Cell Detection And Tracking

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***Abstract*—The automated cell detection and tracking is of concern. The paper mainly focuses on the morphological operations and watershed algorithm to segment the cells from the background and comes up with the tracking strategy which applies the Euclidean distance and overlapping degree as metics to find the matching candidates. The tracking skill could detect the mitosis.**

***Index Terms*—cell, detection, segmentation, watershed, tracking, mitosis**

I. INTRODUCTION

With the increasing requirement of biology research, microscopes having video systems are used to record the motion of cells. However the size of images recorded by microscopes usually is very large, because these pictures are sequences of motions of cells under the screen and each sequence have hundreds of images. This is difficult to correctly analyse the motion of many different cells at the same time and this progress is boring and tedious. Therefore, automatic tracking and analysis correctly is the main purpose of this project and is becoming a more and more significant issue in biology.

There are two main parts of this project: segmentation and tracking. In the segmentation section, we need to segment cells. The quality of the images may be the main challenge to achieve this goal. Some reasons may lead to low-quality images. Firstly, the illumination of images may be different in different images and the illumination may not enough or too bright that have affected the cells self. Secondly, the noise in the picture also can result in the low-quality picture. Therefore, to get clear segmentation, we need to solve the above problems. In the tracking section, we need to draw the trajectory. The main challenge of this task may be the various characteristics of cells motion and mitosis. [1]

There are three data sets used in the project. The first data set is called “DIC-C2DH-Hela” including four sequences. Cells in this data set are larger than the other two, including many cells tissue, such as cytoplasm. This will be a big obstacle in segmentation. The second and the third datasets are named “Fluo-N2DL-HeLa” and “PhC-C2DL-PSC” respectively. Compared with the first one, their cells are much smaller and easier to be segmented.

In this paper, the second section shows the related techniques from previous research, and then describes the detail of proposed method. The fourth section demonstrates the experimental setups and the method used to evaluate.The results and discussion are the following. The last two sections will be the conclusions and the contribution of group members respectively.

II. LITERATURE REVIEW

Segmenting and tracking biological cells in time-lapse microscopy images is one of the most common and important computer vision tasks in cell biology. Segmenting cells in an image is a challenging process because of the huge quantity and it is hard to track the cells that are not separated from each other. To solve these problems, Fernand Meyer et al. proposed a mathematical morphology segmentation technique – marker-controlled watershed transform [2], which can be used to separate round cells or nuclei in cell segmentation. However, as the complexity of visualizing objects increases, the proper definition of tagging and segmentation becomes more and more complex. Yann LeCun et al. proposed deep learning [3] to process medical images. This model is supervised and needs to be adjusted using the training data set and the algorithm can segment cells with complex structure and shape, but it is not suitable for the segmentation of objects in contact. Therefore, we choose marker-controlled watershed transform and convolutional neural network (CNN) to proceed with cells segmentation.

D. Suter[4] et al. developed a multicellular automatic tracking method based on a tagged Bernoulli filter,

which can complete cell tracking without data association. Dewan[1] et al. proposed a vision-based method for automatic tracking of biological cells in time-lapse microscopy by combining the motion features with the topological features of the cells, which can accurately track a lot of cells. Therefore, we choose to track the cells by combining the characteristics of cell motion and topology(including cell registration, Euclidean distance[1] and overlapping degree).

Tracking methods based on Bayesian probabilistic framework are suited for particle tracking in fluorescence microscopy[5], but the detection of mitosis is problematic. A template-matching-based tracking method can be helpful to detect the mitosis, so we choose this method to proceed with the detection of mitosis.

III. METHOD

A. Detection module

The importance of detection is significant. It is the foundation of any further tracking or computation. To achieve better performance, techniques such as morphological processing are often applied. To deal with complex cellular structures, deep learning could be more accurate [6]. The method for detection in this project is mainly based on morphological image processing. which includes various non-linear operations with kernels. The kernel stands for different sized structuring element which usually convolves through the whole image, subtracting or filtering out certain features of regional areas. The non-linear operations are especially effective on the binary image.

a. Segmentation

The main task of detection is segmentation. According to the different images of diverse pixel diversity distribution, we may alter some morphological operation order. We first convert the image to be grayscale and then applied top-hat operation. The top-hat operation could well subtract the small objects in the large-scale background and correct the illumination[1]. It is implemented by subtracting the opening of the image from the raw image. For the image in which the intensity distribution is narrow, the illumination correction could be directly by opening operation without subtraction. After the illumination correction, the image is clear enough to find all the cells, but for the watershed, we need to generate a binary image as a mask. To reduce the intensity variation, we apply Gaussian filtering, which smooths and blurs the image with Gaussian kernel instead of a filter with equal coefficients. The images then should be normalized to restore a wide range of intensity distributions. As shown in diagram Fig 1 (a) and (b) , the histogram explicitly shows the intensity distribution is improved by normalization, which replaces the small bump with several peaks.

After the pixel values are separated, we need to threshold the image to get the binary image. The simplicity of thresholding is prone to error making[7]. We have two approaches for different image patterns, one is to find the unique value and use the first intensity value as thresholding, and the other one is Otsu's algorithm. The previous approach is more applicable to those images with only a few intensity values, and the latter one is more general and practical in most problems. To help the watershed algorithm get higher accuracy, we implement a series of dilation and erosion operations, which contributes to separating the touching boundary between two or more cells.

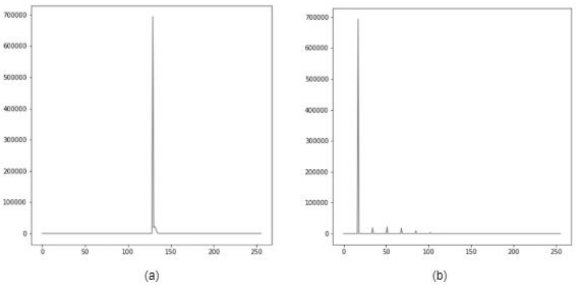


Fig.1 (a) hisogram of raw image (b)hisogram of normalized image

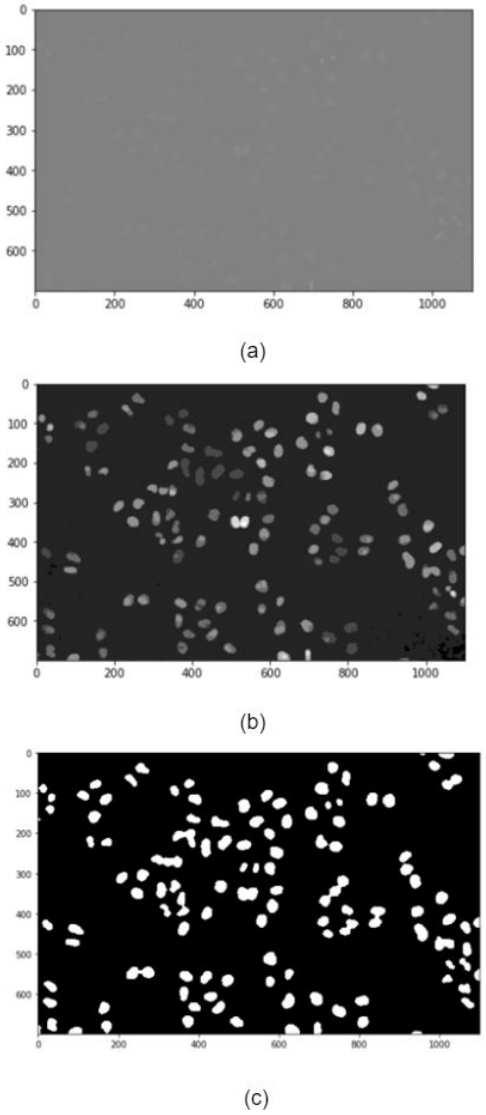


Fig. 2. Illustration of morphological operation . (a) raw image (b) illumination corrected (c) binary image after dilation and erosion

b. Watershed

According to the definition in the dictionary, the watershed is a dividing ridge between drainage areas. The core idea is considering the various intensity as hills and valleys. And through building dams between the peaks, filling in the water, the plane of the low-lying area will be elevated, so the objects are divided by these 'dams'. Although this algorithm could distinguish single or touching object very well, it is impossible to avoid over-segmentation due to the false local minima. To recover this, based on the original algorithm, we modify the Distance variable, which is the distance to background, to have it normalized and blurred. The advantage of this implicitly reflects in the labelled image that the gradient and edges are more reasonable, thus the probability of over-segmentation is decreased. From Fig3 (a) and (b) , the false local minima are relieved through the post processing of Distance.

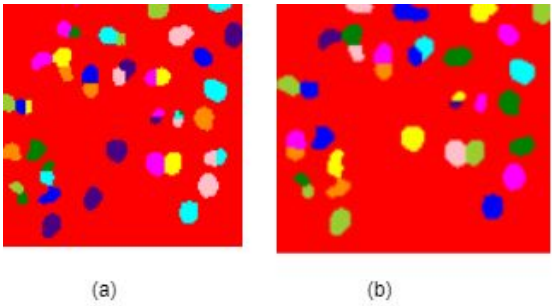


Fig 3. (a) The original label effect (b) The processed label effect

B Tracking module

The tracking progress should be able to track each detected cell in case of motion and mitosis in consecutive images. We set up a cell registration class to record all the cells with their attributes such as velocity. This contributes to future cell matching and motion analysis.

In the main part of tracking, we use Euclidean distance and overlapping degree as metrics to find the most possible candidate cell between two consecutive images. Euclidean distance is defined as the length of the straight line between the centroids of pairwise cells. The overlapping degree is referred to Dewan and Ahmad's approach[1], which represents the degree of overlapping area between two cells. The lower the value, the more likely that two cells are the same.

In our model, we iteratively compare the current image and the previous image, and then according to the distance matrix of each pair of cells, two possible candidates in the previous image are chosen. To further improve the validation of them, we compare the candidate of the lowest overlapping degree to these two, if it matches any of them, it turns out to be the final matched object and we record its id into current cell's attribute. Otherwise, if there is no matching, we establish a flag distance which filters out the candidate cell whose distance is out of range, in case of overmatching.

The whole strategy finally selects only the possible candidate cells, and two or more cells are likely matched to the same cell in the previous image. This is where mitosis happens, and through traversing the matching list, we can easily find the divided cells and locate their previous mother cell. Once the location is done, the successive cell motion or mitosis can be analyzed, we save their speed at that time point, total distance, net distance and confinement ratio which is the ratio

between the total distance and net distance to the cell's attributes.

IV. Experimental Setup

The experiments are conducted on three datasets, they are DIC-C2DH-HeLa, Fluo-N2DL-HeLa, PhC-C2DL-PSC respectively. The cells in each dataset have different size, shape and illumination, which make the images more complex. What's more, there are 4 video sequences obtained by time-lapse microscopy technology in each dataset and the image resolution in each sequence is (700*1100), and the all images are made up of three colour channels. .The processor of the computer we used in the working environment is Intel i5 2.3GHz with 8GB 2133Hz LPDDR3 RAM. And Jupyter notebook has been applied to compile and run our code, and the installed binaries include Python 3.7.4,scikit-image 0.15.0, opencv 3.4.1, scipy 1.3.1.

To verify the effectiveness of our detecting and tracking method of biological cells, the quantitative evaluation we applied is to calculating parameters True Positive(TP), False Positive(FP), False Negative(FN), Precision(P), Recall(R). The parameter P can be calculated by TP/(FP + TP), which is the ratio of the number of true detection of cells to the total number of detected cells. The parameter R is calculated by TP/(TP + FN), which means the ratio of the number of true detection of cells to the total number of cells observed in each image. In addition to above five parameters, we counted manually the number of cells in each sequence as the ground truth.

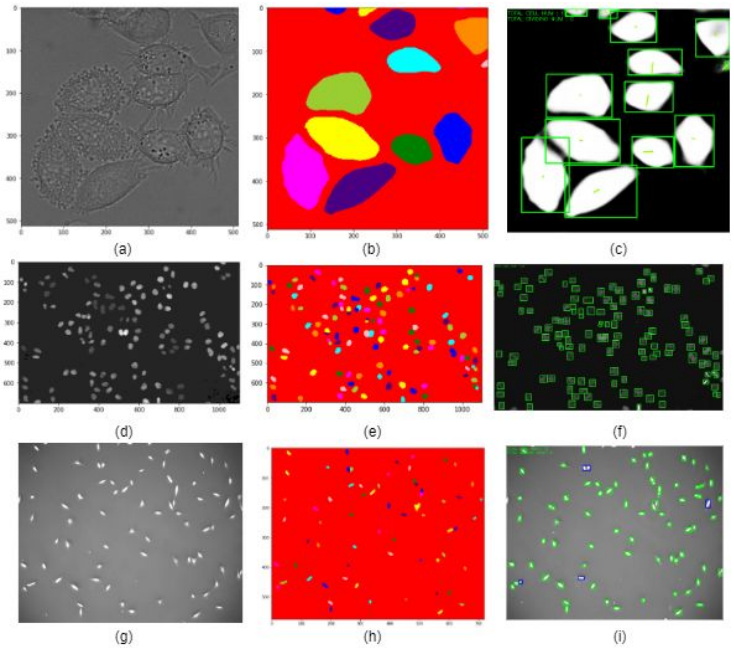


Fig. 4: Results of detection using the proposed method. (a) is the input image t000 of sequence 1 of the DIC-C2DH-Hela. (b) is the segmentation result of (a) using Deepwater CNN model. (c) is the detection result of (a). (d) is the input image t002 of sequence 2 of the Fluo-N2DL-Hela. (e) is the segmentation result of (d) using watershed method. (f) is the detection result of (d). (g) is the input image t003 of sequence 3 of the PhC-C2DL-PSC. (h) is the segmentation result of (g) using watershed method. (i) is the detection result of (g).

Data	Ground truth	TP	FP	FN	P(%)	R(%)
DIC-C2DH-Hela	446	391	10	45	97.51	89.68
Fluo-N2DL-Hela	453	336	62	55	84.42	85.93
PhC-C2DL-PSC	468	332	70	66	82.59	83.42

Table I Performance evaluation of cell detection

V. Results and Discussion

Several images of three datasets of time-lapse microscopy are used to implement some experiments for checking the effectiveness of the proposed method. We use both qualitative and quantitative evaluation of the proposed method to present the results of the experiment.

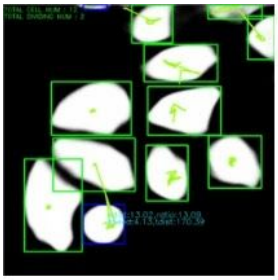
As the classical morphological operation cannot work on complex structure, the dataset DIC-C2DH-HeLa is only tested on the tracking module, not detection module. We use Deepwater CNN model[6] to get the segmentation results of dataset DIC-C2DH-HeLa.

A. Evaluation of Detection

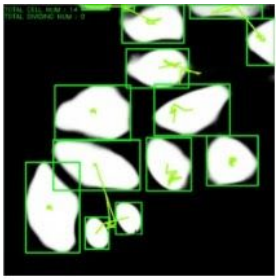
Fig. 4 shows the graphical demonstration of the output of our cell-detection method. As we can see from Fig. 4(b), Deepwater CNN model performances well on the DIC-C2DH-Hela, every cell is clearly segmented, hence we obtain a good detection result shown in Fig. 4(c). From Fig. 4(e) and Fig.4 (h), we notice that the proposed method for the Fluo-N2DL-Hela and the PhC-C2DL-PSC could segment most cells, but there are cells who are extremely close to each other, which the proposed method is unable to separate correctly.

For the quantitative evaluation, we calculate the ground truth, TP, FP, FN, P and R. We record 446, 452 and 468 ground truth from randomly picked frames in the dataset DIC-C2DH-Hela, Fluo-N2DL-Hela and PhC-C2DL-PSC. From Table I, we notice that the parameter P and R for the DIC-C2DH-Hela are 97.51% and 89.68% respectively, which are outstanding results.

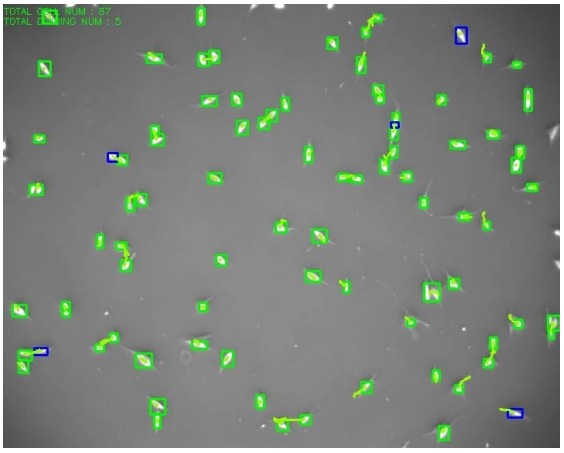
This results also proves the robustness of the proposed method for detection for the DIC-C2DH-Hela. The results also means that CNN deep learning model is extremely good at dealing with cells with complicated internal structures. The parameter P and R for the Fluo-N2DL-Hela are 84.42% and 85.93% respectively, which are higher than same parameters for the PhC-C2DL-PSC, as the size of the cells in PhC-C2DL-PSC are smaller and the miss detection occurs more frequently. The results also shows that the proposed method top-hat followed by watershed performances good on the Fluo-N2DL-Hela with low contrast images and the PhC-C2DL-PSC with small sized cells.



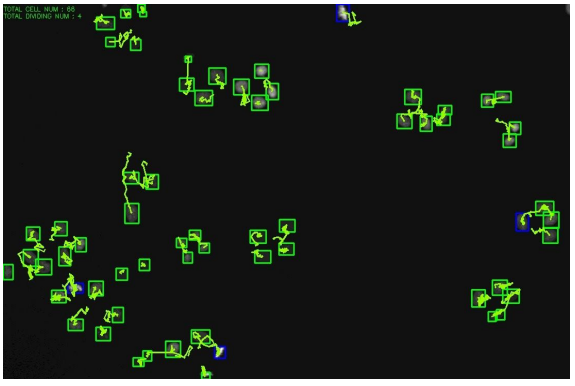
(a)



(b)



(c)



(d)

Fig.5 (a) is the tracking result of image t010 of sequence 1 of the DIC-C2DH-Hela (b) is the mitosis captured result (c) is the tracking result of image t037 of sequence 1 of the Fluo-N2DL-Hela. (d) is the tracking result of image t027 of sequence 3 of the PhC-C2DL-PSC

Data	Frames observed	Tracking detection	Mitosis detection
DIC-C2DH-Hela	20	247/301 (82.21%)	3/4 (75%)
Fluo-N2DL-Hela	10	349/443 (78.78%)	18/25 (72%)
PhC-C2DL-PSC	10	591/786 (75.2%)	42/58 (72.41%)

Table II Accuracy of Tracking and Cell Dvision

B. Evaluation of Tracking

To demonstrate the qualitative results of the proposed method for cell divisions and tracking, we check one frame from each dataset by visual. Fig. 5 shows the results of cell tracking and divisions for three datasets. It is shown in the Fig. 5(a) and (b) that after the cell division event is detected, our method could relocate the new cells divided from the old cell in the next frame. In Fig. 5(c) and (d), most of tracking of cells are recorded correctly and most of cell mitosis actions are detected and are marked with blue bounding boxes, which means that our method based on Euclidean Distance and overlapping degree performances well in all three datasets.

For the quantitative results of our method for cell tracking and division, we record the accuracy of the track detection and the accuracy of cells division. We compute the ratio of the number of tracking and the total number of true track segments in the observed frames to evaluate the accuracy of the track detection. Then we record the ratio of number mitosis events and the number of actual cell division events observed in the frames to obtain the accuracy of the cell division detection. We manually count the actual the number of the track segments and the cell division events.

The Table II shows the results of accuracy of track detection and detection of cell division. It is seen from the table that the proposed method for cell tracking and cell division performances well on all three datasets, the accuracies are all over 70%. As the size of the Fluo and PhC cells are smaller than DIC cells, and there are more cells in one frame for Fluo and PhC, the accuracies of the track segments are lower

than that for DIC cells, which means that when the number of cells in a single frame is large, our method tends to performance less well. There are only several mitosis events in the 20 frames of DIC datasets, since the DIC cells are large and there are 10-20 cells in one frame of the DIC dataset. Due to the time limit, we only evaluate the accuracy of cell division detection in 20 frames. If the frames observed could be increased, the statistics for evaluating track segments detection and mitosis events detection would be more accurate. On the other hand, it is shown in the table that the accuracy of mitosis detection for PhC cells is 72.41% , with the actual mitosis events occurring 58 times in total, which shows that our method for detecting tracking and mitosis events based on the Euclidean distance and overlapping degree is robust.

The proposed method can also clearly show us the information mentioned in the task 3. When you click the cell in the picture, you can see the details of the cell at that point., as shown in the Fig.5 (a).

VI. Conclusion

In this project, we tried our best to find a perfect strategy to segment and track cells under different circumstances. In some cases, the proposed method can segment cells from background correctly and box cells one by one clearly. In some cases, the method cannot perform well, but the CNN model trained by others works well in these cases. To minimise the effect on other tasks, this CNN model was used to segment in these cases. However, the tracking section has a good effect on all cases. Except for the situation that interruption of detecting cells, the trajectories of these cells will be intermittent. In addition, we also can get the detailed information of each cell, such as speed, distance and confinement ratio of their motion. It is beneficial for biologists to avoid spending redundant time analysing the features of cells. And then they can progress further research directions.

In the future, we need to analyse our own segmentation strategy to find the reason why it is not suitable for all cases and improve the proposed method for better results. As for tracking, the main challenge is the intermittent trajectories. To solve this problem, we need to analyse the situations that cells cannot be detected and then try different methods to find the best way.

VII.Contribution of Group Members

Chunyang Jiang: Chunyang took charge for completing the report, especially introduction and conclusion. She is also involved in modifying code.

Huanyun Fan: Huanyun was in charge of completing the report, especially literature review. She is also involved in modifying code.

Shaohong Xu: Mainly responsible for the code in the task 1, 2 and 3. He also wrote method and abstract section of this report, and modified all the structures. He also presented the demo.

Yuehui Chu: Yuhui took charge for the code in the task 1 and the section of experimental setup in the report.

Ziyi Wang：Mainly responsible for the code in the task 1 and the result and discussion part of the report. He also contributes many idea and effort to complete the project

REFERENCE

[1] Dewan et al., Tracking biological cells in time-lapse microscopy: an adaptive technique combining motion and topological features, IEEE Transactions on Biomedical Engineering, vol. 58, no. 6, pp. 1637-1647, June 2011

[2] Fernand Meyer and Serege Beucher, “Morphological segmentation,” Journal of Visual Communication and Image Representation, vol. 1, no. 1, pp. 21–46, sep 1990.

[3] Yann LeCun, Yoshua Bengio, and Geoffrey Hinton, “Deep learning,” Nature, vol. 521, no. 7553, pp. 436–444, may 2015.

[4]R. Hoseinnezhad, B.-N. Vo, B.-T. Vo and D. Suter, "Visual tracking of numerous targets via multi-Bernoulli filtering of image data", Pattern Recognition, vol. 45, no. 10, pp. 3625-3635, 2012.

[5] I. Smal,W. Niessen, and E.Meijering, “Bayesian tracking for fluorescence microscopic imaging,” in Proc. Third IEEE Int. Symp. Biomed. Imag.:Nano to Macro, Arlington, VA, Apr. 2006, pp. 550–553.

[6] Lux F , Matula P . Cell Segmentation by Combining Marker-Controlled Watershed and Deep Learning[J]. 2020.

[7] Meijering, E., Dzyubachyk, O., Smal, I. and van Cappellen, W., 2009. Tracking in cell and developmental biology. Seminars in Cell & Developmental Biology, 20(8), pp.894-902