Commentary: Tracking Biological Cells in Time-Lapse Microscopy

Introduction

When traditional cell analysis methods fail to obtain the expected results, the use of real-time cell analysis methods usually results in many new biological discoveries. Through the development of various fluorescent proteins and nanocrystals and breakthroughs in optical microscopy technology, it has been possible to image dynamic processes in real-time at the cellular and molecular levels. However, the increase in the amount of image data, the larger the resolution, the variability of cells, and the various noises caused by image sampling, have brought huge difficulties and challenges to the automatic tracking and analysis of cells.

In fact, it is impossible to manually and accurately track a large number of cells in thousands of cell sequence images. Not only because it will cost too much time but also because of the need for reliability. Therefore, there are high requirements for the automation of cell analysis, and it has attracted more and more research attention.

But there are many challenges in automated cell tracking derived from different image acquisition techniques such as low image quality and low signal to noise ratio and topological issues such as complex structure and unpredictable movement of cells. If we can solve these problems and achieve high reliability in cell tracking, current medical researchers, doctors, and patients with corresponding diseases can benefit from it.

Before this paper was published, there were mainly three types of cell tracking methods. The first category is a method based on model evolution, which has low accuracy in some complex situations. The second category's methods are driven by segmentation, this method is limited by tracking and detection error, and it will increase the complexity of the system if we want to solve this problem. The last type of method is based on the Bayesian probability framework. If there is not enough data, this method cannot complete the cell tracking task, so we cannot use these methods to track cells in a phase contrast microscope.

This paper proposes a new idea and solves the tracking of biological cells in time-lapse microscopy problems by combining the motion features with the topological features of the cells. First, they use a top-hat filter to detect the cell center. Besides, they introduced a robust measure of dissimilarity between the cells, combining motion features with topological features. At last, they propose a method to recover trajectory caused by some error. All of these can improve the accuracy of cell detection and tracking and finally achieve better results.

Methods

There are three main modules in the proposed method: detection, tracking, and trajectory recovery. Each part of the author has chosen an appropriate computer vision method to achieve the goal. I think all methods can perform very well, but based on the knowledge I have learned, and the literature reviewed, there may be some better ways. I will explain my points in the next paragraph.

In detection modules, the author chooses a top-hat filter to reduce shading artifact, which is caused by non-uniform illumination, camera sensitivity, and some other reasons. Then, the author decides to use the top-hat filter to reduce shading artifact which is caused by non-uniform illumination, camera sensitivity, and some other reasons.

$$\hat{A} = A' - open(A') = A' - max\ (min(A'))$$

Because the result of the open operation is to enlarge the cracks or local low brightness areas. After subtracting the open operation image from the original image, the resulting effect image highlights the cell area than the surrounding area of the original image and can increase the dynamic range of the intensity between the cell and the background [1]. Therefore, Top-hat operation is suitable in this kind of illumination correction.

Then, the author chooses a Gaussian filter to reduce noise peaks, which can minimize image noise and reduce details. After Gaussian filter, h-maxima transformation in mathematical morphology, which is used to suppress pixels above a certain intensity [2]. It can extract local maxima value related to target objects from the gray image. By reading related questions, I think $Extended - Maxima\ Transformcan$[3] gets better segmentation results. The function as below:

$$E\ MAX_h(f) = R\ MAX[H\ MAX_h(f)]\ [3]$$

In tracking modules, the author chooses E_{color} as a measure of the variation of color between two nuclei, because cells may change rotation frequently. Hence, I think $perceptual\ hash$ [7] may be a better choice because it can indicate the distance between two blocks more clearly. Meanwhile, Cell movement is prone to overlapping problems and changes in size at any time, experiments show that the algorithm can successfully handle the occlusion problem and adjust the target size in time [4].

In trajectory recovery modules, divided cells into three categories and calculate the similarity between two cells by the Template Matching method with normalized. If they are similar and belong to the right classes, they can connect the trajectories of cell fragmentation caused by cell entry or entry, segmentation errors, or mitosis in the field of view. But there may be some better choice than standard Template

Matching methods such as SIFT [8] and O-DAISY [9], which are rotation-invariance and scale-invariant. Rotation - invariance and scale-invariant should be important in cell tracking because cells may move or spin at any time.

Results

The author compared their method in two-part: detection and tracking. In general, their result looks great in 2011 when deep learning is not prevalent. I will summarize and comment on the results of the two parts separately.

In the detection part, the author shows their detection method worked well in a murine embryonic sequence and Hela cell sequence in Fig 7. And then compare with the watershed segmentation method, which is a standard but good-preference method. In the watershed segmentation method, there are undersegmented and oversegmented problems. But in the proposed method by this paper, it can work well, which is represented in fig 8.

Then the author compared their method with Hybrid Merging [5], Compactness [6], Watershed in 700 random choose frames, and measuring by detection accuracy of the number of the cell nucleus in the segmentation result. The result shows that the proposed method got the highest precision and recall and reduce nearly a half error rate than the other three methods.

The above data shows that the proposed method does have a more significant advantage over other popular algorithms in terms of accuracy of segmentation in 2011. These data will prompt potential users to follow their method, but it may be more detailed if the analysis of the effect of cell size or type on detection accuracy is made.

In the tracking party, the author measures the performance by two indexes: tracking accuracy and mitosis accuracy. The tracking accuracy is measured by the ratio of the number of valid cell motion trajectory detected in the test data set to the total number of actual cell motion trajectory. The accuracy of mitosis detection is measured by the ratio of the total number of effective mitotic events detected in the test data set to the actual number of mitotic events in the test data set. The proposed method gets 85.38% and 82.66% as tracking accuracy and mitosis event accuracy on three murine embryonic sequences and a Hela cell sequence.

The author also compares tracking accuracy between the proposed method, the Hybrid merging method, and the compactness method under different cell numbers. Fig 11 shows that the proposed method gets the highest tracking accuracy with about 100 cells and the slowest rate of decrease in accuracy as the cell grows. This indicates that in most cases, potential users should choose their method because of excellent performance.

At last, the author compares computation times of the proposed method, the Hybrid method, and the compactness method. The proposed method is near double than of the Hybrid method and a bit longer than the compactness method, but I think this is a worthwhile trade-off. If people have specific requirements for accuracy and not strict requirements for computation time, the proposed method is the right choice. Still, if people have rigorous requirements for computation time, they may need to modify this method or choose other methods.

Conclusions

This paper presented an automatic cell detecting and tracking method, which introduces a cell-detection method based on h-maxima transformation and fitting of an ellipse for the nucleus shape and tracking by topological features and motion features of cells. This method solved low accuracy problems caused by non-uniform illumination, complex cell structure, irregular cell motion. It shows high detection and tracking accuracy in several different cell sequences.

This paper has several strengths. First of all, the author focuses on the segmentation of cell nuclei to avoid cell morphology and partial overlap of cells caused by detecting the entire cell. Then, the top-hat filtering and h-maxima transformation with the adaptive threshold ensure the accuracy of segmentation. After that, cellular motion features and the topological features constitute a dissimilarity measure which performs well in complex situations. Finally, the trajectory recovery method provides a new idea to solve problems such as segmentation errors.

However, there are still some weaknesses. First of all, the tracking accuracy of this algorithm depends to a certain extent on the accuracy of the segmentation algorithm, and the accuracy of the segmentation algorithm determines the upper limit of the accuracy of this tracking algorithm. If there are very complex and noisy sequences, the accuracy of segmentation will decrease, and the accuracy of tracking will also decrease accordingly. Secondly, the method of estimate the cost of matching between cells in consecutive frames can be improved because rotation-invariance and scale-invariant are important in matching two cells, such as we can use $perceptual\ hash$[7] with Hamming distance instead of the variation of color. At last, Template Matching is not the best choice in the trajectory recovering, maybe some method with rotation-invariance and scale-invariant invariance is a better choice such as SIFT [8].

Some remaining issues still need to be addressed nowadays in cell segmentation and tracking. Firstly, how to achieve cell tracking issue with low computing cost to ensure real-time tracking. Then, how to improve tracking accuracy with a considerable number of cells. Last, how to improve the robustness of the cell recognition and tracking model to ensure that the model can adapt to more cell environments. I think all of these can be future research directions.

References

1. Bright, D.S. and Steel, E.B. (1987), Two‐dimensional 47top hat filter for extracting spots and spheres from digital images. Journal of Microscopy, 146: 191-200.
2. Halkiotis, S., Botsis, T., and Rangoussi, M. , “Automatic detection of clustered microcalcifications in digital mammograms using mathematical morphology and neural networks,” Signal Processing, vol. 87, no. 7, pp. 1559–1568, 2007.
3. Qin, Y., Wang, W., Liu, W., & Yuan, N. (2013). Extended-Maxima Transform Watershed Segmentation Algorithm for Touching Corn Kernels. Advances in Mechanical Engineering.
4. Ai-hua Chen, Ming Zhu, Yan-hua Wang and Chen Xue, "Mean shift tracking combining SIFT," 2008 9th International Conference on Signal Processing, Beijing, 2008, pp. 1532-1535
5. J. Yan, X. Zhou, Q. Yang, N. Liu, Q. Cheng, and S. T. C. Wong, “An effective system for optical microscopy cell image segmentation, tracking and cell phase identification,” in Proc. IEEE Int. Conf. Image Process., Atlanta, Oct. 2006, pp. 1917–1920.
6. X. Chen, X. Zhou, and S. T. C. Wong, “Automated segmentation, classification and tracking of cancer cell nuclei in time-lapse microscopy,” IEEE Trans. Biomed. Eng., vol. 53, no. 4, pp. 762–766, Apr. 2006.
7. W. Zhen-kun et al., "A Robust and Discriminative Image Perceptual Hash Algorithm," 2010 Fourth International Conference on Genetic and Evolutionary Computing, Shenzhen, 2010, pp. 709-712.
8. Lowe, D.G. Distinctive Image Features from Scale-Invariant Keypoints. International Journal of Computer Vision 60, 91–110 (2004).
9. J. Fischer, A. Ruppel, F. Weißhardt and A. Verl, "A rotation invariant feature descriptor O-DAISY and its FPGA implementation," 2011 IEEE/RSJ International Conference on Intelligent Robots and Systems, San Francisco, CA, 2011, pp. 2365-2370.