Cell Tracking Project Report

I. Introduction

This project consists of three tasks about cell detection and their status/motion analysis as well as three data-sets for experiment.

For the first step, we need to detect and mark all the cells by boundary boxes within the image sequences and figure out the cells' count for each image frame. We also require to draw the past trace for each cell. The second task is to investigate cell division and mark the dividing cells by distinguishing boxes from the ones in task 1. The counts of dividing cells are also required. The final task is to extend the program to analysis the motions of a specified single cell which include its real-time speed, total distance travelled, displacement and confinement ratio. This project is intended to be done in Python programming with our methods.

Three various data-sets are provided while each of them contains four sequences of image frames. In the first data-set (DIC-C2DH-HeLa), cells have the largest size among all the data-sets and most of them stick close together. They also shows a similar gray scale with the background which is full of noise. Although the bodies of cells are complicated, the boundaries of them are relatively clear and potential to be used for segmentation. Notice that before a cell intends to divide, its boundary becomes more obvious in thickness and contrast. The cells change the shape of themselves enormously over time in contrast to their small displacement. The cells in the second data-set (Fluo-N2DL-HeLa) are difficult to find due to the small size of cells and small gray scale difference between cells and background. The gray scale of their body is evenly distributed and when a cell intends to divide, it will become much brighter. The cells look like they prefer to get together to form small clusters all the time and just move within a small area around their group. The images in the last data-set (PhC-C2DL-PSC) illustrate that the cells' intensities differ sufficiently from the background whose gray scale is not uniformly distributed. The size of the cells is the smallest one among the data-sets. In spite of their slow movement, they are quite active to divide. The whole image is filled with new born cells at last despite there are few cells at the beginning.

II. Literature review

With a steady development of computer vision techniques, a variety of technologies have been applied to image processing and trajectory analysis. According to [1], thresholding, watershed transformation and deformable models make cell segmentation easier for researchers. Thresholding is the most popular technique when segmenting cells because of its simple operation. One of the shortcomings of thresholding is that it tends to make errors when the gray level difference is not obvious. Besides, the watershed transform is also very commonly used, however, it is sensitive to noise and can easily lead to over-segmentation. Therefore, researchers like Zimmer C have paid attention to the results of deformable models in cell segmentation. [2] One of the deformable models called the level-set method which can easily capture topological changes in cell segmentation. Even though many techniques are used in cell association in each time frame of a sequence, we mainly focus on consecutive frames.

The performance of deep learning in analyzing biological images has made great progress in recent years. Because of its superior performance, deep learning is widely used in segmentation, classification and target detection. In terms of segmentation, deep learning algorithms are less error-prone than classical segmentation algorithms and also demonstrate a higher accuracy rate in identifying objects. [3] In deep learning field, there two widely used segmentation methods, which are U-Net and DeepCell. Both methods can identify cell boundaries to separate merging and splitting cells and deal with lost cells with high accuracy. DeepCell is one of CNN-based strategies and contains seven convolutional layers with ReLu activation function and batch normalization. Another CNN-based strategy is U-Net which contains two structures: encoder and decoder. The encoder is used for down-sampling and the decoder is used for up-sampling, forming a u-shaped structure. U-Net can perform not only effective but also accurate segmentation when dealing with small cell datasets and adapt to different application scenarios. However, the deep learning algorithm has some shortcomings. These algorithms need enough dataset for training and ground-truth corresponding to the original image. Excessive performance on the training data leads to another problem called overfitting. In addition, failure to optimize the hyperparameter may cause the entire project to be very time-consuming.

When studying cell tracking, the recent trend has shifted from deterministic approaches to probabilistic model-based approaches, which abandon the idea of matching every particle and every frame. Generally, cell tracking methods can be divided into the contour evolution method and probabilistic model for two or more frames of images to establish the association. Probabilistic approaches can better deal with the uncertainties caused by cells, such as their splitting and merging. In terms of performance, the tracking by the detection method with probability is better than the tracking by the contour evolution method even in cell division events. [4] There are several tools can detect mitosis. According to [5], mitosis events can be addressed by fitting two Gaussian mixture models (one with 2 components and the other with 3 components). In addition, [6] have stated deep learning algorithms can also help detect cell mitosis.

III. METHODS

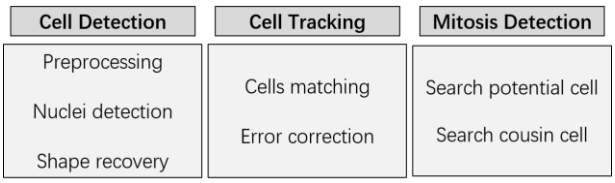


Fig. 1. Structure of proposed method

The proposed method is intuitive and consists of three parts and each of them solves the problem of cell segmentation, cell tracking and mitosis detection, respectively. The process is shown in Fig. 1.

A. Cell Segmentation

In cell detection and tracking, we choose top-hat filter followed by a mathematical tool called h-maxima transformation as well as Gaussian filter to preprocess all the images for Fluo dataset and PhC dataset. Due to the different background light of the images, when using the threshold method to convert the picture, some dark background areas will be lost. Applying top hat transformation can solve this problem by doing illumination correction and remove some small noise. Thus, the top-hat filter not only highlights the brighter areas around the image outline, but also retains more important information. In segmenting cells, nucleus has the maximum value of the region in the cluster because histogram shows that nucleus is brighter than its surrounding. Gaussian filtering followed by h-maxima transformation can detect nucleus without detecting the whole cells, thus, we use nuclei to represent the cells. The purpose of using a Gaussian filter is to achieve a uniform overall intensity distribution and generate a unique local maximum in each cell. In addition, the noise at the top of the peak can be suppressed by it. H-maxima transformation can suppress all intensity lower than or equal to the threshold of h. [7] The original image is reconstructed by dilation of A from A-h. According to the article wrote by Dewan, [8] the h parameter has no serious effect on the final segmentation result.

In the cell segmentation stage, we used a new watershed-based method combined with the previous step. The original watershed's sensitivity to the noise made it error prone. The new watershed method combines with top-hat filtering can solve this problem because this filter is applied to reduce noise before using the original image in watershed. Then dilating the image after noise removal can obtain the background image. Finding the centroid of the cells can not only give the number of cells with higher accuracy, but also can be used as a foreground image when we apply watershed transform without using distance transformation. After watershed segmentation, the cells of image can be segmented with high accuracy in consecutive time frames, even the image with high density. Fig. 2. shows the intermediate images until segmentation. Fig. 2(a) is the image after top-hat filtering and Fig. 2(b) is its binary image. Fig. 2(c) is the nuclei image obtained after h-maxima transformation. Fig. 2(d) gives the profile of each cell through watershed method. Fig. 2(e) is the desired segmentation result.

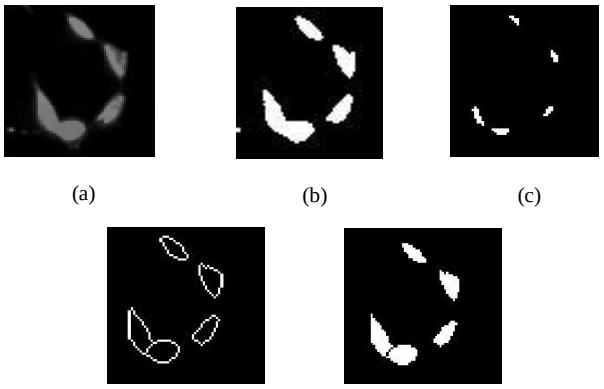


Fig. 2. Process of segmentation. (a) image after top-hat filtering. (b) binary image of (a). (c) nuclei image segmented from (b). (d) the profile image obtained by using watershed method with (b) and (c). (e) the segmented cells with original shape and area.

B. Cell Tracking

Connected Component can show the number of cells, label corresponds to which contour is for the current pixel, centroids correspond to the cell, coordinates, and contour area. Starting from the second frame, each frame of image establishes a distance matrix with the previous frame. Fluo-N2DL-HeLa only uses distance, but we add area feature to improve accuracy for PhC-C2DL-PSC. The Hungarian algorithm [9] is used to find the minimum weighted matching between the current and previous frames. When the moving distance of a cell is greater than a certain range, we regard it as a mismatched point and add it to the list of abnormal points. The trajectory of the two frames of pictures can be obtained by connecting the corresponding points of the two frames. A global variable is used to store the path and cell information in a complete sequence.

C. Mitosis Detection

The methods to detect mitosis are based on the results of matching from last step. They are slightly different for Fluo and PhC datasets on the selection of reference cell and search window. The common assumption here is that those unmatched and mismatched cells in the current frame are more likely to be a daughter cell. Such set of cells is called potential cells (PC1) in both methods for two datasets. Since there is an obvious change in intensity of cells when they tend to divide in Fluo dataset. The cells in current frame which have a significant intensity change compared with their matching cells from last frame are considered as potential daughter cells as well for Fluo dataset, and they are called PC2. The intensity difference is chosen by analyzing all the situations in dataset.

For the method to detect mitosis in Fluo dataset, each cell in PC2 is selected to be the center and build a search window to look for those cells in PC1. As the change of intensity is the most representative feature of mitosis in Fluo. Such feature is assigned the highest priority to filter potential mitosis. The size of the search window is based on the size of the cells and the regular cell spacing to ensure the window could cover the other daughter cell and is not too large to cover irrelevant cells. Within the search window,

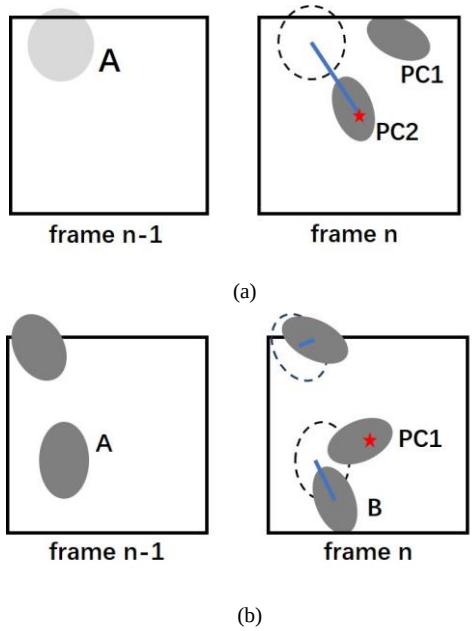


Fig. 3. Mitosis detection within search window. (a) shows the process for Fluo dataset. (b) shows the process for PhC dataset.

whenever finding a cell from PC1, we frame the two daughter cells in red and rewrite the previous frame to mark their mother cell in red as well. Fig. 3(a) illustrates the detection process, cell A in frame n-1 changes its intensity significantly to PC2 cell in frame n. Then within the search window a PC1 cell is found. PC1 cell and PC2 cell in frame n are considered as the daughter cells of cell A in frame n-1. For those cells who has a significant change in intensity but cannot find a cousin within the search window, we treat them as normal cells.

Each cell from PC2 is selected to be the center for the search window and a correctly matching cell is desired to be found as its cousin in PhC dataset. Because of the slow movements of the cells, daughter cells are normally connected or very close right after they are divided from their parent. Also, their short moving distance may verify those short-distance matchings are more likely to be correct. Thus, cells from PC1 are used to filter potential daughter cells at first in PhC dataset. The search window has much smaller size, but it could still contain more than two cells which interrupt the detection of mitosis. As the cousin cells should stay close right after dividing, the nearest correctly matching cell within the search window is chosen to be the cousin of the center one. Fig. 3(b) shows the process, cell A in frame n-1 divides into PC1 and cell B in frame n. Cell B is matched with cell A. The search window for PC1 covers two correctly matching cells and B is the nearest one to PC1. Thus, PC1 and cell B from frame n are the daughter cells of cell A in frame n-1. If there are no correctly matching cells found within search window or there is one but not close to the PC1 cell. The PC1 cell is more likely to be the new incoming one that moves very fast. As the observation, such cells will move out of the frame shortly and seldom has mitosis activity. These cells are normally ignored for mitosis detection.

D. Motion Statistic

In the part of task 3, we designed program that can conduct speed, total distance, net distance and ratio of each cell in every time frame. User can view all these data for each cell at any time in current viewed time frame. Depending on the way that these data can be calculated, we decide to build a dictionary for each type of data and maintain these dictionaries when detecting and tracking cell.

1) Speed: The speed here is in unit pixels/frame, thus, the speed of a cell is the number of pixels it moved between frames. It can be calculated as the distance between the centers of matching cells in consecutive frames.

2) Total distance: Total distance for a cell is the length of its past trace. This can be updated by adding the speed to the total distance of the matching cell from last frame. For the cells first appear, this parameter starts from 0.

3) Net distance: It represents the displacement of the cell from its first occurrence. Thus, we need to record the coordinates of its first occurrence position. The position coordinates can be transferred when matching.

4) Confinement ratio： It is the ratio between the total distance and net distance obtained above.

IV. EXPERIMENTAL SETUP AND EVALUATION

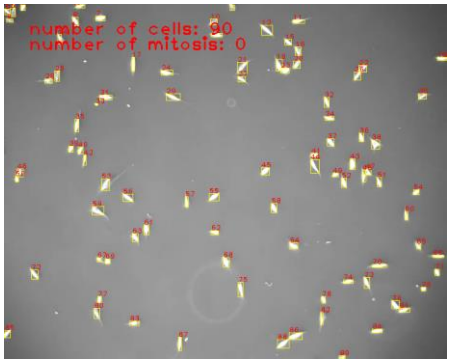
Experiments are conducted with three various datasets of time-lapse microscopy, each of them contains four sequences. We develop program in Python and run on a MacBook Pro with 2.7GHz Intel Core i5 processor and 8G RAM. Since datasets have various illumination conditions and cell types which includes size, shape, and motion habit. We write three separate programs for each of the dataset and share a single UI program to show images and read the cells' motion information.

We provide qualitative evaluation for the full functions for Fluo and PhC datasets as well as the cell segmentation for DIC dataset. Quantitative evaluation for the cell detection and mitosis detection is provided based on 356 results from Fluo and PhC datasets.

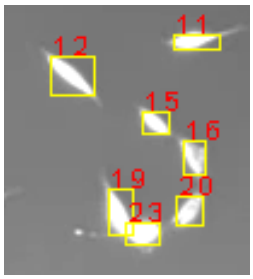
A. Evaluation of Cell Detection

Fig. 4 shows the segmentation result examples of images from Fluo and DIC datasets. We could find all the cells are detected and framed in Fig. 4(a) which is the first frame of a Fluo sequence. Even the images from this sequence has an uneven background intensity and small size of cells. Fig. 4(b) is an enlarged image of a small area of Fig. 4(a). It illustrates that the overlapped cells no.19 and no.23 are segmented correctly. As Fig. 4(c) shows, most cells from DIC sequence can be detected but with some errors.

We count the ground truth of 356 frames from Fluo and PhC datasets and provide quantitative evaluation of cell detection with False Positive (FP), represents for those cells that we have detected but are not correct, True Positive (TP), those cells we have detected and they are correct, and False Negative (FN), those actual cells but we cannot detect. Precision and Recall are calculated from these parameters. Precision is the ratio between TP and the sum of TP and FP. This parameter may tell us the portion of cells that we detected are correct. Recall is the ratio between TP and the sum of TP and FN. This parameter tells us the portion of actual cells that we succeed to detect. Both high values for Precision and Recall may illustrate the strength of a system.



(a)



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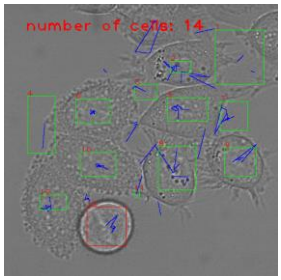


(c)

Fig. 4. Illustration of cell detection through our method. (a) Detection result for Fluo dataset. (b) Enlarged image of a small area from (a). (c) Segmentation result of DIC dataset.

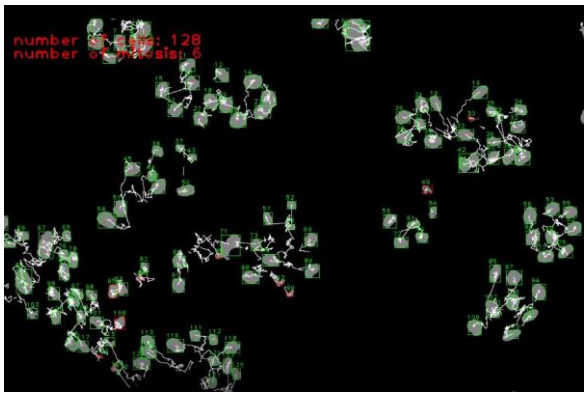


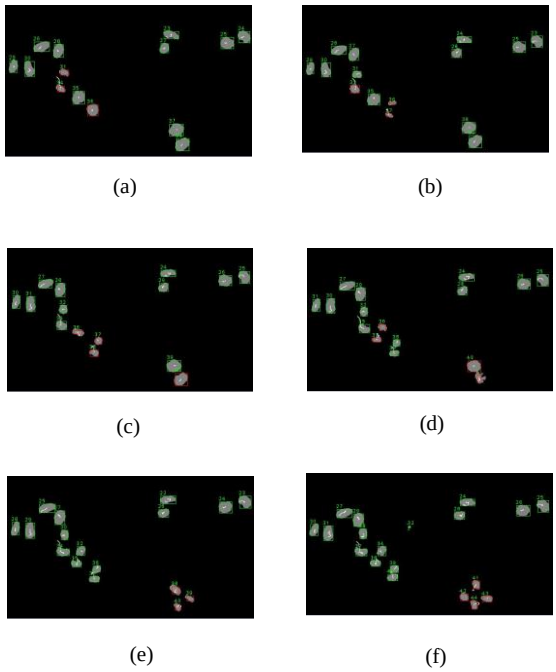
Fig. 5. Illustration of cell tracking

B. *Evaluation of Cell Tracking*

The qualitative evaluation of tracking is through the time-lapse video which shows the past traces of each cell for around 100 frames. Fig. 5. shows the last frame of the sequence so that we could see all the past trace of each cell. Especially for those highly active cells, their continuous trace illustrates the success of detection to their movements. The correct updated motion information for task 3 could also verify the tracking result of our method.

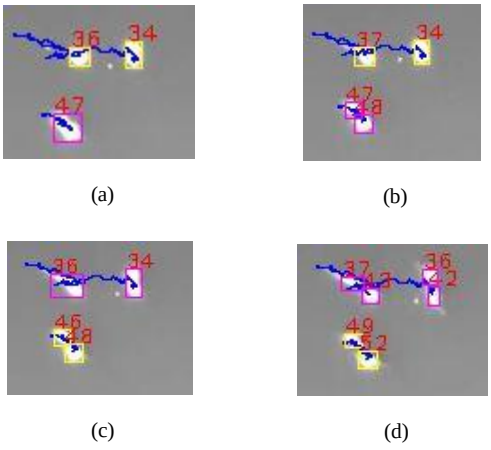
C. *Evaluation of Mitosis Detection*

As mitosis are framed in red bounding box in our method, they can be easily evaluated through our result images. Fig. 6. shows two sequences of mitosis activities from Fluo and PhC datasets, respectively. From Fig. 6(a)-(f), multiple mitoses are detected from their start to end. While the red frames from Fig. 7(a)-(d) illustrate the correctness of our mitosis detection method. The method works even when



(a) (b) (c) (d) (e) (f)

Fig. 6. Illustration of mitosis detection in Fluo dataset. (a) to (b) are six consecutive frame sequences during the time multiple cells divide.



(a) (b) (c) (d)

Fig. 7. Illustration of mitosis detection in PhC dataset. (a) to (d) are four consecutive frame sequences during the time multiple cells divide.

the cells are small and move slowly, as well as they are still connected right after the mitosis. Similar quantitative evaluation for mitosis as that for cell detection will be given in details in the next section.

V. Results and Discussion

The qualitative results are illustrated in the last section, in this section, we will provide detailed statistics to provide quantitative evaluation. Table I illustrates the statistic of cell detection with parameters TP, FP, FN, Precision (P) and Recall (R). Table II gives those parameters of mitosis detection. We use 356 frames in total from Fluo and PhC datasets. Fluo dataset provides sequence 1 and 3, PhC dataset provides sequence 1 and 2.

TABLE I
EVALUATION OF CELL DETECTION

	Seq.	counts	TP	FP	FN	P(%)	R(%)
Fluo dataset	Seq1	92	7797	326	39	95.99	99.50
	Seq3	92	11693	282	19	97.65	99.84
PhC dataset	Seq1	71	6577	571	121	92.1	98.19
	Seq2	101	8595	884	50	90.67	99.42
Average		356	34662	2063	229	94.38	99.34

TABLE II
EVALUATION OF MITOSIS DETECTION

	Seq.	counts	TP	FP	FN	P(%)	R(%)
Fluo dataset	Seq1	92	449	349	6	52.27	98.68
	Seq3	92	397	838	82	32.15	82.88
PhC dataset	Seq1	71	69	49	5	58.47	93.24
	Seq2	101	55	61	21	47.41	72.37
Average		356	970	1297	114	42.79	89.48

It is seen from Table I that our method of cell detection achieves both high values for precision and recall. The average precision is 94.38% while recall is 99.34%. This illustrates the robustness of our method of cell segmentation on different types of cell images. The recall value remains high above 98.19% all the time while the precision for PhC dataset is between 90.67% and 92.1%. Our method over segments some cells if they are complicated in their intensity. Thus, our method may think the uneven part of a cell is another cell. There are plenty of parameters that are set depends on the specified dataset, e.g., the threshold for removal of background, the kernels' size of morphological operations. The choice of these parameters may influence the results a lot.

From Table II, we could see the recall is within a satisfying range with average 89.48% and as the number of cells increases, its value decreases. However, the precision value is very low with an average 42.79% and none of the dataset has a great value for precision. Compared with the normal values of recall. We could find most of the actual mitosis, but our method also treats many normal cells as they tend to divide. The over detection is more severe for Fluo dataset. As we use intensity change as the first-round filtering to detect mitosis but with not enough conditions to remove the false judgements. Many cells indeed change their intensity a lot during a short time even their shape which is also a feature for these cells before their division. But our method keeps them as the mitosis in the results.

Except for the cell segmentation and mitosis detection, the correct past traces of cells are mostly drawn in the result images. We could have a clear view to find that cells like to stay in a cluster for Fluo dataset and cells in PhC dataset prefer to divide rather than move fast. Fig. 8. shows the motion information is correctly record and shown for each cell. Their motion information is reset right after their mitosis and daughter cells are treated as new cells. Fig. 8(a)-(c) illustrate the update of cells motion. Fig. 8(d) and (e) illustrate that the motion information of daughter cells is reset and restart to update.

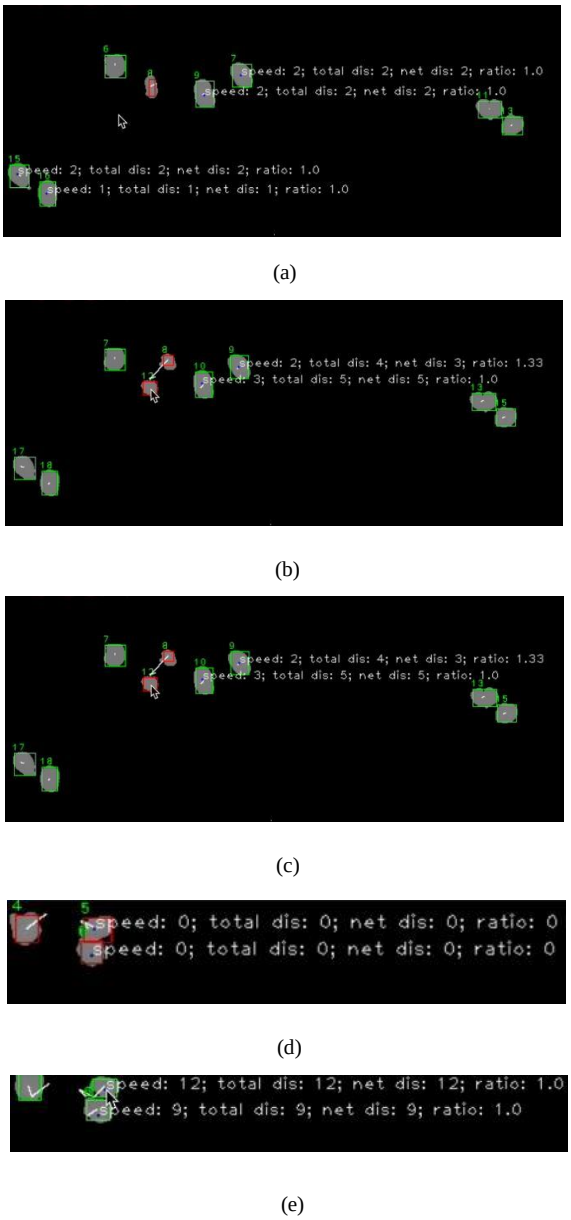


Fig. 8. Illustration of cells' motion information. (a)-(c) shows the update of cells' motion. (d)-(e) illustrate the reset of newborn cells

VI. CONCLUSION

We have proposed a method to implement full functions of all three tasks for Fluo-N2DL-HeLa and PhC-C2DL-PSC cell microscopy sequences, including cell segmentation, cell tracking and mitosis detection. A series of preprocessing is applied before segmentation, starting from a top-hat filtering, h-maxima transformation, and removal of small objects. A watershed technique is used based on the intermediate nuclei image to generate the exact profile of each cell. So that the method could segment and label the cells with their original shape and area even though many of them overlaps. The segmentation result could provide a precise description of cell morphology and a solid base for matching process. Then, Hungarian algorithm is used with parameters of cell distances and areas to find the minimum

weight matching to track cells through frame sequences. Those unmatched and mismatched cells are treated as the source to search their cousin cells and detect mitosis activities. For different image sequences, the choice of cells for first-round filtering of potential daughter cells varies according to their different features. Mitosis events are framed in different color meanwhile the counts of cells and mitosis are printed in the result images. The motion statistic of each cell for any time is recorded and updated according to their movements and mitosis. A UI program is developed for users to read the cell information by simply clicking on the cell that they are interested in.

However, due to the high segmentation error and the small number of occurrence of cells in each frame for DIC dataset, further implementations cannot be extended to it. The profile of some of the cells can be extracted but the noise from background is hard to remove. Normal morphological operations can hardly segment these cells precisely. So, deep learning approach is probably feasible if the ground truth is provided.

Both cell detection and mitosis detection results show a high recall which means we could find most of them and prove the correctness of our method. But the low precision of our mitosis detection results tells us we have treated some normal cells as mitosis as well. That is because our checking condition is not strict enough to correct those mistakes. And the parameters we used for matching are still not enough to give a very high accuracy and complete matching. The error from matching may enlarge the error for mitosis. Thus, more effective parameters for matching and more strict conditions for filtering mitosis are required to be developed in the future work.

VII. CONTRIBUTION OF GROUP MEMBERS

Qingshuai Feng is the team leader. He holds the weekly meeting, design method for detecting mitosis and give presentation at demo.

Haoran Luo (Coder) is responsible for coding the part of analyzing cell motion. The PowerPoint of final presentation is finished by him as well.

Huiya Zhao (Tester) is responsible for testing the code, providing the quantitative evaluation of results and making demo videos.

Jiling Yang (Coder) is responsible for coding the part of detection and tracking cells.

Wenxuan Yan (Tester) is responsible for testing the code, giving feedback, and doing evaluation.

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