Automated Cancer Cell Dynamics Monitoring

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***Abstract*—This paper presents the application for automated biliary cancer cells (M213) segmentation, tracking and cell dynamics monitoring. This kind of application is very helpful for medical technology researches. The biliary cancer cells (M213) are grown up in specific culture dish to observe cell behavior. Researchers require to investigate the behavior of each monitored cell for finding novel methods in biliary cancer treatment. Hence, this application was developed to support the requirement of medical researchers. In this research, the cell segmentation process is firstly performed. Top-hat and bottom-hat filter technique is applied to reduce undesirable noise and also perform image enhancement. Then, the concavity detection, circle fitting and circle candidate techniques are applied to the cluster of cancer cells for automated cell dynamic monitoring. Finally in tracking process, three features of a cell or a cell cluster: displacement, area and deformation are employed for finding adjacency cost matrix between each cell cluster. Then, standard Hungarian method is applied to perform cell matching along the sequence of images.**

***Index Terms*— biliary cancer cells (M213), tracking, cluster, automated cell dynamics monitoring.**

I. Introduction

Group of living biliary cancer cells (M213) are recorded in microscopy video for medical technology research. Researchers monitor them for a long period of time to observe their appearances. They try to develop the methods for monitor properties of cells such as position, size, velocity, and their behavior in each sequence of images. Automated cell segmentation, tracking and analysis is very helpful to describe biological processes of these cancer cells.

Group of monitored cells are both individual cell and cluster of cells. Each cell and each cluster are focused and tracked for monitoring their dynamics and appearances. Manual observation and analysis of these cells is a tedious and time consuming task. Mistakes may occur because the observation is performed on a very long sequence of images [1]. Therefore, the automated monitoring and analysis is developed for supporting this requirement.

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Group of these biliary cancer cells are naturally grown up in specific culture dish to observe their behavior, without using cell staining technique. The color of cancer cells and their food are in original and natural colors. Thus, the proper image acquisition and processing technique together with a controlled condition is required to reduce problem during image segmentation, and also reduce the problem due to poor quality of video recording which may causes multiform illumination and other imaging artifacts

In addition, the multiple touching of cells and huge cells overlapping degree cause a very complicated tracking process. In this paper, we present method for automated segmentation, and tracking group of living biliary cancer cells (M213) including analysis of cluster. Top-hat and bottom-hat filter technique is introduced to decrease multiform illumination and image artifacts problem [2]. Furthermore, topological features tracking such as displacement, area and deformation along with minimum weighted matching are provided to track these cells along the sequence of images [3]. Moreover, analysis of these cells and report of cell dynamics are also displayed on a developed program.

II. System Overview

The proposed method in this application consists of three main steps: detection, tracking and automated cell dynamics monitoring. In detection step, we employed top-hat and bottom-hat filter to decrease processing troubles which caused by poor quality of CCTV camera that record through microscopy above the culture dish. After that, the next step is divided into 2 portions: Processing for a single cell and Processing for a cluster of cells. A cluster can be discriminated from a single cell by using average cell size threshold. Each of single cell's center is found and localized. Then, the concave points are searched around the contour of each cluster to identify the position of each single cell and to estimate the number of single cells in each cluster. In tracking part, we match features these cells then track them along images sequence. Finally, report of cell dynamics is created. In tracking step, features of these cells are brought to the matching process, then track them along the sequence of images. Finally, report of cell dynamics is created.

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III. Method Details

A. Image Pre-Processing

One constraint of the culture system, the color of these cells is similar to their food in the background. In addition, undesirable noise appears due to low sensitivity of CCTV. These cause problem in threshold value selection. We provide top-hat and bottom-hat filter to increase the contrast of the captured image in pre-processing step. This step can be functioned as (1) and (2).

$$I_{top} = I \,\&\sim imopen(I, se) \tag{1}$$

$$I_{bot} = imclose(I, se) \,\&\sim I \tag{2}$$

and, the image enhancement is formulated as (3).

$$I_f = (I_{top} + I) - I_{bot} \tag{3}$$

where I is input image, se is circular structure element with defined radius, *imopen* is morphological opening operation, *imclose* is morphological closing operation and I_f is filtered image.

This image enhancement can raise the contrast between foreground and background before performing global threshold in the image to detect locations of those cells for using in the next step.

B. Cluster Processing

In any image, it has both single cell and cluster. So, we can determine that it is a single cell or cluster by using its size. The object that has number of pixels more than 3000 is a cluster. This rule is retrieved from manual observation of average single cell size. Then, we localize centroid of all single cell in each image. In the part of cell cluster, we want to know the number of cells in each clusters. Unfortunately, most clusters in each image are very complicated due to their overlapping. The problem will occur, when we grant h-maxima transform along with any simple techniques [4]. So, we provide circle processing technique to solve this complexity. Then we can estimate the number of cells in each cluster as well. Fig. 1 is the example of cells in our observation.

(a) (b) (c)

Figure 1. Illustration of example of cell. (a) Single cell. (b) Touching cell. (c) Multi touching cell.

Concavity Detection: The main purpose of concavity detection is to segment cluster into single cell by using concave points [5].

First, we smooth the contour of overlapping cells by polygon approximation algorithm to remove small scale of oscillation along the contour [6]. Then, the corner points (p_c) are identified after polygon approximation process. Both convex and concave points can be located by this technique. In our algorithm, only the concave points are focused and searched by using angle between vectors of each points (p_c) and then find cross product of them. So, the concavity can be calculated as (4) and (5).

$$a_{(p_{pre}, p_c)} = \tan^{-1}((y_{pre} - y) / (x_{pre} - x)) \tag{4}$$

$$a_{(p_{next}, p_c)} = \tan^{-1}((y_{next} - y) / (x_{next} - x)) \tag{5}$$

where, x and y are the coordinates of corner point (p_c). (x_{pre} , y_{pre}) are the coordinates of previous corner point (p_c). (x_{next} , y_{next}) are the coordinates of next corner point (p_c). Using these angles to find concavity as (6).

$$Concavity = \begin{cases} |(C)| & ; \text{if } |(C)| < \pi \\ \pi - |(C)| & ; \text{else} \end{cases} \tag{6}$$

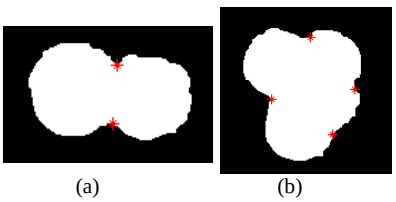
where C is $a_{(p_{pre}, p_c)} - a_{(p_{next}, p_c)}$. We defined constants a_1 and a_2 to indicate that the point (p_c) is a concavity by equation (7). The defined value of a_1 and a_2 come from manual observation along sequence of images.

$$a_1 < Concavity(p_c) < a_2 \tag{7}$$

After the corner points (p_c) and their concavity values are identified, then the cross product of vector is employed to reject a convex point. The point that has positive cross product value is confirmed to be concave point. In addition, the point that has negative cross product value is convex point. The cross product (cp) can be found as (8).

$$cp = |\vec{U}||\vec{V}| \sin\theta \tag{8}$$

where $\vec{U}$ is $\overrightarrow{p_c p_{pre}}$, $\vec{V}$ is $\overrightarrow{p_c p_{next}}$ and θ is angle between vector. We observe only sign of cross product for reject convex point. Some example of concavity detection are displayed in Fig. 2 (a) and (b).



(a) (b)

Figure 2. Illustration of example of concavity detection. (a) One touching cell. (b) Multi touching cell.

Circle Fitting: Almost all of the cells in our data have circle-like shape. So, a circle can be approximated to each cell. After the concave points are located, the circle fitting method is provided to estimate the number of cells and location of all cells in each cluster.

Circle Candidate: After aforementioned processes, a problem that occurred during the research is the number

of computed concave points more than the actual. This causes undesirable circle fitting into the cluster.

So, we provide some constraints to reject these circles. The real circle should satisfy the following two rules:

- Distance between center of circle should not be too small (9).

$$\|c_1 - c_2\| > D_{threshold} \tag{9}$$

- Area of circle should not be too small (10).

$$Area > A_{threshold} \tag{10}$$

Two above rules acquire more accuracy in circle fitting and the number of cells estimation.

C. Tracking

In living biology cell system, a large number of cells have no pattern in moving. Researchers pay attention to their movement and their behaviors. In this application, we provide an adaptive technique combining motion and topological features tracking method to track these cells [3]. This method is an integration between motion and topological feature to get comprehensive and retention in tracking in a long image sequence. We provide displacement, area and deformation features to explain the movement of these cells.

The displacement feature is defined as distance between centers of cell u and v (11).

$$F_{displacement}(u,v) = \frac{\left\|\overrightarrow{c_u^n c_v^{n+1}}\right\|}{\sqrt{H^2+W^2}} \tag{11}$$

where, c_u^n and c_v^{n+1} are the centers of u and v in the frame n and $n+1$ respectively. H and W refer to height and width of image. The displacement of the same cell on two adjacent images must be a small value.

In topology feature section, we use area feature to identify the size of each cell. The area difference of the same cell on two adjacent images must be a small value. The area difference between cell u and v in the frame n and $n+1$ respectively is defined as (12).

$$F_{area}(u,v) = \frac{\left|A_u^n - A_v^{n+1}\right|}{\sqrt{H^2+W^2}} \tag{12}$$

where, A_u^n and A_v^{n+1} are areas of cell u and v in the frame n and $n+1$ respectively. H and W refer to height and width of image.

To compute the deformation, we use eccentricities of ellipses which calculated by $Q=P^2/(4\pi A^2)$, where P and A are perimeter and area of ellipse. This feature is defined by (13).

$$F_{deformation}(u,v) = \frac{\left|Q_u^n - Q_v^{n+1}\right|}{\sqrt{(Q_u^n)^2+(Q_v^{n+1})^2}} \tag{13}$$

where, Q_u^n and Q_v^{n+1} are the eccentricities of u and v in the frame n and $n+1$ respectively. The difference of deformation of the same cell on two adjacent images must be a small value. We use these three parameters to find the cost of matching between cells in consecutive frames. We provide weight bipartite matching to find the total cost using following equation (14).

$$\begin{aligned} F^{n,n+1}(u,v) = {} & \alpha_1 F_{displacement}(u,v) + \\ & \alpha_2 F_{area}(u,v) + \\ & \alpha_3 F_{deformation}(u,v) \end{aligned} \tag{14}$$

$F^{n,n+1}(u,v)$ is the total cost of matching between cell u and v. In addition, α_i is a scalar constant in range of [0 1] and i is the number of feature. The next process is to find the minimum weighted matching. We provide standard Hungarian algorithm [7] to perform matching (15).

$$M^* = \arg\min_m \left\{ \sum_{u,v \in M} F^{n,n+1}(u,v) \right\} \tag{15}$$

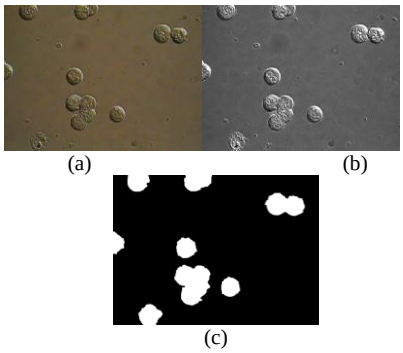
This minimum weighted matching method is used for matching these cells in frame n and $n+1$ along the sequence of images.

IV. Experimental Result

In our application, we get input image data from CCTV camera that record through microscopy that be installed above biliary cancer cells (M213) specific culture dish in Medical Technology research lab at Chiang Mai University, Chiang Mai, Thailand. The video has resolution 640x480 pixels, frame rate 20 fps and 188944 frames. This application is developed by using MATLAB.

A. Evaluation of Image Pre-Processing

Fig. 3 is a result after pre-processing step. Fig. 3 (a) is an example frame of image sequence. Then, we provide top-hat and bottom-hat filter to increase contrast between background and foreground. The result is presented in Fig. 3 (b). The morphological operation is applied to detect foreground object. The result is displayed in Fig. 3 (c).



(a) (b)

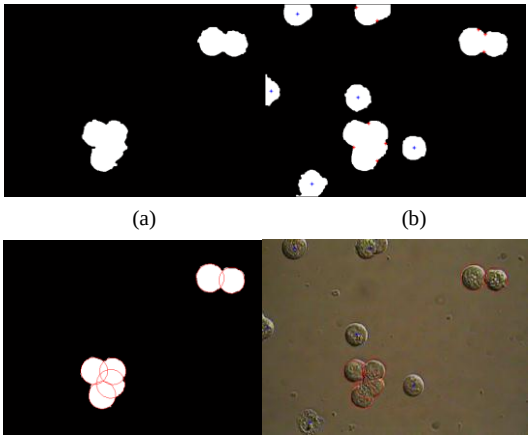
(c)

Figure 3. Illustration of Pre-processing step. (a) A frame in biliary cancer cell sequence. (b) Image after perform top-hat and bottom-hat. (c) Threshold image

B. Evaluation of Cluster Processing

Fig. 4 (a) is a result after cluster threshold. We decide that the object that has number of pixel less than 3000 is a

single cell. Then, the center of each single cell is identified. After that the single cells are separated out and only clusters are processed next. The concave points along the contour of each cluster are located by using proposed methods. The example result is displayed in Fig. 4 (b). The circle fitting is performed after the concave points are identified. The result is indicated in Fig. 4 (c) and (d).



(a) (b)

(c) (d)

Figure 4. Illustration of cluster processing. (a) Binary cluster image (b) Single cell localization and concavity finding in each cluster. (c) Circle fitting in each cluster. (d) Input image combine with our application.

	1	2	3	4	5	6	7	8
1	0.0137	0.5928	0.7318	1.1400	0.6399	0.8580	0.8078	1.3597
2	0.5787	0.0032	0.6242	1.1243	0.3609	0.3910	0.5441	1.0643
3	0.7193	0.6198	0.0035	0.8279	0.3557	0.6189	0.4288	1.0966
4	1.1324	1.1174	0.8198	0.0108	0.7873	0.9745	0.8073	0.7584
5	0.6544	0.3890	0.3265	0.7771	0.0321	0.3504	0.2994	0.8533
6	0.8485	0.3887	0.6161	0.9809	0.3825	8.3623e-04	0.5874	0.7106
7	0.7983	0.5447	0.4198	0.8042	0.2548	0.5755	0.0128	0.8668
8	1.3562	1.0664	1.0988	0.7622	0.8823	0.7167	0.8776	0.0067

(a)

	1	2	3	4	5	6	7	8
1	1	0	0	0	0	0	0	0
2	0	1	0	0	0	0	0	0
3	0	0	1	0	0	0	0	0
4	0	0	0	1	0	0	0	0
5	0	0	0	0	1	0	0	0
6	0	0	0	0	0	1	0	0
7	0	0	0	0	0	0	1	0
8	0	0	0	0	0	0	0	1

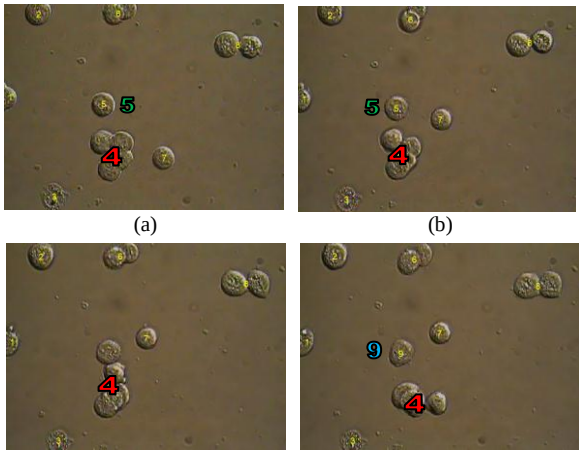
(b)

Figure 5. (a) Illustration of example of adjacency matrix of matching costs. (b) Matrix of matching.

C. Evaluation of Tracking

We label all of these cells in each image and then, we use three features to find total cost of matching. The total cost is returned as adjacency matrix $u \times v$ where, u is the number of object in frame n and v is the number of object in frame n+1. Fig. 5 (a) is an example of adjacency matrix after calculate total cost of features. The next step is to find minimum weighted matching from adjacency matrix between frame n and n+1 by use standard Hungarian algorithm. Fig. 5 (b) is an example of matrix of matching.

Fig. 6 presents tracking of the cells' movement in sequence of frames. We noticed that the number of cells in frame 50,000th decreased from 8 to 7, because cell number 5 moved to attach to cell number 4. Then in frame 100,000th, they moved apart. We noticed that the number of cells increases from 7 back to 8. And, we define it as a new cell, as show in Fig. 6 (a)-(d).



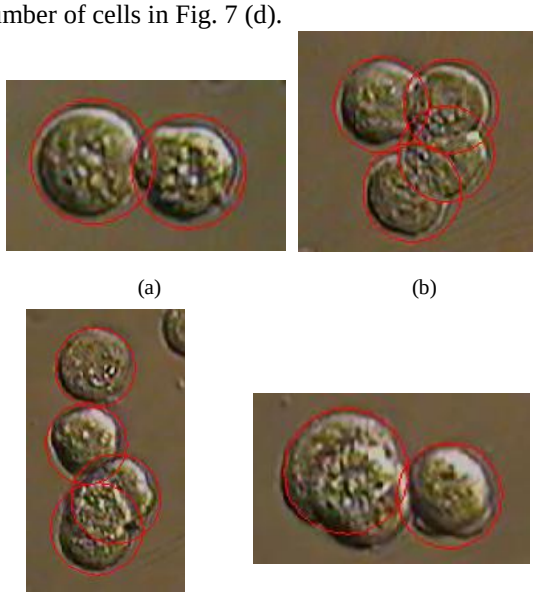
(a) (b)

(c) (d)

Figure 6. Illustration of tracking. (a) First frame in biliary cell (M213) sequence. (b) Tracking of cells in frame 10000th. (c) Tracking of cells in frame 50000th and the detection of aggregation. (d) Tracking of cells in frame 100000th and the detection of secession.

D. Report of Dynamics Evaluation

We created report of cell behavior for all of the captured images. The position, velocity, speed and moving direction of cells are informed to the medical technology researcher. Fig. 7 (a) and (b) are example of good cells' position identification, which will generate the correct report of cell dynamics. However, the mistakes could occur in this step. Some clusters have complicated overlapping as illustrated in Fig. 7 (c)-(d). In Fig. 7 (c), there are five single cells in this cluster, but our method can identify only four single cells. And only two cells was indicated instead of four which is the correct number of cells in Fig. 7 (d).



(a) (b)

(c) (d)

Figure 7. Illustration of identification. (a) Simple touching cell identification (b) Multi-touching cell in first frame. (c) Multi-touching cell in frame 60000th. (d) Multi-touching cell in frame 100000th.

V. Conclusion

We have presented an application of automated segmentation, tracking and cell dynamics monitoring of biliary cancer cells (M213) in a video sequence. Our collected data was supplied from Center of Excellence for Molecular Imaging (CEMI), Department of Radio Logical Technology, Faculty of Associated Medical Sciences, Chiang Mai University, Chiang Mai, Thailand. These cells are grown up in the culture dish and recorded through CCTV camera which connected through microscopy.

In segmentation step, we provide top-hat and bottom-hat filter to increase contrast of the image, to reduce the problem during segmentation which caused by the similar shade of cells and their food and poor quality of CCTV. In tracking step, we provide the combination of topological and motion feature to find cost of matching. Then, the Hungarian method is used to find minimum weight matching. From the experiment, good efficiency in cells tracking is achieved.

In cell dynamics monitoring, we grant polygon approximation technique to approximate contour of the cluster. Then, concave points around the contour are located for using in circle fitting method. Finally in cell dynamics monitoring, the report of dynamics of single cell and cluster such as position, the number of cells in cluster, mobility of single cell in cluster, velocity and movement trajectory is generated. This generated report would be helpful to the specific researcher in biliary cancer cell.

In future, we plan to study more about cell behavior of the focused cells to create more useful report for specific researcher.

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