Commentary: Tracking Biological Cells in Time-Lapse Microscopy

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Introduction

The reviewed paper addresses the problems of segmentation errors and uneven movements in biological cell tracking in time-lapse microscopy. The issue of segmentation error could be represented in poor image quality when an image is acquired in a controlled circumstance. The problems of topology would be shape deformation, close contact, an overlap of cells, and events like mitosis. Moreover, cells' uneven movements challenge the modelling of motion, and the changes in capturing speed also make the computation more complex. Traditional methods such as Kalman or particle filters cannot be applied, on account that there is no way to gain prior knowledge of a cell's motion. Additionally, considering the smoothness of a cell's action, the nearest neighbor constraint is not always satisfied.

The author aims to decrease the segmentation error, to match cells correctly through frames, and to implement the recovery of broken cell trajectories. The realization of these increases the accuracy in cell correspondence, and the construction of the cell proliferation trajectory relies on cell recognition precision. Fixing the trajectory can help detecting a cell's vanishment is out of death or mitosis and reducing segmentation errors.

If this paper authentically solves the segmentation error and uneven movements, biological researchers who focus on cytopathy and cell division would be beneficiaries. For the cytopathy domain, researchers have the capacity to track that given a specific dose of chemicals or some stimuli, how does a cell evolve. As for cell division, novel patterns or secrets of life evolution could be explored. Moreover, drugs that target cells can be tracked on their effects through a time series.

Methods

The approaches utilized in the reviewed paper are summarized in three modules: detection, tracking, and trajectory recovery.

For detection, a morphological top-hat filter is applied to reduce shading artifacts, and noisy peaks resulted from uneven illumination. With inversion and morphological opening operation first, the processed image is subtracted from the inverted unprocessed image. As the result, illumination becomes uniform, and shading artifacts are removed. This algorithm performs outstandingly on the input images as the output images are clean, and cells are well segmented. The reasons for the top-hat filter application are given below. An observation of the original images reveals that the background is white, and noises as well cells are black. Since erosion removes white dots in black backgrounds, an inversion operation is applied to flip the colors of elements in input images. The morphological opening operation has the effect of removing tiny objects and smoothing the edge of significant objects. Hence, it is wise to remove noises and to improve the shape of cells by using the opening operation. As for the subtraction, it reduces the intensities of background and highlights the regions of cells. Based on the threshold segmentation conducted on the output of the top-hat filter in the discussed paper, the top-hat filter is considered as an excellent practice for raw cell image processing, with the merits of simple execution and ideal output quality. Compared to a popular method, which is a combination of Fast Fourier Transform Band-pass filter and watershed transformation for automated cell segmentation in [1], the top-hat filter outperforms in the efficiency of processing. Nevertheless, there is one concern for the top-hat filter usage that if after the morphological opening operation, the cells have close intensities as the ones of the inverted unprocessed images, in the difference of subtraction, the cell parts could hardly be recognized. The author does not include this extreme case in his paper.

To find out the regional maximum for each cell, Gaussian filtering is utilized to search the unique local maximum in a cell and to suppress noises. Gaussian filter is a classic method for noise removal and detail extraction. Undoubtedly, it is a good choice here.

After applying Gaussian filtering, $h-$ maxima transformation is utilized to get the regional maximum of each cell by suppressing regional maxima which are lower than h. Then, each cell is fitted into an ellipse for nuclei localization. Compared to typical bounding box methods, an ellipse is closer to the shape of a cell, reducing bias in tracking. Besides, this method has an advantage of fast speed, which makes it surpass the Keypoint Graph-based Bounding Boxes method in [2], though both of them have good accuracy.

When it comes to tracking, the author introduces displacement ($E_{displacement}$) as well as skewness ($E_{skewness}$) to cellular motion, and color compatibility ($E_{compatibility}$), area overlap (E_{area}) as well as deformation ($E_{deformation}$) to the cellular topology. A combination of these parameters $E^{n,n+1}(u,v)$ is used to estimate the cost of matching between two cells u,v in two consecutive frames. In the next step, the values of $E^{n,n+1}(u,v)$ are utilized in the minimum weighted matching of a bipartite graph. The method's idea is more or less like the particle filtering, which also tracks objects using dynamic models. Moreover, the assumptions of this method imply the tracking problem has the structure of inference on a hidden Markov model. The consideration of these five parameters is reasonable based on the natural characteristics of cells. And the complexity of computing these parameters is rather simple, as to the extended RankBoost machine learning method used in [3]. Nonetheless, the base assumption of the matching algorithm in the reviewed paper is rather ideal. It

cannot match exceptional cells that move fast between consecutive frames.

To recover the trajectory breaks due to mitosis and segmentation faults, a template-matching-based tracking method is introduced. Its execution identifies cells by their identity numbers and frame numbers. This method goes backward through the image sequence and recovers the missing IDs to join the breaks in trajectory. By doing this, the newly generated cells from mitosis could be detected, and the original cell trajectory gets fixed so that we can keep tracking the child cells. In the process of fixing breakpoints, we need to match a new cell to a previous trajectory. In this case, γ the correlation coefficient at the location (p, q) is introduced for matching reference. But this tracking method is not used when a cell is close to the boundary of the image. This recovery method is presumed from a mathematic way, and this idea is practical for the majority of cases because this method is machine learning free, which means it is not affected by the training data. Better still, compared to training a Support Vector Machine to detect mitosis events in [4] to get high accuracy, the recovery method saves a lot of time by conducting posterior processing but getting a similar level of correctness. In contrast to the math methodology in [5], which searches the "nearest cell" by similarity in intensity, area, etc., the mathematic in the discussed paper is more comprehensive in the cell morphology, indicating more cases are supposed to be successful. In general, this trajectory recovery method is reckoned as an excellent technique to perfect cell tracking. However, we should still point out that the mathematic method is not segmentation error free. Exceptional segmentation errors still exist to make the technique conduct wrong matches.

Results

For cell detection, it is evaluated qualitatively and quantitatively. This paper compares the proposed method's segmentation with the outputs of the watershed algorithm to show the qualitative evaluation that the proposed method increases the contrast and has a more accurate segmentation. In quantitative evaluation, 700 frames are extracted, and cells are manually counted. The author calculates the parameters in the confusion matrix, such as true positive, false negative, etc., to evaluate the method. And it turns out that the method has an accuracy of 91.74%. This conduct is assumed to be convincing enough for potential readers to apply top-hat filter and $h-$maxima transformation, because the number of sample frames is big enough, and the accuracy is comparatively high. However, in the qualitative evaluation, more segmentation methods, such as thresholding, mean shift, and UNet neural network could be included to compare with the proposed method. Therefore, readers can have a more comprehensive understanding of how well the proposed method is.

When it comes to cell tracking, it is also evaluated in qualitative and quantitative aspects. In the qualitative results, four frames in a murine embryonic sequence are extracted, and the number of cells as well as the detection of mitosis are shown on the four images. Furthermore, the spatial-temporal trajectory of the cell proliferation of the first 700 frames is shown. The plot of the trajectory does not have any breaks, which means the recovery method is successful. As for quantitative evaluation, the accuracy of cell tracking is represented as in a given image sequence, the ratio of perceived valid track segment count to the total of actual track segments. Also, the representation of the accuracy of mitosis perception is the ratio of the number of mitoses detected to the total of actual mitosis events. The averages of cell tracking accuracy and mitosis event perception accuracy are 85.38% and 82.66%, respectively. Generally speaking, these accuracies are acceptable but could be impractical when the accuracy requirement is high. In comparison with the mitosis detection accuracy (99.1±0.8 %) of the HCNN model in [6], if a potential reader is pursuing accuracy instead of efficiency, training an HCNN model should be preferred. However, if time consumption is also an important factor to be taken into account, the proposed method in the discussed paper is acceptable as it does not require a long-time neural network training.

Conclusions

The commented paper presents a rather simple method to segment cells and to conduct cell tracking. This paper has its strength in splendid cell segmentation in a simple time complexity. By using the top-hat filter and $h-$maxima transformation, it achieves a segmentation accuracy of more than 90%. This combination of techniques could be an inspiration for people who want to make enhancement on their outputs from the watershed algorithm. Also, the proposed tracking method is easy to execute, and its mathematic principle is supposed to work for the majority of cases. The advocated tracking method solves the uneven movement problem, which cannot be solved by traditional methods like Bayesian inference because prior knowledge of motion does not contribute to the posterior movements. However, the accuracies for tracking and mitosis detection are not high enough is the weakness of the proposed method.

Before the issues are assumed to be solved, this paper is recommended to discuss more about the extreme cases of cellular topology, since the general mathematic for the cell matching part might fail in some special situations. Whether the parameters of displacement, skewness, color compatibility, area overlap, as well as deformation would be adequate to summarize the cellular topology is needed to be confirmed.

To enhance the proposed method, further research on the mathematic in deep learning about cell tracking and mitosis detection is encouraged. If utilized in precise applications, accuracies less than 90% are not acceptable. Attempts to improve the accuracies have the potential to address the problems efficiently mentioned in the introduction part. Furthermore, researches of combining top-hat filter, $h-$maxima transformation, and deep learning to make a more precise segmentation are recommended. This combination has a promising expectation of more than 95% accuracy.

References

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