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Computer Vision Based Tracking Of Biological Cells-A Review

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Abstract

With the advent of highly advanced optics and imaging system, currently biological research has reached a stage where scientists can study biological entities and processes at molecular and cellular-level in real time. However, a single experiment consists of hundreds and thousands of parameters to be recorded and a large population of microscopic objects to be tracked. Thus, making manual inspection of such events practically impossible. This calls for an approach to computer-vision based automated tracking and monitoring of cells in biological experiments. This technology promises to revolutionize the research in cellular biology and medical science which includes discovery of diseases by tracking the process in cells, development of therapy and drugs and the study of microscopic biological elements. This article surveys the recent literature in the area of computer vision based automated cell tracking. It discusses the latest trends and successes in the development and introduction of automated cell tracking techniques and systems.

1. Introduction

Wide spread advancements have been achieved in the techniques pertaining to object tracking and detection forthe purpose of surveillance. The idea of computer vision based biological cell tracking is a very recent development, analogous to the approach of object tracking for surveillance. However, in contrast to object tracking in surveillance where the objects monitored are massive, for example a vehicle or a person, cell tracking involves cellular objects which are micro or nano scale entities. A fundamental property of any real-world object is that it extends in both space and time. This is particularly true for living organisms, which, by definition, require the passage of time for their metabolism, growth, reaction to stimuli, and reproduction.

Full understanding of any animate entity therefore necessitates studying not only its spatial (anatomic) but also its temporal (dynamic) properties [1].The past decade has seen an unprecedented data explosion in biology. It has become evident that in order to take full advantage of the potential wealth of information hidden in the data produced by evens a single experiment, visual inspection and manual analysis are no longer adequate. To ensure efficiency, consistency, and completeness in data processing and analysis, computational tools are essential. Of particular importance to many modern live-cell imaging experiments is the ability to automatically track and analyze the motion of objects in time-lapse microscopy images [2].

Reliable visual tracking of multiple objects is an indispensable task in many emerging computer vision applications such as automatic video surveillance and robotics. In a multiple object setting, not only the states of the objects vary with time, but the number of objects also changes due to objects appearing and disappearing [3].Advances in optics and imaging systems have enabled biologists to visualize a living specimen's dynamic processes by time-lapse microscopy images. However, the image data recorded during even a single experiment may consist of hundreds of objects over thousands of images, which makes manual inspection a tedious and inaccurate option [4].

Due to the advent of Nano and molecular microscopy and imaging techniques, there is a steep

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rise in the amount and complexity of experimental data available now, which has presented new challenges for data analysis and management. Manual data analysis and multi object tracking in a huge population of cells is practically not possible as it is very time consuming, limited number of parameter scan be analyzed, and analysis procedures are tough to reproduce exactly. Therefore, it has become impertinent to develop automated techniques which can reliably and accurately support biological research.

Automated analysis of cell behaviors in time-lapse microscopy images is critical for efficiently studying and for research and discovery in biology and medicine. Automated cell tracking can capture minute data accurately and efficiently for example, monitoring cell events like migration (translocation), proliferation (through a division mechanism called mitosis), differentiation (by which cells acquire more specialized functions), quiescence (inactivity) and apoptosis (death).Automated systems for visual-tracking of cell populations in vitro have enormous potential for stem cell biology and stem cell engineering because these systems allow for high throughput analysis of time-lapse microscopy images whereas manual analysis is often intractable[5].

Computer-vision based automated cell tracking has vast applications in cellular biology and medical science which includes discovery of diseases by tracking the process in cells, development of therapy and drugs and the understanding and study of microscopic biological elements. A significant application is monitoring the healing of wounds. The cells tracking system developed are capable of tracking individual cells during the process and accurately compute the decreasing size of the middle wound area and the cell densities. This data is useful for treatment of wounds. Another application is tracking of cells in stem cell cultures, a research area which promises to revolutionize medicine. To meet the demand of clinical applications, a sufficient number of cells are needed during medical treatment. Therefore, stem cell cultures are developed in laboratories to support the requirement of medical therapy. The metrics of stemness requires that we capture the spatiotemporal history of each cell's fate in a population. The system developed by Kanade et al [4], efficiently computes the lineage trees of individual cells and provides the required metrics.

Biology has by now unquestionably developed into a multidisciplinary field, and it seems that the joint optimization of all aspects of biological experimentation (sample preparation, image acquisition, image analysis, data modeling, and statistics) is best achieved by a close collaboration between biologists, chemists, physicists, mathematicians, statisticians, as well as computer scientists, all the way from experiment planning to the ultimate interpretation of the results[2].Infact, in the past three decades there has been an exponential rise in the research focus and developments in the area of cell and particle tracking.(Figure 1).

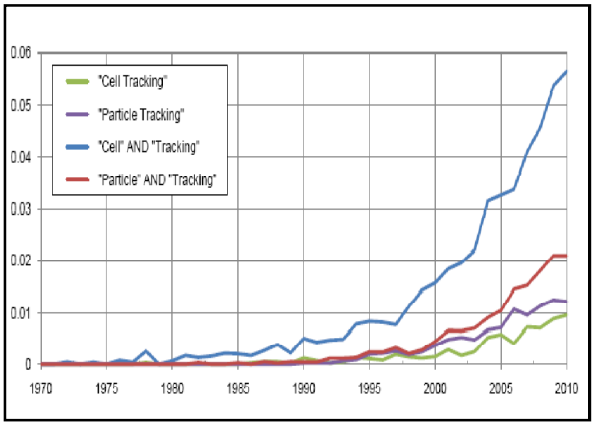

Fig: 1. Percentage of publications in the PubMed database. The horizontal axis indicates the publication year and the vertical axis indicates the combination of words in the title and/or abstract. The graph depicts an exponential rise in the publication [2].

This article briefly surveys the latest trends in computer vision based automated cell tracking experiments. Mostly, research work published between 2010 to 2013 has been reviewed in this paper.

The rest of the paper is organized as follows: the next section introduces the fundamental algorithms incorporated in the computer vision system for cell tracking. The subsequent section is a compilation of the latest developments and the state of art achieved in automated cell tracking. The following sections provide an outline of the computer vision based systems developed for various types of cell tracking and analyze the open questions, scope for future work; and conclusion.

2. Fundamental Algorithms

The algorithms used in automated cell tracking can be broadly classified into one of the following four categories: Particle filtering based approaches, Model-based evolution approaches, Detection-based association approaches, Sequential tracking approach and Segmentation & Association based approach [4], [5], [6], [7], [8].

Particle filtering approach is used for multiple object tracking. In multiple models such as measurement models and dynamic models need to be

known before estimating the posterior distributions of an object's current states.

Model based evolution approach first creates the object to be tracked and gradually updates the changes in its appearance. Another model-based approach is Active contour methods. This approach works well even for different shaped objects.

Detection based association approach include intensity thresholding, gradient (edge) detection, morphological operations and watershed algorithms. However, due to intensity variations, image noise and artifacts from the optical system, intensity thresholding are not effective. In this approach the idea is to segment and locate the cells and then link those objects. Edge detection shows better performance than thresholding. However, on low contrast images edge detection is error prone. The disadvantage of watershed algorithm is that it is prone to noise and often over segments the image.

Sequential tracking approach use particle filtering with spatiotemporal information which was used by Smal et al. [9] and Li et al. [10], Zhou et al.[11] presented an orientation adaptive mean shift optimization into particle filter framework in their research on tracking of sperm cells. Ray et al. [12] employed sequential Bayesian framework to establish cell correspondences. Ryoo and Aggarwal et al. [13] proposed a computationally efficient algorithm for tracking under severe occlusion. Many of the sequential tracking methods described above are computationally intensive and hence suffer from the problem of scalability.

Segmentation-based frame-by-frame association approach is also effective for automated cell tracking. Al-Kofahi et al. [14] use adaptive thresholding method, and then, resolve the association between two frames by optimizing probabilistic objective functions based on distance measures. The disadvantage of this method is that multiple cells merge into a cluster creating occlusion during tracking. Besides, in this approach, cells entering or leaving the field of view were not considered.

Recently, several cell tracking algorithms have been developed which are independent of tracking results. For example, Li et al. [10] proposed cascade Adaboost algorithm and Liu et al. [15] introduced the Hidden Conditional Random Field Model. However, these approaches do not indicate the time of completion of cell division and they are computationally intensive. Active Contour model [10], Watershed method [16], Adopting Motion Model [10] has been widely used for cell tracking [17].

3. State Of Art Of Computer Vision Based Cell Tracking

In this section, the latest algorithms and cell tracking techniques, which have been adapted to develop an automated cell tracking system, are discussed, with a focus on the application.

In their research work, Kanade et al. [18] proposed a tracking system which was based on a track-compilation and track-linking algorithm to track the cell in frame-by-frame manner and link the track trajectories so discovered to develop the entire lineage tree. Interactive Multiple Model Filters (IMM) are also employed in this process as they have shown better performance than Kalman Filter in tracking biological events.

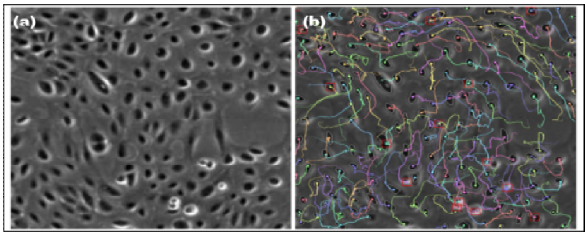

Fig: 2. Tracking results of Amnion Epithelial (AE) cells for quantitative analysis. (a) Original image (b) Image with cell trajectories generated. Red rectangles indicate mitotic cells [18].

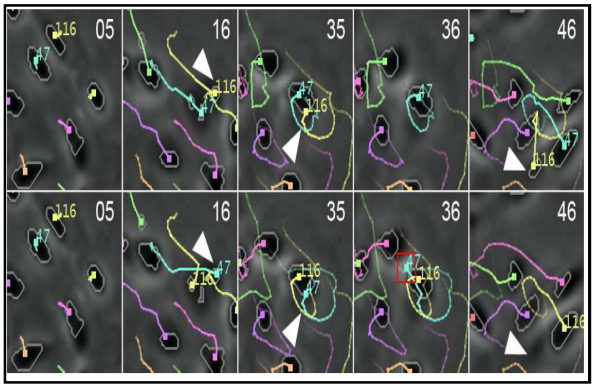

Fig: 3. Depicts how the system overcomes occlusion. Top row: The system tracks accurately 116 which were completely occluded by 47.Bottom row: Result of the previous system, shows that 116 was overlapped by 47 and disappeared during tacking. [18]

Li et al. [10] have efficiently tracked stem cells, by developing a machine learning approach which computes an energy term in the level-set based cell tracker and enhances the tracking performance. Bilateral filtering has been successfully used in cell detector to reduce noise level and an edge based

approach is employed to capture different phases in mitosis.

Yang et al. [19] used graph cut method for segmentation in their research work on tracking brain cells, as this approach guarantees to obtain globally optimal solution.

Huh et al. [20] states that automated mitosis detection algorithms can be categorized into tracking based method and tracking-free method.

Park et al. [21] researched on single mRNA tracking in live cells and adopted particle tracking by nearest-neighbor approach, Kalman Filtering and Multiple Hypothesis Tracking (MHT) algorithms for automated tracking of mRNA in cells.

Kanade et al. [4] pursued their work further and presented an image restoration and segmentation method which uses a quadratic optimization function to restore the original image eliminating the artifacts like shadows, shading and halos. Thresholding gives excellent performance while segmenting the restored image. Additionally, a three step approach is also presented for detection of cell division (mitosis), which includes creating patch sequence, extracting features and finally detecting whether the patch contains a mitosis occurrence and the specific location of occurrence.

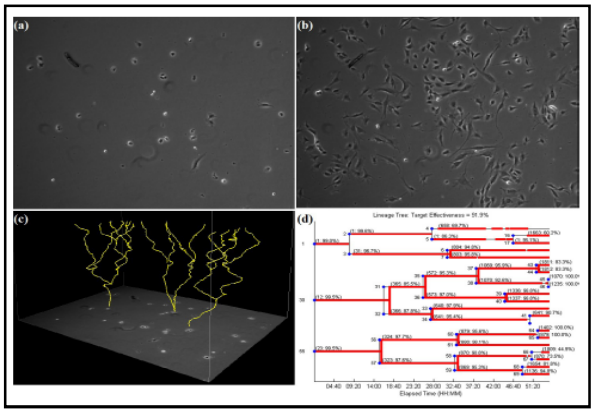

Fig: 4. Depicts the process of cell lineage construction.(a) At the beginning of the experiment (b)At the end of the experiment(55 hours later) hundreds of cells (c)Trajectory of three cell generated (d) Lineage tree constructed[4].

Scherf et al. [22] presented an algorithm to automatically compute characteristics of single cells in a highly dense population and construct its entire lineage history. This algorithm also employed level-set segmentation for object detection and watershed algorithm was applied to alleviate the problem of cell occlusion.

Padfield et al. [8] introduced the graph theoretic minimum cost flow framework to resolve the data association which was accurate in cell tracking. However, this method failed for overlapping cells. As a solution to this problem Bise et al. [7] proposed the contour tracking method based on partial contour matching which could detect multiple overlapping cells. This technique uses contour shapes of cells and clusters and successfully incorporates it to track overlapping cells of Human Central Nervous System stem cells during migration and proliferation [7].

Kang et al. [23] proposed a novel method for tracking nuclei of C.elegans during embryogenesis. The algorithm consists of two aspects: tracking of nuclei by using a simple spherical mask, and detection of cell division by tracking multiple objects locally and detects the new born sister cells at a later point of time when its existence becomes more clear in order to avoid false detection. This algorithm shows accurate and robust tracking result even in low Signal-to-Noise Ratio (SNR) images.

Yuan et al. [24] have mentioned that Mean-shift algorithm is a fast and powerful object tracking method and has been successfully applied to surveillance and segmentation system. To alleviate the problem of disappearing, merging, and splitting objects Jaqaman et al. [25] designed an algorithm, the linear assignment problem (LAP).Yang et al. [26] proposed an algorithm for reliable tracking of large-scale dense antiparallel particle motion based on the Kalman filter. These algorithms did not work well with low Signal-to-Noise Ratio (SNR). Subsequently, Smal et al. [9] presented a powerful algorithm using particle filtering which was capable of tracking objects in noisy image sequences. However, this method uses generic particle filtering (GPF), which is computationally expensive. Sargin et al. [27] and Koulgi et al. [28] described different methods to track the elongation, shortening and gliding of microtubules employing an arc-emission hidden Markov model and a graphical model-based algorithm, respectively. The limitation of this approach was that it only works well for small displacements. The latest development in the field of object tracking is the use of kymographs. Kymograph is a significant method developed as it represents the movement information in a single graph. However, in this when the object moves rapidly, discontinuous trajectories are created and the method fails to give the desired performance.

Yuan et al. [24], in their work on tracking axons, they utilized particle filtering approach. Here the idea was to dynamically limit the spatial area to the shape of the axon. The trajectory of the axon movement is developed with a piecewise polynomial function formulated using cubic spline interpolation.

Huang et al. [29] developed an online system for 3-D tracking and monitoring of cells in a suspension.

This method combines a 2-D tracking method based on an enhanced version of online MILBoost visual tracking algorithm (to obtain X–Y positions) followed by a region-based autofocusing algorithm (to obtain Z-position).This system obviates the use of fluorescent dyes, thus avoiding photo toxicity.

Hoseinnezhad et al. [3] presented a novel method of visual tracking of multiple targets in a sequence of image without explicit target detection. This algorithm is based on Bayesian muti-target filtering solution developed from the random finite set (RFS) framework, known as the multi-Bernoulli filter. This method gives best results when applied to numerous targets with similar visual pattern. Also, it accounts for targets coming in and going out of the scene.

Magnusson et al. [30] have proposed an algorithm for multiple targets tracking for tracking cells in microscopic images. This is method Viterbi algorithm is applied to the image sequence and global data association is achieved because here data from the whole image is used to make local decisions about construction of the cell tracks. The algorithm includes a scoring function to rank tracks and uses Viterbi algorithm to iteratively search for the highest scoring tracks. To evaluate the algorithm experiments were performed on 115 image sequences of Muscle Stem Cells (MuSCs) and the precision and recall rate (called mitosis branching correctness) computed by the algorithm was observed to be to 0.78 and 0.75 for mitosis (cell division event) 0.74 and 0.62 for apoptosis (cell death event), as compared to Huh et al. [20] the recall rate was 0.65.

Huh et al. [31] proposed an automated mitosis detection algorithm based on a temporal probabilistic model for validation of mitosis event detection. The algorithm was evaluated on model achieved 97.4% precision and 96.6% recall on 14 cell populations of Hematopoietic Stem cells (HSC) and it was observed that 97.4% precision and 96.6% recall was achieved.

Chatterjee et al. [6] proposed an automated system of tracking human monocyte cells in a video sequence in which first the image is preprocessed to eliminate any background noise and the position of centroids of the cells is obtained. Then the Maximum Cardinality Minimum Weight Bipartite Matching is applied to create the cell trajectory.

Mkrtchyan et al. [32] developed an efficient technique for fully automated registration of image and applied it to images of shoot apical meristem (SAM) of Arabidopsis to evaluate its performance and observed that this algorithm improves cell lineage and data statistics. Image registration is the process of transforming different sets of data into one coordinate system. Data may be multiple photographs, data from different sensors, times, depths, or viewpoints. Registration is necessary in order to be able to compare or integrate the data obtained from these different measurements. They adopted Iterative Closest Point algorithm (where a set of landmark point pair correspondences are matched between two images and then the images are aligned so as to minimize the mean square error between the correspondences.), with slight modification. In this case IPC uses features of the local neighborhood area to mark the corresponding landmark pairs for image registration.

Baker et al. [33] proposed an algorithm that performs accurate cell tracking and identifies the correct cell path eliminating occlusion events. This algorithm incorporates four modules: cell and sibling identification, cell tracking, occlusion detection and correction and correlation analysis. Here contour-based segmentation is used.

Konda et al. [34] worked to address the challenges of tracking a population of cells which exhibit variations in density, dynamics and behavior. They proposed an Event Indicator Function classifier (ElF) for identifying cell tracking errors and phenotypes. The EIF is designed to specifically perform the following functions: Detection Errors: missed-detection and false detection (fp) errors, Tracking Errors: data-association ambiguity which may leads to cells ID swapping and other errors, Cell Phenotypes: mitosis (division) and apoptosis (death).

Hagwood et al. [35] evaluated performance of four popular segmentation algorithms: K-means, Canny, Watershed and Otsu, on the basis of their misclassification error (rate of misclassification of a pixel into cell category or background category). Experiments were performed on as sequence of images of A10 rat smooth muscle cells and NIH-3T3 cells and concluded that Canny and Watershed were robust and effective for a variety of samples.

Table: 1. Comparison of algorithms based on their cumulative distributive function of their misclassification error. The experiment is performed on two set of samples: A10 rat muscle cells and NIH 3T3 cells. Long, medium and short depicts the exposure time to illumination [35].

	Long	**Medium**	**Short**
A10	Canny, K-means, watershed all similar	Canny, watershed similar and better than K-means	Watershed slightly the best
NIH 3T3	K-means the best	Watershed slightly the best	Watershed slightly the best

4. Automated Cell Tracking Systems

An Automated Cell Tracking System for simultaneously tracking thousands of cells using phase-contrast time-lapse microscopy was introduced by Kanade et al. [18]. In this system five modules were integrated: cell detector, cell tracker, dynamic filter, track compiler and track linker to achieve spatiotemporal linking of cell paths. The system performance was evaluated and achieved accuracy in the range of 85.9 % and 92.5 %, which is 9 % higher in comparison to the previous system.

Li et al. [10] designed a computer vision based cell tracking system that is capable of tracking the behavior of a large population of cells including cell movement, division, quiescence, death and differentiation. In this system the challenges like increasing cell densities during population expansion, cell entering/leaving the field of view and discriminating between overlapping cells, have been effectively overcome.

In Bise et al. [17] a system for cell tracking is presented which is based on contour-based cell tracking. The system has been used for detailed analysis of Human Nervous System stem cells and achieved 97% accuracy. In this system each image sequence undergoes three steps: preconditioning and segmentation, cell-blob correspondence and separation of overlapping cells.

Yang et al. [19] presented a Tracking and Segmentation Framework to overcome boundary ambiguity problem and used it to extract 2D contour boundaries from Serial Block Face Scanning Electron Microscopy (SBF-SEM) image stacks. In this approach graph cut technique was used to obtain the globally optimal solution of an energy function which consists of the flux of the gradient vector fields, the image gray-scale intensity, and the distance function. This energy function can solve the boundary ambiguity problem occurring in densely packed EM images.

A Real-time Cell Tracking System was developed which can measure cell migration routes under cell culture conditions.The results are single cell path (x, y) during migration, cell size, migration distance, migration speed, real-time pictures and so on. This system is applicable to all kinds of researches related to cell migration such as cell angiogenesis, chemo taxis, and moreover cancer metastasis [36].

An automated system capable of quantifying cell proliferation metrics in vitro in real time was developed by Kandade et al. [4] which supported online biological experiments. Three cell image analysis algorithms: image restoration, mitosis detection and error-tolerant data association was inducted into a public website. Researchers all across the world can upload their cell images for processing. The system's performance was tested to be very efficient. Magnusson et al. [30] describes the above system as arguably the most advanced system at this point in time.

Bise et al. [7] proposed an automated method to obtain cell trajectories and lineages which was proven to be more efficient than the previous method that used level-set technique. Experimental results on a challenging data set show that the proposed method significantly improves the tracking performance including target effectiveness, track purity; mitosis branching correctness by globally associating tracklets.

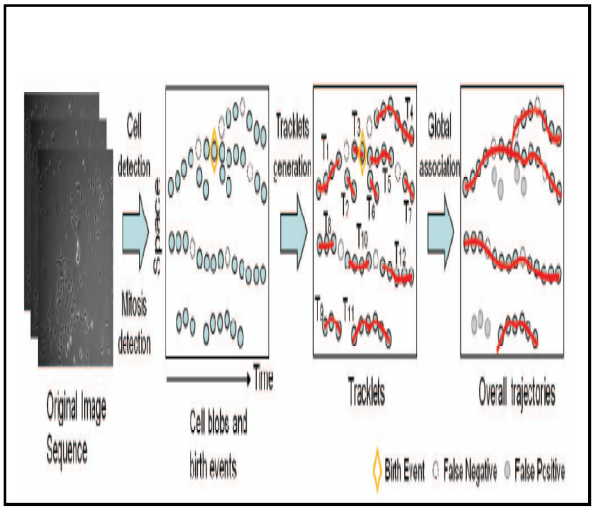

Fig: 6. System developed based on global data association. False Positives are the non-mitotic cells that are detected as cells in the lineage. False negatives are non-mitotic cells that correctly escape the detection [7].

Software for precise tracking of cell proliferation, TADOR was developed for multi-target cell tracking, is based on active contour model that eliminates the locally optimal solutions, hence this method does not face the challenges of signal fluctuation and morphological changes. By applying TADOR to the analysis of cultured cells whose nuclei had been fluorescently labeled, cell division and cell-cycle progression on coverslips was tracked over an extended period of time [37].

Cell Quantification Framework in Brain Tissue was developed to automatically quantify fluorescently labeled molecules in brain tissue images of rats. The technique applied is based on morphological segmentation followed by depth-dependent detection applied to a stack of microscopic images. This development proved to be a significant advancement

in neurological studies for accurate automatic counting systems [38].

Chateerjee et al. [6] proposed a novel automated method and a multi target tracking system to track human monocyte cells in video microscopy which involved the matching and linking with the help of bipartite graph and several cost functions. This system is highly scalable and works wells for entry and exit of cells. Additionally no user input is required during processing.

Automated Landmark Based Image Registration Method developed by Chowdhury et al. [39].The authors proposed a method which could automatically estimate landmark point pairs in densely packed SAM tissue and register the images. This method was rigoursly tested on various data sets and it was shown that cell lineage tracking and division tracking process was improved significantly.

5. Future Aspects

The technique of automated cell tracking has achieved an unprecedented success and demand in the biological and medical research arena in a short span of time, however, there remain several voids and challenges that need to be addressed in the future research work. The foremost challenge is meeting the high processing demand, tracking in varying density of the cell culture (with cells dividing/dying, leaving/reentering the field-of-view), and managing cell tracking despite the complexity of the cellular topologies (shape deformation, close contact, and partial overlap).

Although the computer vision based cell tracking systems are quite efficient in monitoring cellular events, they have certain limitations also.

In the work of Chatterjee et al. [6], the proposed future research is to include the intensity and shape information of the cells in the tracking paradigm to achieve a higher tracking accuracy. Another direction of future work will be to capture splitting and merging of cells in more complex scenarios.

Li et al. [18], plan to incorporate more effective segmentation algorithms and graphical models to cope with more complex cell shapes and intercellular interactions.

Li et al. [10] aim at developing a novel machine-learning approach to detect mitosis events without segmentation, and a more reliable cell detector with bilateral filtering. Li et al. [5] intend to extend their work to quantifying quiescence and the identifying cell differentiation using cell shape changes or fluorescent biomarkers. Additionally, improvements on robustness and processing speed of the system are currently in progress.

Mathiasen et al. [40] while reviewing the techniques for imaging stem cells in cardiovascular tissue to assess their in vivo efficacy analyzed several challenges such as development of methods for monitoring stem cell grafts non-invasively, with sufficiently high sensitivity and specificity to identify and map the fate of transplanted cells, an imaging method that permits longitudinal tracking of implanted cells for months to years allowing long-term follow-up of tissue function and donor survival. Also, the imaging technique should provide high spatial resolution and the capability of tracking cells without affecting the cells or the target organ.

Huang et al. [29] intend to further include mitotic detection phase in their tracking algorithm and add additional tracking procedures. Also the computational performance of the algorithm can be improved.

Konda et al. [34] in future propose to enhance ElF classifier by including additional parameters such as cell size, age, intensity, mode probabilities, etc. A significant proposed work is to develop a human computer interaction framework, where human operator can input as a feedback for any manual correction of error.

6. Conclusion

This paper reviews the existing trends in computer vision based automatic cell tracking with a focus on the developments published after 2010. In the view of the data explosion occurring in the field of biology and biomedical science, there is a critical demand for developing automated techniques to cater the data management and analysis of the massive data that is being vigorously created in the experiments. Automation in cell tracking will allow biologist to focus on their research objective rather than facing the challenge of data interpretation and analysis, in order to facilitate breakthrough research in science.

This paper presents useful pointers to existing literature of automated cell tracking and also analyzes the future perspective.

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