Commentary: Tracking Biological Cells in Time-Lapse Microscopy

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Introduction

The paper aims to present a new method which can help to track biological cells. We track biological cells to understand different organisms and how they multiply and interact with each other. This helps us in developing new medicines and medical processes.

Due to the size, delicate nature of the cells and limited imaging technology we face low signal to noise ratio which produces low contract images. The process of cell detection and tracking can be done manually but it is tedious and error-prone especially when the cell count increases rapidly. Hence, automatic cell detection and tracking techniques must be adopted. Low contrast images are difficult to study without any processing. Moreover, to track the cell, we must associate them in all the frames of the time-lapse footage. Current processes are producing a result which is decent when a small number of cells are present in the image. But their detection decreases dramatically when the number of cells in the image increases. Current techniques also fail to detect cells in a cluster which results in either false detection or under segmentation. Also, current methods like mean shift and active contour do not work desirably to detect mitosis.

The author aims to solve the problem of cell detection and cell tracking. With the proposed method will be able to detect cells in a cluster and will be stable even when the number of cells increases. Also, the proposed method tries to detect the cell division process (mitosis) and track the new cell's movement. The proposed method will also help in rectifying segmentation errors while tracking of new or old cells.

Using the new technique would enable researchers to have a better understanding of cells. They would be more effectively track cells, detect mitosis, and understand their various events of the cell lifecycle.

Methods

The writer has divided the methods used to detect and track cells into three sections Detection, Tracking and Trajectory recovery. For the detection of cells, the first step is to remove the noise and correct any illumination error which occurred due to dust or imaging error. This is done by inverting the image and performing erosion and dilation. Then the processed imaged is subtracted with the original image. This process reduces noise and the halos around the nuclei of the cells. It also increases the dynamic range of the image. Improved dynamic range helps in picking a threshold which will enable to detect the cell from the background.

The next step in detection is the segmentation of nuclei. The author uses the Gaussian filter along with h-maxmina transformation. After correcting the illumination and applying thresholding we may still see some close by cells are under segmented. The writer has focused on the cell nucleus as the point of reference for the study rather than the complete cell structure. And since the nucleus is brighter than the halo around it, we use h-maxima algorithm to detect the nucleus of the cell. We use a Gaussian filter on each of the cells to neutralize the noise peak in the image and in the later stage of tracking individual cells. Also, a threshold h is selected to plateau the top of the regional maxima by h. The parameter h is determined by the difference in contrast of background and cells. The regional maxima are defined by a set of pixels with uniform grey-level values. Since we can not reach a point of higher elevation (intensity) without going lower than the current position, hence this transformation helps in separating nucleus intensity with the background. I feel that using the h-maximum algorithm is a good way to segment nucleus. But the only downside to this algorithm would be that it is focused on the nucleus of the cell and does not work well with other parts of the nucleus which may be required for some research problems. For example, the neuron cell has a long tail which may need to be studied and the proposed algorithm would not perform well in segmenting tail.

The last step in detection is nuclei localization. The author uses the elliptical curve fitting for this step. The nucleus changes shape during frames. This is primarily due to noise and cytoplasm changing. Thus, to approximate the nucleus of the cell we try to fit an ellipse to detect the nucleus of the cell.

The next step in the process is to track the cellular movement. The major hurdle in tracking a cell is that cell changes its topology features with mitosis, cell movement and time. To solve this problem writer uses multiple topological features. The first feature is displacement, the distance between the centre of the nucleus of the cells. Second is skewness which is defined by the deviation in the direction of the cell motion. The third feature is the colour. Colour is calculated from the cell nucleus and compared with the nucleus in the next frame. The fourth feature is the area, which measures the overlap between the areas of the two nuclei in a different frame. And the last feature is the deformation which is calculated by comparing the eccentricities of the ellipses which were used to get the nuclei of the cell. All these features are then assigned some weights summed together to form a function which is minimized.

The result of the function is written in an adjacency matrix with size NxN where N is the size of the detected nucleus. A threshold is calculated, and we reject any value which is greater than the threshold to reject errors due to incorrect segmentation or false matching. Then the adjacency matrix is used as the edge weights for a bipartite graph. Where one part consists of all the cells from one frame and the other part of

the graph consist of the next frame's cells. Hungarian algorithm is used to match one to one matching of cells in two different frames. Since it uses one to one matching hence it will not consider mitosis. To overcome this problem, the writer uses the next technique, template matching-based tracking.

For tracking the writer has proposed to initialize the cells with an ID. And then categorize the cells into 3 categories, A, B and C. **Category A** will have the cell that has ID in the nth frame and (*n+1)*th frame but not in the *n-1*th frame. **Category B** has cells which have ID in the *n*th frame and (*n-1)*th frame but not in (*n+1*)th frame. And last **Category C**, the category has cells which have ID in *n*th cell and (*n-1*)th but not in (*n+1*)th frame. The other possibilities are considered as errors. We may observe a break in the trajectory of the cell due to mitosis, hence, to recover the trajectory we use a template of the ellipse which we learned from the previous techniques. We then use this ellipse and for each cell falling in category A, we try to fit the ellipse in the previous frame within a certain radius search space. We do this till we find a cell in category B or Category C. This algorithm is not performed to the cells which are close to the boundary as they are expected to leave or just enter the frame, this can be considered as a weakness of this approach.

Results

For cell detection, the author has analyzed his proposed method with both qualitative and quantitative methods. For the qualitative method, the writer runs the different algorithm with the same images and observes that the watershed algorithm was not able to perform as good as the proposed method when there is some pattern in the nucleus. Watershed would over segment it, but the proposed methods would be able to segment it correctly. For quantitative analysis of the result, the author takes in some frames from 4 different sets of time-lapse images and count the number of cells nuclei for all of them manually. The results are compared with the different algorithms and his proposed new method with the manual counted cells. With previous calculation, different values such as true positive (TP), false positive (FP), false negative (FN), precision (P), and recall (R) are calculated. Here precision is defined by TP/(FP + TP) and recall is defined by TP/(TP + FN). The proposed method demonstrated up to 12% increase in precision and more than 3% better recall score as compared to previous methods.

For evaluation of cell tracking, the author again took the same cell samples. The author presented both qualitative and quantitative evaluation techniques. For the qualitative approach, the mitosis process was manually detected between the frames and was compared with the cell tracking results. And for the quantitative evaluation, cell tracking and mitosis detection both were calculated manually and computed by the proposed method and results were compared. Also, the results from different methods were compared with the proposed method which showed that the new method is more stable even if the number of cells increases drastically.

If feel that the author has done enough analysis to convince the potential adopters to adopt this new technique as he took the help of qualitative and quantitative approaches which signify the robustness of his proposed approach. Assignment of weights to detect cell association makes it easier for the method to be adapted to different types of cells. But the algorithms have been tested on only 2 types of cells (3 sequences of murine embryonic cell and 1 sequence of HELA cells). But the result would have been more confidence-inspiring if we had 3 or 4 different types of cell sequences as to generalize the algorithm for different types of cells with different structures. The author also compares the computation viability by calculating the time to process each frame with a different algorithm and proposed algorithm. Though the proposed method is slower overall it produces a better result overall. It is to be noted that overall, the proposed method is faster, but the recovery step is the bottleneck and it is the most computationally intensive step in the proposed method.

Conclusions

Overall, the author has proposed a new method to automatically detect cells and track their movement through time including the process of mitosis. The author has been successful in proving the credibility of the proposed method using qualitative and quantitative methods of assessments. The proposed algorithms prove to be more efficient in detecting cells and their movement but overall is a bit slower in the process.

The strength of the method is that it uses h-maxima algorithm to detect the generally brighter nucleus of the cell. It is immune to closely pack cell structures and able to detect cells better than the other methods discussed in the paper. Also, the method of template-matching-based backwards tracking solves the issues of cell division and tracks the cell much better than previous techniques.

The weakness of the discussed method would be for detection of the cell, it only focuses on nuclei of the cell and assumes that other parts of the cell are not the point of concern which may not be the case. Also, the evaluation of the results from the proposed method only considers two types of cells. It would have been considered more robust if more types of cells would have been used for the evaluation of the proposed method. This would validate the usefulness of the proposed method even further and generalize the algorithm for wider range of uses.

For future research work, the template-matching-based backward tracking is the bottleneck for the algorithm as it is the slowest part of the algorithm, it can be improved. Also, with increase adoption and ease of use of neural netrowks and deep learning techniques, they can also be considered a potential technique for solving similar problems.