Tracking of biological cells in time-lapse microscopy images

Group Name: CV_Stars

Anrui Wang
University of New South Wales
z5183159

Zhao Wang
University of New South Wales
z5219645

Rongtao Shen
University of New South Wales
z5178114

Haoran Zhang
University of New South Wales
z5186017

Shen Yan
University of New South Wales
z5196202

1 Introduction

Tracking of biological cells under a time-lapse microscope is one of the most common and important computer vision tasks in cell biology. Specifically, measurements of cell movement and mitosis under different conditions are necessary in cell analysis. Manual analysis of these images, however, is too complicated. It is tedious for human observers to track cells over long periods of time and get reliable results.

Therefore, it is necessary to implement computer vision methods to automatically detect and track cells and then conduct a quantitative analysis of cell motion. Automatic cell tracking is a multi-target tracking problem and has many additional challenges compared to general target detection. First, the signal-to-noise ratio (SNR) of images is usually lower due to sampling conditions. In addition, from the perspective of topology, cells in the image tend to overlap closely and look similar, making it difficult to be detected. Finally, cells may suddenly disappear during the process of tracking, due to cell division and other reasons, leading to a more complex tracking problem.

In our project, we carried out cell detection as well as mitosis detection via the methods of image pre-processing, feature extraction, target detection, target tracking, and motion detection of the target images. Finally, we analyzed the different parameters of cell motion.

During the detection process, we drew bounding boxes for all identified cells and made special marked bounding boxes for mitotic cells. During the tracking process, the movement of each cell was traced and drawn on the image. Finally, we analyzed the cell speed at the time point, total distance traveled, net distance, and confinement ratio of the cell motion.

The rest of this article is organized as follows. Section II briefly introduces the related work in the literature of cell biology and computer vision. The proposed methodology is described in section III. Section IV contains experimental setup and section V discusses the results of experiments. The last section summarizes our project.

2 Literature Review

Due to the development of the ability to visualize cells and subcellular dynamic processes in the past few decades, researchers have made considerable research effort on cell tracking. Based on the topic of our project, we found some related work which provides different methods of the problem. Relevant work can be found in [1], [2], and [3].

In the first paper, the author presents a method for cell tracking in time-lapse microscopy by combing motion and topological features [1]. The author proposed several challenges facing the automation of cell tracking, which result from different image acquisition techniques, complex cellular topology, and uneven motion of the cell [4]. This paper provides a method combing motion and topological features to solve these challenges. This method can be divided into three parts: detection, tracking, and recovery of trajectory. The detection part uses the h-maxima transformation for nuclei segmentation and morphological top-hat filter for illumination correction. In the tracking part, this paper collects motion parameters including skewness, displacement, color compatibility, area overlap, and deformation. The trajectory part introduced a template-matching-based tracking method to recover a broken trajectory. The evaluation and experiment data indicate that this method is robust and accurate. With high accuracy and stable performance, this method can be an appropriate solution for cell detection and tracking problem.

The second paper provides a method that combines marker-controlled watershed segmentation and convolutional neural network to analyze images of densely clustered cells [2]. The author trained two CNNs while the first one predicts cell markers and the second one predicts the image foreground. Moreover, researchers conduct a process to transform CNN prediction into a watershed marker function and segmentation function. Based on the test result, this method had stable performance and reached the top scores both in cell segmentation and detection. Due to the limitation of giving dataset, we didn't use CNN to solve our problem, but this paper indeed provides us another solution to deal with the issue.

The third paper presents a method using a deformable template approach to segment cells [3]. This method contains three stages. The first stage is to collect evidence about the location of cells on the image. The second one is to calculate an elliptical approximation of the location by using evidence from the first stage. The third stage is to improve cell boundaries by using deforming models. This method can be used under severe noise conditions, which inspired us on how to deal with the noise in our dataset.

3 Methods

3.1 Image pre-processing and segmentation

There are different image datasets given in the project. And it is obvious that different groups of images share different shapes, sizes, and intensities. So given different

features of the datasets, different image pre-processing methods and segmentation methods have been applied in the experiment.

3.1.1 Fluo-N2DL-HeLa

The most important feature of images in the Fluo-N2DL-HeLa group is the low-intensity contrast. The pre-processing method is the mean image normalization which makes the raw image subtract the mean intensity of it and then divides the standard deviation. In this way, the high-contrast image has been created and it is also the image on which the counters and bounding boxes of the cells would be drawn.

In terms of the method of cell detection, some morphological methods and watershed algorithms have been applied in this dataset. Firstly, the morphological open operation has been used to get rid of the white noise. And then the sure background of the image could be found by the dilate operation on the previous image. Next, in order to find the core area of the cells among those connected cells, the distance transformation was used. After that, the unsure region was found by the subtraction between the background and foreground. And all these three kinds of regions were marked for the watershed algorithm. Finally, the cells were segmented by watershed and the result was passed to the tracking module.

3.1.2 PhC-C2DL-PSC

The features of images in the PhC-C2DL-PSC group are high-intensity cells and high contrast between cells and background which means the threshold can divide the cells and background very well in the images without noise. So there are only two methods applying for image pre-processing and image segmentation. Firstly, the erode and dilate operations have been used to get the background of the image. Then the raw image was used to subtract the background image. Secondly, the contrast stretching and threshold work very well to segment cells in this group of image datasets.

3.2 Tracking Module

As in a biological system, it is very difficult to track all the cells in the image. This is because the cells can move around and interact with a large number of cells within the system. Things can be harder for some cells which can divide very quickly after many times of mitosis. Because many cells could connect to other cells very closely in the limited space. Another challenge is to tell the difference between mitosis and normal cell tracking. In view of the above difficulties, three features from the domain of cellular motion and topology have been used in cell tracking and mitosis detection. From the domain of cellular motion, we chose displacement. From the domain of cellular topology, area ratio and color have been applied.

For cell tracking, what we used is the combination of displacement and area. The feature displacement is defined as the Euclidean distance between the centers of the bounding box u and v where u is the cell bounding box in the t-1 timestamp and v is the one in the t timestamp. The feature area ratio is defined as the ratio of the minimum bounding box area to the maximum area of the 2 cell in the time stamp t and t-1, which is calculated by the expression W1*H1/W2*H2.

In terms of how to track cells in the previous time stamp, for each cell in time stamp t, we traverse all the cells in time stamp t-1 and try to find the one which has the minimum displacement and satisfy the area ratio threshold. And we also have a minimum area threshold, like 60 pixels, to prevent the matching between small cells and some large material in the biological system.

For mitosis detection, we changed the shape of the cell boundary from the bounding box to a circle once the mitosis has been observed and the feature that has been employed is color. It is chosen on the given fact that the cells would become brighter when the mitosis happens. So color is used to monitor the brightness change. The feature color is defined as the intensity of the gray-value image. In this case, if a cell's intensity become significantly larger than the average intensity of all the cells then the cell would be in the mitosis stage.

For the images in PhC-C2DL-PSC dataset, intensity alone cannot detect mitosis successfully due to the low difference in intensity between the cells. So another method was introduced to assist detecting mitosis. When performing cell tracking, if there is another cell that is of similar displacement to the minimum displacement and the 2 cell is of similar area, then the 2 cells should be the result of a mitosis.

3.3 Analyse Cell Motion

In order to get the motion features of cells by double-clicking mouse, the first step is to apply the Tkinter package to create a window and capture the coordinate of the mouse click. After that, the selected cell could be found by traverse the coordinates of bounding boxes center and get the one with the nearest Euclidean distance.

After getting the information of the selected cell, the motion feature can be calculated directly from the hash table of the cells that have been built in the tracking module.

The speed of the chosen cell is the answer to 3-1 which is defined as pixels/frame. So, the speed can de directly computed as c['distance']/c['frame'] where c is the hash table of the selected cell and c['distance'] is the sum of the distance of adjacent frames and c['frame'] is the total frames from the first time point to current time point.

It is clear from the previous paragraph that c['distance'] is the answer to 3-2 which is the sum of the distance of adjacent frames from the first time point to the current time point.

The net distance is the answer to 3-3 which is defined as the Euclidean distance between the coordinates in the first time point and the current time point. The coordinate of the current time point comes from the nearest cell center of mouse-click and coordinate in the first time point is recorded in the hash table and is passed frame by frame in the tracking module.

Finally, the answer to 3-4 which is the Confinement ratio is the ratio between total distance(answer to 3-2) and net distance(answer to 3-3).

4 Experimental Setup

4.1 Experimental Setup

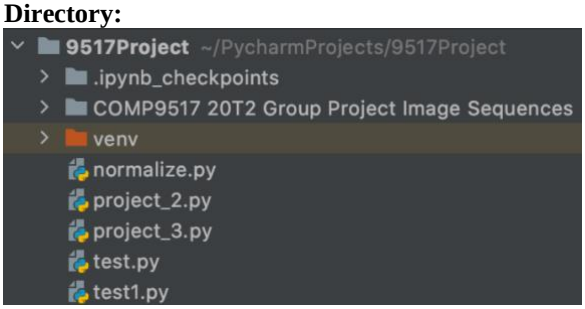
Dataset: DIC-C2DH-HeLa, Fluo-N2DL-HeLa, and PhC-C2DL-PSC

Running equipment: MacBook Pro with 2.4 GHz Intel Core i5 and with 16 GB RAM

Environment: Python 3.8

Python package: OpenCV 4.2.0, NumPy 1.18.1, Tkinter and PIL

Directory:



4.2 Evaluation Methods

The main task for this project requires to segment the cells, track their trajectory and detect if they are dividing so 2 metrics have been chosen to evaluate our method, cell segmentation and mitosis detection. Since there is no ground truth for either of those tasks, we need to manually count the number of cells in an image and check if they are dividing. However, there are too many images and the project need to be completed in limited time with a limited resource so not all the images can be examined. So we choose only a subset of the images and do the evaluation on that subset. We also record the average processing time as it would be a decisive factor when the sequence length grows larger.

For cell segmentation, we randomly select 120 images from the Fluo-N2DL-HeLa cell sequences and 60 images from PhC-C2DL-PSC cell sequences and use them as the testing set. We test the cell segmentation part of our model based on the output of the cells' number and manually check the bounding box. We count the number of real cells, the number of cells that haven't been detected, and the number of misclassification. Based on those numbers, we compute precision and recall.

For mitosis detection, we randomly select 150 images and count the number of real mitosis and the number of mitosis that haven't been detected and compute precision and recall based on our detection.

5 Results and Discussion

5.1 Datasets

Our project is conducted on three datasets, respectively DIC-C2DH-Hela, Fluo-N2DL-Hela, and PhC-C2DL-PSC, which is taken from the international Cell Tracking Challenge (CTC).

These three datasets are different from each other. As for the first dataset, there are 84 frames in the first two sequences and 115 frames in the last two sequences. Each frame is represented by a grayscale image of size 512 x 512. The characteristic of this dataset is that the size of the cell is relatively big, and the contrast of each frame is not good. In addition, most cells in this dataset are transparent and we can see there are many particles or small substances existing in the cytoplasm, which increase the complexity of cell segmentation and cell detection. As for the second dataset, there are totally four sequences in this dataset and 92 frames of size 1100 x 700 in each sequence. The characteristic of this dataset is of low contrast. As for the third dataset, there are 426 frames in the first two sequences and 300 frames in the last two frames. The size of each frame is 720 x 576. The number of cells in each frame is relatively large and the number of cell divisions is large.

5.2 Preprocessing Results

In the whole project, our group does a good performance on last two datasets which are Fluo-N2DL-Hela and PhC-C2DL-PSC and almost complete all the tasks in the project specification, but for the DIC-C2DH-Hela dataset, we still have some problems on preprocessing the frame in this dataset, therefore it does not achieve a good performance so that we can do the cell detection and cell tracking.

The preprocessing results of the datasets are shown below in the following images. We will show our results according to the different datasets. For the Fluo-N2DL-Hela dataset, the image (a) below is the original image, we can see the contrast of this original image is really low, it is hard to distinguish the cells from the background. The image (b) below is the image after normalization, we can see the contrast of this image has been improved but the intensity of some cells is still low and hard to detect. Therefore, we use an image threshold method and watershed algorithm to deal with the image (b) and get the image (c). The image (d) is the enlarged image of the image (c), we can see image (c) has a good performance on the cell segmentation.

(a) (b) (c)

(d)

For the Phc-C2DL-PSC dataset, the image (e) is the original image, we can see the intensity of the cell is high, but it still exists some shading artifacts. Then we use top-hat filter and contrast stretching to get the image (f) which has good contrast. The image (g) below is the thresholding image of the image (f), we can see the quality of this image is relatively good, but it still exists some cells which are not segmented.

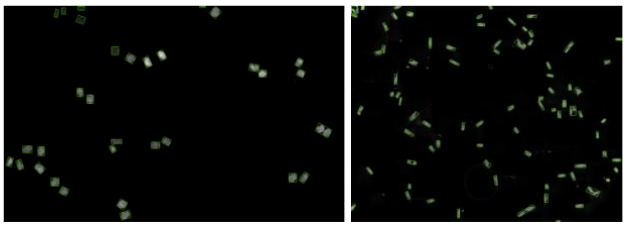
(e) (f) (g)

(h)

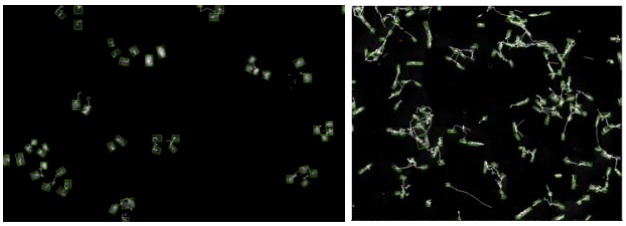
5.3 Task 1 Results

There are totally three parts in task 1 and we will show our results one by one through pictures and figures. As we can see, there are some pictures below showing the results of task 1.

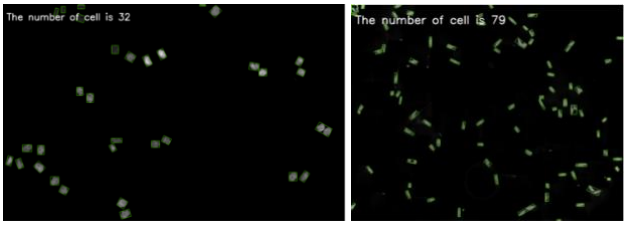
In the first part picture, we can see our method can detect all the cells and draw a bounding box successfully but for some under-segmented cells, some errors may occur on drawing a bounding box.



In the second part picture, we can see our method can draw a trajectory for each cell. The main challenge for this part is the recovery of a trajectory, for some cells with the high speed, our method is hard to draw a complete trajectory for this cell.



In the third part picture, we can see our method can print the real-time count of the cells detected in each image of the sequence on the top left side of the picture and the accuracy of the printing depends on the accuracy of drawing the correct bounding box.



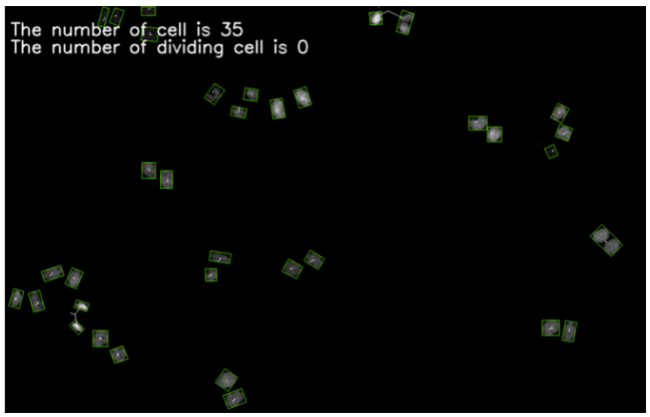
We can see in Table I below the precision of the dataset Fluo-N2DL-HeLa for cell detection is about 95.4% and the recall is about 97.9%. The precision of the dataset PhC-C2DL-PSC for cell detection is about 92.0% and the recall is about 90.6%. The average precision of these two datasets is 92.3% and the average recall is 94.1%. The overall performance of the cell detection and segmentation part is good, which has relatively high precision and recall.

TABLE I. RESULTS OF CELL DETECTION

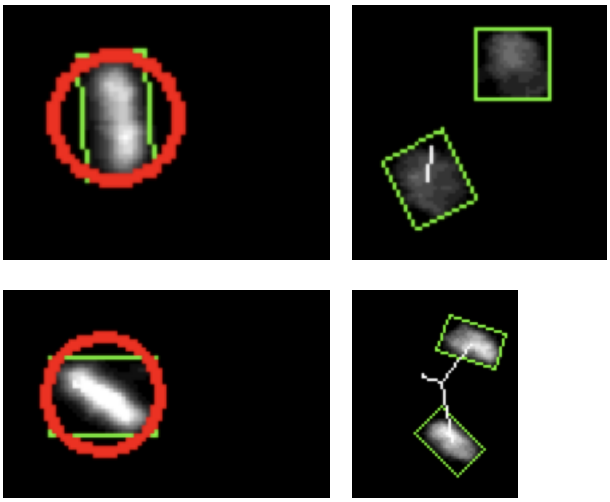
Dataset	Fluo-N2DL-HeLa	PhC-C2DL-PSC	Average
Precision	95.4%	92.0%	92.3%
Recall	97.9%	90.6%	94.1%

5.4 Task 2 Results

In task 2, there are totally two parts that need us to detect the cell division and print the real-time count of the cells that are dividing at each time point. As the picture is shown below, these three frames are consecutive. In the first frame, we can see our method can detect the cell which is in the process of dividing. After detecting this cell, our method can draw a red bounding box whose shape is a circle. In addition, we can also see our method will print the number of dividing cells on the top left of the image window. In the second frame, we can see our method will continue to track the two daughter cells as new cells after cell division. In the third frame, since there is no cell in the process of the dividing, therefore, the number of the dividing cell is 0, which is printed on the top left of the image window.



The pictures below are the detail image for the mitosis detection, we can see our method can successfully detect the mitosis of the cell and draw the red circle when the cell is in the process of the division



According to Table II below, we can see the precision of the dataset Fluo-N2DL-HeLa for mitosis detection is about 60.8% and the recall is about 84.9%. The precision of the dataset PhC-C2DL-PSC for mitosis detection is about 63.9% and the recall is about 47.1%. The average precision of these two datasets for mitosis detection is 62.0% and the average recall is 68.2%. The performance of the dataset PhC-C2DL-PSC for mitosis detections is not good, maybe in the future, we will add some features to increase the accuracy.

TABLE II. RESULTS OF MITOSIS DETECTION

Dataset	Fluo-N2DL-HeLa	PhC-C2DL-PSC	Average
Precision	60.8%	63.9%	62.0%
Recall	84.9%	47.1%	68.2%

5.5 Task 3 Results

In task 3, our method is to use a double click to select a cell. After selecting a cell, on the right-hand side of the window, our method can show the coordinates of the selecting cell, the speed of the selecting cell, total distance, net distance and confinement ratio in turn. In the picture below, the selecting cell is selected by the red rectangle bounding box, and the information of this selecting cell is printed on the right-hand side of the image window. The information of the selecting cell will do the real-time updates until we re-select a new cell.

5.6 Discussion

According to the results above, we can see that our method can almost complete the tasks in this project and has a relatively good performance and relatively high accuracy on cell detection, cell tracking, and detecting cell division for the last two datasets. A good image preprocessing, cell segmentation, and the choice of different parameters are basics of the cell detection and tracking, therefore, sometimes some errors may occur due to the under segmented cells and the choice of parameters. For the first datasets, we do not show our results in the results part because we do not find a better method to preprocess the image and the performance of the cell detection and tracking is not good for the first dataset. Maybe in the future work, we will try to use deep learning method to deal with the first dataset.

6 CONCLUSION

In conclusion, our method can be implemented successfully on Fluo-N2DL-Hela and Phc-C2DL-PSC dataset and achieve all the project tasks including drawing bounding boxes for each cell, drawing the path for each cell, doing the mitosis detection and printing the number of cells and dividing cells. In addition, our method can also analyze the cell motions and get information about selecting a cell by double click the cells in the program window. The precision and recall of the cell segmentation and detection part are relatively high. However, we do not do well on mitosis detection for the dataset Phc-C2DL-PSC. That's may because of the high density of the cell in the pre-processed image. Further study can be done to add extra features to mitosis detection since currently we only use 1 or 2 features which is not enough according to our result. Additional features like intensity, shape, texture may increase the precision and further testing should be done to confirm their contribution.

For dataset DIC-C2DH-HeLa, using our method to pre-process the images in this dataset is hard to get a good cell segmentation image for us to track cells and analyze the cell motion. Therefore, maybe in the future work, our group may use the deep-learning method or some models to pre-process the images in this dataset, thus achieving the good segmentation performance.

7 CONTRIBUTION OF GROUP MEMBERS

Anrui Wang: Write code for cell tracking and mitosis detection in Task 1, 2, and write the code for Task 3. Writing the evaluation and conclusion part of the report.

Haoran Zhang: Contribute to the image pre-processing, cell tracking, and cell counting part in Task 1 and Task2. Writing method description.

Zhao Wang: Contribute to the image pre-processing and part of cell tracking, Writing the introduction part of the report.

Shen Yan: Contribute to the image pre-processing in Task1 and mitosis detection in Task 2, Writing literature review in the report.

Rongtao Shen: Write Code for image pre-processing, cell detection and printing the number of cells in Task 1, Writing results and discussion part of the report.

REFERENCES

[1] Dewan, M.A.A., Ahmad, M.O. and Swamy, M.N.S., 2011. Tracking biological cells in time-lapse microscopy: An adaptive technique combining motion and topological features. IEEE transactions on biomedical engineering, 58(6), pp.1637-1647.

[2] Lux, F. and Matula, P., 2020. Cell Segmentation by Combining Marker-Controlled Watershed and Deep Learning. arXiv preprint arXiv:2004.01607.

[3] Garrido, A. and De La Blanca, N.P., 2000. Applying deformable templates for cell image segmentation. Pattern Recognition, 33(5), pp.821-832.

[4] Debeir, O., Van Ham, P., Kiss, R. and Decaestecker, C., 2005. Tracking of migrating cells under phase-contrast video microscopy with combined mean-shift processes. IEEE transactions on medical imaging, 24(6), pp.697-711.

[5] E. Meijering, O. Dzyubachyk, I. Smal, W. A. van Cappellen. Tracking in cell and developmental biology. Seminars in Cell and Developmental Biology, vol. 20, no. 8, pp. 894-902, October 2009.

[6] E. Moen et al. Deep learning for cellular image analysis. Nature Methods, vol. 16, no. 12, pp. 1233-1246, December 2019.

[7] Y. Li et al. Detection and tracking of overlapping cell nuclei for large scale mitosis analyses. BMC Bioinformatics, vol. 17, no. 1, p. 183, April 2016.

[8] E. Meijering et al. Methods for cell and particle tracking. Methods in Enzymology, vol. 504, no. 9, pp. 183-200, February 2012.