Commentary: Tracking Biological Cells in Time-Lapse Microscopy

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Introduction

Nowadays, automated cells tracking and analysis are necessary because human analysis is hard or even impossible to be accurate with a large number of different cell sequences. Automated method has already been achieved. However, the cells cannot be detected accurately because of different acquisition technology, complex topological features and uncertain cell motility and mitosis. So automated track always faces segmentation error and problem of searching correct cells in consecutive frames. The existing methods can be into three categories. The first one is a model-evolution-based method, which needs another function to solve the merging of boundaries when the cells are close. And the second one is a segmentation-driven-based method, detecting cells in each frame and then relating them between all the frames. But in this method, all the errors caused by segmentation and detection will influence the accuracy of tracking. The third one is probability-based method, which lacks the ability to analyze different cells using one model. Using different models for different events will cause the low efficiency. All these methods have problems needed to be solved. This paper provides a method using top-hat filter and h-maxima transformation for detection, combining motion features and topological features for tracking and using h-maxima transformation and match-based backward tracking method for correct trajectories. This method improves the accuracy of tracking cells and reduces the possibility of segmentation errors. It will improve the efficiency of follow-up biological science research and provide a new way of thinking for the later development of cell tracking technology. Also, this method may be useful for disease diagnosis and follow-up treatment.

Methods

In the part of detection, using top-hat filter for nonuniform illumination caused by shading artifact and undesirable noisy. The top-hat filter can obtain the difference between the original image and its opening, but still open to erase something, which can get a smoothing background. Some cells may cannot segmentation because of overlap and indistinguishable edges. The author segments nucleus instead of the whole cells. Using Gaussian filter followed by h-maxima transformation to find the regional maximum for each cell as a nucleus, where Gaussian filter can generate a unique maximum inside a cell. The h-maximum transformation is used to get a reconstructed image with the same value with the original image for the non-maximum pixel but h less than the original image for the maximum pixel. After subtracting the original image and reconstructed image, we can simply get the maximum parts as nuclei and remove all other parts of cells. All the method used in the detection are good choices. Because they can significantly solve the problem of segmenting cell clusters and overlapped cells. Improving the detection accuracy and reducing the segmentation errors.

But only the nuclei are not enough in cells tracking. They may change because of noise and cytoplasm in different frames. The writer used least square method to fit the ellipses with the segmentation result to avoid the change of the shape. In the part of tracking, different frames have different locations of the same cells. The topology of cells also changes over time. The features are already used for tracking in the existing methods are displacement and skewness. Displacement is detecting the closest position and skewness is getting the deviation of direction. But it is difficult to correct matching error only using there two features. The author combines cellular topology features together with features mentioned before to reduce the matching errors in this paper. Using color and the overlapped area between two nuclei and eccentricities of the ellipses for nuclei to estimate the cost of matching cells in different frames. Regarding cells with the minimum weighted costs as the same cells. This method is a good choice to solve the problems caused by cell motion and the shape changes of nuclei. Using topology is a good way to reduce the possibility of false matching between different frame. But the way used to find minimum weights of these parameters is bipartite graph, which can only used for one-to-one matching and do nothing for dividing cells. Although this overcome can be recovered using the method of trajectory, it reduced efficiency.

In the part of trajectory, what needed to be done is connecting trajectories. But the trajectories may break because of leaving or entering a cell or perhaps errors caused in the previous methods. The author introduced a method called template-matching-based tracking method to overcome mistakes caused by segmentation and mitosis. In this method, giving each cell an ID and current frame number. Firstly, remove the IDs that only exist once as an isolated point. Then dividing all cells into three categories using the exist state of the frames before and after. Then getting a search window and moving it frame-by-frame in the backward directions. Updating the intensity values of the template unless it matches a cell of another track segment. And performing matching with corresponding cells in the previous frames for the break recovery. This method is a good choice to identify different cells and determine the state of cells in consecutive frames which is useful to recover the break of trajectory caused in tracking and segmentation methods.

Results

To evaluate detection, the author compared the method to watershed method, hybrid merging method and compactness method. Watershed method often over segmentation due to noise and local discontinuity. So segmenting cell sequences with the variations in illumination, low contrast, cell clustering and cell overlapping will causes the low accuracy of segmentation using watershed. Two other methods are also not enough to solving under or over segmented regions. The author presented a quantitative evaluation way to compare these methods intuitively. Choosing 700 frames randomly from four different sequences and counting the cells manually. Then calculating the accuracy and recall of detection. It is obvious that both these two parameters of present method are highest, which means the present method increases the performance of detection using top-hat filter and h-maxima transformation and works well in bad image status.

To evaluate tracking, the author used a cell sequence with mitosis events and showed the trajectories of cells. It can be seen that mitosis can be successfully detected. Accuracy is also an important evaluation index. Using the same four sequences in detection evaluation which have complex cell motions and getting correct track detection and mitosis detection manually. Then calculating accuracy for present method and other existed methods. We can see that present method do a pretty good and stable job than the other three methods. It is because this method is based on both motion and topological features. Changing the weight of different features will improve the performance for different images. However, using the same dataset for different methods may cause different time complexity. And the time cost of the present method is higher than others because in the trajectory step the method need to do a recovery to overcome the error from the previous step.

The findings in evaluation of detection are sufficient to convince potential users to adopt the proposed method. But the defects can still be found during evaluation of tracking. We know that time cost will increase as the quantity of dataset increase. So using a huge dataset and comparing its time cost and accuracy for both detection and tracking with other methods is a good way to show it is worth to waste time for the performance.

Conclusions

The paper presents a method of detecting and tracking cells in a time-lapse microscopy. This method can solve the problems caused by nonuniform illumination, cell clustering, cell overlapping, cell motions and cell mitosis. In the segmentation step, using top-hat filter and h-maxima transformation are good choice to solve illumination problems. And choosing nuclei as the segmentation objects are very useful to solve cell clustering and overlapping. All these methods in segmentation will convince potential users to adopt the propose method, it can indeed improve segmentation accuracy and efficiency. In the tracking method, combining cell motion features with topological features increase the accuracy of tracking. But the way to get the weights of different features is bipartite graph, which cannot be used for mitosis. And this error needs to be recovered in trajectory step which increase the time complexity at the same time.

But this method still has some weaknesses. Firstly, evaluating this method requires manual tracking of many tiny structures in numerous noisy images, in other words, large numerous testing will be impossible. Secondly, in this method, tracking step is based on detection. Each particle is detected in each frame. And then linking cells together between different frames. The performance of this method is closely related to detection method. Once the accuracy of detection decreased because of complex motions and highly cluttered images, the detection errors cannot be recovered during the next steps. The performance will sharply decrease.

This method does not completely resolve the problem of tracking cells. But it provides a good idea to solve the problem. It is very useful for disease diagnosis, follow-up treatment and other biological research.

References

[1] Rezatofighi, S. H., Gould, S., Vo, B. T., Vo, B. N., Mele, K., & Hartley, R. (2015). Multi-target tracking with time-varying clutter rate and detection profile: application to time-lapse cell microscopy sequences. IEEE transactions on medical imaging, 34(6), 1336-1348.