighbourhood relationship among the cells is dependable in the tracking process even when the density of the cell is high [15]. A cell is called inactive cell if the cell moves in a short distance between two consecutive images [25]. Therefore, an active cell can be described as cell that moves a long distance from frame to frame. The neighbourhood relationship is used to track the active cell while overlap method is used to track the inactive cell. A graph is used to describe the relationship of the cells. Cell ID, cell's size and cell's location are recorded in a vertex subset. The cells are separated in different region according distance limitation and are ensured through correlation calculation. An overlap method was introduced to track the cell based on overlapping regions in two successive frames. The tracking result is revised by detecting the cell in two contiguous images.

A global data association which use tree structure to define the relationship of the cells is introduced [26]. Each tree is a cell family that includes the ancestor and its descendents. The algorithm assumes that the cells among the family will not overlap with one another. If the time and space distances of the cells in previous frame with the considered cells in current frame are less than thresholds, then it treats the cells as a translation candidate. If birth event is detected and the cell appears in the frame, then parent and children relationship will be labelled [11].

There is another tracking approach which also tracks the cell with mitosis detection. In the beginning, the one-to-one correspondences are determined

based on spatial distance and feature similarity. The feature distance is calculated by the combination of Euclidean and spatial distance. Three consecutive frames are used to find the correspondence and increase the robustness, compared to the traditional way which is using only two frames. Final cost is measured by sum up the distance between the previous frame and current frame, and between the current and next frame. After that, mitosis likelihood measure is used to identify the mitosis events. Size and mean intensity of parent and child cell nuclei are the measuring components of mitosis likelihood measure [4].

4.0 COMPARISON OF CELL MIGRATION TRACKING TECHNIQUES IN PREVIOUS WORKS

Table 1 shows the strong point(s) and future work(s) of different techniques in different papers. From the papers, it can be concluded as below:

1) Human judgment (threshold value, transform distance) should be eliminated.
2) Pre-processing is very important to determine the cell detection result.
3) Cell detection needs consider the changes of cell such as overlapping, clustering and proliferation, including dead cells.
4) Frame by frame segmentation can help to link the cell by using the position of the cell within the frames.
5) Back-tracking can increase the accuracy in cell tracking process.

In consequence, some suggestions are provided for future research:

1) All cell changes shall be considered in cell detection process.
2) The features of cell should be identified and the combination of features can be used to detect the cell.
3) Back-tracking can be used while tracking the cell, not only as a correction step in the end of the tracking process.

Table 1 Cell migration tracking techniques review

Title	Techniques	Dataset	Remark	
Segmentation and tracking of neural stem cell [25]	Segmentation based on fuzzy and watershed segmentation, level set algorithm to construct geometric active contour model and first frame is validated interactively. Active, inactive, dividing and clustered cells are tracked individually. Backtracking step is used to correct the errors occur in initial frame for tracking step.	Captured from a computer-controlled microscope which attached to a cell culture system.	Future	More advanced shape analysis should be used to give more accurate results in splitting the cell in segmentation stage and the backtracking step can be used in segmentation stage.
			Weakness	Cluster cell can't be separated accurately.
			Strength	Backtracking step helps in tracking all cells.
Tracking of migrating cells under phase-contrast video microscopy with combined mean-shift processes [19]	Combination of mean-shift processes with nested kernels to detect the configurations of gray level of image. Do not focus on border detection but position of the centroids.	Captured under phase-contrast microscope, video camera and acquisition board.	Future	Automated initialization of cell centroid should be adopted in different frame to detect new cells enter the view of field.
			Weakness	Manual detection of cell is required for initialization detection.
			Strength	Able to track large numbers of cell even when cell proliferation, overlapping and changing of shape occur; short computation time.
Reliably tracking partially overlapping neural stem cells in DIC microscopy image sequences [9]	Contour shapes of cells is identified and updated from frame to frame in the tracking process. If the cell is overlapped, then it will undergo separation process by using partial contour matching.	Data is captured every 5 minutes by using camera with Zeiss Axiovert 135 TV microscope to produce DIC microscopy images. Each image size is 640x512 pixels and the number of images is 800 frames.	Future	Enhance the current algorithm to track random numbers of overlapping cells.
			Weakness	Unable to differentiate more than 4 overlapping cells.
			Strength	Able to track the cells that are partially overlap from the initial contact to separation.
The segmentation of overlapping milk somatic cells based on improved watershed algorithm [26]	The main purpose of the technique is to separate the overlapping cells to count the numbers of cells and further analyse its characteristic. Therefore, improved watershed technique is proposed to solve over-segmentation problem of traditional watershed algorithm. At first, one of the distance transform algorithm, called chamferingm is used in preparing to obtain the seed points. The redundant seed points will be merged if both seed points are in short distance.	Institute of Animal Science and Medicine	Future	More criteria to be included in merging the seed points.
			Weakness	Under-segmentation occurs after solving the over-segmentation problem.
			Strength	Success to solve over-segmentation of traditional watershed algorithm even when the overlapping cells do not have obvious boundaries.
Tracking of Cell Populations to Understand their Spatio- Temporal Behavior in Response to Physical Stimuli [27]	The authors propose image understanding methods by observing the characteristic of cells. Adaptive thresholding is used to produce binary image and segment cluster from the background. Bigger groups of pixels, which are more than 300 pixels, are considered as cell. Then the boundaries and holes of the cells are closed to give more accurate result in segmentation. In tracking process,	Princeton Instruments D1299421 camera is mounted with microscope. The image size is 1300x1030 pixels and there are 82 images for each image sequences. Once image contains 15-30 cells approximately	Future	More cost functions can be considered in order to cope with different condition in tracking.
			Weakness	Tracking accuracy decreases when cell density is increasing.
			Strength	High accuracy in detection and segmentation processes. It also provides fast interpretation of cell behaviour.

	iterative probabilistic data association algorithm is combined with auction algorithm and two-phase batch algorithm to solve data association problem.			
Analysis of segmentation and tracking algorithms for time lapse microscopic progenitor cell images [18]	H-maxima transform and watershed algorithm are used to detect the cell while multiple matching object method is used to track the cells from frame to frame.	The image sequences are captured by CCD camera with time-lapse videocassette recorder. The images produced are 480x640 pixels and total of 700 frames. The depth of the image is 8 bits.	Future	Cell shapes and division pattern can be use to increase the accuracy in segmentation and back tracking can be implemented to identify incorrectly rejected of cells in previous frames.
			Weakness	Tracking result is not accurate due to labelling the false negative cell in previous frame as a new cell in current frame.
			Strength	Merging the over-segmentation parts increase the percentage of true negative.
Cell nuclei and cytoplasm joint segmentation using the sliding band filter [16]	Sliding band filter detects the rugged topology to detect the cell. Overlapping problem is corrected and the shape of the cell is regularized to improve the estimation of cell shapes.	Drosophila Kc167 dataset(400x400 and 512x512 pixels) and simulated cell culture dataset(950x950 pixels)	Future	The robustness in identifying a cell correctly should be increased. Not only shape but other characteristic of the cell should be considered.
			Weakness	Parameters used in experiment are determined by the user, it may lead to different result if the dataset is different. Incorrectly rejected the cells also decrease the percentage of precision and recall.
			Strength	Detect the cells even when it is a noisy image.
Significantly improved precision of cell migration analysis in time-lapse video microscopy through use of a fully automated tracking system [28]	Cell centroid extraction, Kalman filter cooperate with unique neighbour algorithm to detect a cell from frame to frame while estimates the state of cell. Monitoring module was used to handle cell proliferation and cell moving problem.	-	Future	Testing in different kinds of cell images to obtain bigger view for the algorithm.
			Weakness	The algorithm may only suitable for certain cell type since the predefined threshold value is based on cells' size and cells' growth behaviour.
			Strength	Can handle cell proliferation and cell moving problem.
Automated and semi-automated cell tracking- addressing portability challenges [29]	Centroid of cell is used to detect the cell. Linking of the cell from frame to frame is done by automatic calculating gating distance from frame to frame. A semi-automated algorithm is proposed to correct the error in post-processing stage.	5 videos are used in evaluation: - ak - hex.6 - hex.16 - hex.22 - square The numbers of the cells in the videos are in range of 6-35.	Future	Automatic detection of cell's status may use in post-processing.
			Weakness	Instead of full automated, semi-automated approach is used to make the correction for error detection. This may lead to time consuming if there is a lot of frames need to be corrected.
			Strength	Can handle noise, cell division and cell death. The system is parameter-free.
Cell image analysis- algorithm, system and applications [10]	Image restoration is implemented before processing the image, then thresholding is used to detect the cell. Mitosis and cell overlapping is solved by cell-blob association approach. At the end, cell behaviour is measured by labelling the ID of each cell.	16 image sequences are recorded under four cell culture conditions. Each sequence consists of 1000 images with the size of 1392x1040 pixels.	Future	Trajectory pruning can be used to correct the wrong lineage tree and cell-blob affinity model learning is suggested to be included to increase system performance.
			Weakness	Some of the cells are not tracked due to wrong lineage tree relationship.
			Strength	If the mother is not detected in the beginning, it is able to recover the lineage tress if the

				daughter cells are detected.
Large-scale tracking for cell migration and proliferation analysis and experimental optimization of high-throughput screens [4]	Adaptive thresholding is implemented to segment the cell. Euclidean distance and watershed transform are performed to resolve cell cluster and cell splitting. Tracking stage is performed with mitosis detection to increase the robustness.	4400 image sequences are used as dataset with 1344x1024 pixels in resolution. Each image contains around 100 to 300 cells.	Future	Develop algorithm to resolve the cell overlap on the top of one another and cell behaviour can be interpreted.
			Weakness	Overlapping cell cannot be detected.
			Strength	Detect abnormal mitosis and cell density problem.
Reliable cell tracking by Global Data Association [11]	Global spatio-temporal data association is used to track the cell. Different conditions of the cells are considered. Thresholding is used to detect the cell and frame by frame linking process is performed.	Phase contrast microscope is used to record five sequences with 1040x1392 pixels in resolution. Total of 124621 are annotated in five sequences.	Future	Expert may need to be employed to detect the cells manually or more suitable dataset shall be found.
			Weakness	Partially annotation of cells due to unable in tracking the purity of the result.
			Strength	Correctly detect relationship in lineage tress among the cells after birth event.
A novel cell segmentation, tracking and dynamic analysis method in time-lapse microscopy based on cell local graph structure and motion features [23]	Cell tracking is based on segmentation which able to separate the touching cell by comparing the position of the cells in several continuous frames. The motion features of the cells are considered in local graph structure to track the cells and its end product will be used in analysis of cell morphology and the changing in movement of the cell.	Histone H2AFV cell fluorescence sequence imaging video. Image resolution is 480x640 pixels.	Future	Use more criteria to determine the identity of the cell within frames.
			Weakness	Cell tracking result decreases after getting more cells in one frame.
			Strength	Proposed segmentation technique able to segment 97% of cells.
Cancer cell detection and tracking based on local interest point detectors [30]	Blob like region in the images are considered cell locations by using local interest point detector with the enhancement of scale-normalized Laplacian of Gaussians filter. The tracking step is based on the speed and using SIFT descriptors.	156 brightfield images	Future	Other image enhancement technique may need to use in order to get better image.
			Weakness	Incorrectly rejected due to intensity value of some of the cells are similar with the background.
			Strength	Consider both static and active cells by using constant position and constant velocity as the condition in algorithm.

5.0 CONCLUSION

Automated cell migration tracking involves cell detection and cell labelling. There are many kinds of cell detection methods can be used to detect the cell based on the location of cell nuclei, shape of the cell or other criteria. The changes and status of the cell is the main problem in cell migration tracking. More researches should be conducted to achieve more accurate result.

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References

[1] Gradinaru, C., J. M. Lopacinska, J. Huth, H. A. Kestler, H. Flyvbjerg, and K. Molhave. 2012. Assessment of Automated Analyses of Cell Migration on Flat and Nanostructured Surfaces Abstract : Motility Studies of Cells Often Rely on Computer Software That Analyzes Time-Lapse Recorded Movies and Establishes Cell Trajectories Fully Automatically. *Comput. Struct. Biotechnol. J.* 1(July, 2012).

[2] Milde, F., D. Franco, A. Ferrari, V. Kurtcuoglu, D. Poulikakos, and P. Koumoutsakos. 2012. Cell Image Velocimetry (CIV): Boosting the Automated Quantification of Cell Migration in Wound Healing Assays. *Integr. Biol.* 4(11): 1437-1447.

[3] Meijering, E., O. Dzyubachyk, and I. Smal. 2012. Methods for Cell and Particle Tracking. *Methods Enzymol.* 504(February): 183-200.

[4] Harder, N., R. Batra, S. Gogolin, and N. Diessl. 2011. Large-Scale Tracking for Cell Migration and Proliferation Analysis and Experimental Optimization of High-Throughput Screens. 6th Internat Workshop on Microscopic Image Analysis with Applications in Biology. 2011.

[5] Huth, J., M. Buchholz, J. M. Kraus, K. Mølhave, C. Gradinaru, G. v Wichert, T. M. Gress, H. Neumann, and H. a Kestler. 2011. Time Lapse Analyzer: Multi-target Analysis for Live-Cell Imaging and Time-lapse Microscopy. *Comput. Methods Programs Biomed.* 104(2): 227-234.

[6] Pham, D. L., C. Xu, and J. L. Prince. 2000. A Survey of Current Methods in Medical Image Segmentation. *Annu. Rev. Biomed. Eng.* 2: 315-338.

[7] Zuva, T., O. Olugbara, S. O. Ojo, and S. M. Ngwira. 2011. Image Segmentation, Available Techniques, Developments and Open Issues. *Can. J. Image Process. Comput. Vis.* 2(3): 20-29.

[8] Bise, R., T. Kanade, Z. Yin, and S. Huh. 2011. Automatic Cell Tracking Applied to Analysis of Cell Migration in Wound Healing Assay. 33rd Annual International Conference of the IEEE EMBS. 2011. 6174-6179.

[9] Bise, R., K. Li, S. Eom, and T. Kanade. 2009. Reliably Tracking Partially Overlapping Neural Stem Cells in Dic Microscopy Image Sequences. MICCAI Work. Opt. Tissue Image Anal. Microsc. *Histopathol. Endosc.* 67-77.

[10] Kanade, T., Z. Yin, R. Bise, and S. Huh. 2011. Cell Image Analysis: Algorithms, System and Applications. IEEE Workshop on Applications of Computer Vision (WACV) 2011. 374-381.

[11] Bise, R., Z. Yin, and T. Kanade. 2011. Reliable Cell Tracking By Global Data Association. 1004-1010.

[12] Khadem, M. S. 2010. MRI Brain Image Segmentation Using Graph Cuts MRI Brain Image Segmentation Using Graph Cuts.

[13] Taghizadeh, M. and M. R. Mahzoun. 2011. Bidirectional Image Thresholding Algorithm Using Combined Edge Detection and P-Tile algorithms. *J. Math. Comput. Sci.* 2(2): 255-261.

[14] Ramadevi, Y., T. Sridevi, B. Poornima, and B. Kalyani. 2010. Segmentation and Object Recognition Using Edge Detection Techniques. *Int. J. Comput. Sci. Inf. Technol*. 2(6): 153-161.

[15] Tang, C., L. Ma, and D. Xu. 2011. Topological Constraint in High-Density Cells' Tracking of Image Sequences. In Software Tools and Algorithms for Biological Systems, vol. 696, H. R. Arabnia and Q.-N. Tran, Eds. New York, NY: Springer New York. 255-262.

[16] Quelhas, P., M. Marcuzzo, A. M. Mendonça, and A. Campilho. 2010. Cell Nuclei and Cytoplasm Joint Segmentation Using the Sliding Band Filter. *IEEE Trans. Med. Imaging*. 29(8): 1463-1473.

[17] Sahoo, P. K., S. Soltani, and A. K. C. Wong. 1988. A Survey of Thresholding Techniques. *Comput. Vision, Graph. Image Process*. 41(2): 233-260.

[18] Suresh, R. and N. Jayalakshmi. 2010. Analysis of Segmentation and Tracking Algorithms for Time Lapse Microscopic Progenitor Cell Images. *Signal and Image Processing(ICSIP)*. 453-456.

[19] Debeir, O., P. Van Ham, R. Kiss, and C. Decaestecker. 2005. Tracking of Migrating Cells Under Phase-contrast Video Microscopy with Combined Mean-shift Processes. *IEEE Trans. Med. Imaging.* 24(6): 697-711.

[20] Adanja, I., O. Debeir, V. Megalizzi, R. Kiss, N. Warzee, and C. Decaestecker. 2010. Automated Tracking of Unmarked Cells Migrating in Three-dimensional Matrices Applied to Anti-cancer Drug Screening. *Exp. Cell Res.* 316(2): 181-193.

[21] Bredies, K. and H. Wolinski. 2012. An Active-contour Based Algorithm for the Automated Segmentation of Dense Yeast Populations on Transmission Microscopy Images. *Comput. Vis. Sci.* 14(7): 341-352.

[22] Tse, S., L. Bradbury, J. W.L. Wan, H. Djambazian, R. Sladek, and T. Hudson. 2009. A Combined Watershed and Level Set Method for Segmentation of Brightfield Cell Images. SPIE Symposium on Medical Imaging: Image Processing. 2009. 7259.

[23] Zhu, C., Q. Guan, and S. Chen. 2012. A Novel Cell Segmentation, Tracking and Dynamic Analysis Method in Time-Lapse Microscopy Based on Cell Local Graph Structure and Motion Features. *Pattern Recognition*. 359-366.

[24] Mehmet, S., and S. Bulent. 20014. Survey Over Image Thresholding Techniques and Quantitative Performance Evaluation. *J. Electron. Imaging.* 13(1): 146-165.

[25] Tang, C. and E. Bengtsson. 2005. Segmentation and Tracking of Neural Stem Cell. *Advances in Intelligent Computing.* 851-859.

[26] Norouzi, A., M. S. M. Rahim, A. Altameem, T. Saba, A. E. Rad; A. Rehman; M. Uddin. 2014. Medical Image Segmentation Methods, Algorithms, and Applications. *IETE Technical Review (Institution of Electronics and Telecommunication Engineers, India)*. 31(3): 199-213.