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Modified watershed transform algorithm for cancer cell segmentation counting

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Abstract

In cancer research, the migration-invasion assay is one of the most important experiments used to evaluate the spreading potential of cancer cells from primary site of tumor to other organs. The process needs to read the number of cells spread to target organs on the pictures taken under microscopic in order to evaluate the performance of clinical trails on various cancer treatments. The number is usually counted, manually, under microscope or using Image J software as an assisting tool. However, the cells are difficult to identify since they are usually overlapped, hence, the process requires experienced researchers. Moreover, the process is time consuming and subjective, which could lead to an inaccurate results.

In this paper, an alternative approach to segment and count the number of cells is presented. The proposed algorithm is based on digital image processing technique, specifically, Watershed transform. The studied pictures are from migration-invasion assay bile duct cancer cell line. The results are compared to the numbers reported by three experienced researchers. The results show that the proposed method can achieve 97.01% average on accuracy. The average time of processing per picture is 4.28 minute.

Keywords: Image segmentation, Watershed transform, Cancer cell

1. Introduction

The determination of migration and invasion ability of cancer cells is one of the important tests used to screen the effect of any substances in term of stimulation or inhibition. Therefore, the accurate measurement of the test is of important and need the precision tools. Nowadays, the manual counting migrated or invaded cells or using an assisting software, for example IMAGE J program [1] to determine cells number in the picture of stained cell taken from microscopic image are used as gold standard in cell number determination. The current manual counting is prone to error as the accuracy of the counting is based on the experience, expertise and judgment of researchers. The difficulty of number determination usually occurs with ambiguous cell image such as the clumping of cells. Thus the method that can determine the number of cells using standard parameter will solve this problem and reduce the bias of researcher in the results.

The crucial step of counting objects in image when they are touching or even clumping is object separation. The correctness of counted number depends on the precision of the segmentation method. However, the segmentation algorithm is difficult and separation success rate depends heavily on the original images [2-9]. One of the most widely segmentation algorithm is Watershed transform technique is based on their shape and character resembling the separation of two peaks by water flooding around and then the marking of peak at the highest point of each object. Before separation of objects by watershed transform, the masking of determined objects is needed and then the area of object that needed to be separated from each other is determined. Many current works used variations of watershed to segment objects. In [4], Tulsani, Gupta, and Kapoor used morphological watershed transformation and regional maxima in order to segment and count for blood cells and platelets. In [5], Hari, Prasad, and Rao proposed an efficient geometrical features and segmentation of distance transformed watershed in order to classily RBC, WBC and Platelets in a microscopic image of a blood stream. Their segmentation algorithm was based on auto threshold, chessboard distance and watershed. In [6], Mouelhi, Sayadi, and Fnaiech segmented breast cancer cell by first obtaining cell region using Chan-Vese energy function based active contour. Then the inner edges were identified using a gradient-weighted distance transform. Finally the segmentations were obtained using their proposed methods in order to prevent the common problem of over-segmentation in watershed. Similarly, in [7], Mouelhi, Sayadi, and Fnaiech used a hybrid segmentation method based on fuzzy active contour model and an enhanced watershed method in order to segment cells in stained breast tissue image. In [8], Khorshed, Yousuf, and Jiang proposed an algorithm based on automated mean in order to segment nuclei of the colonic cancer in a stained image. Finally in [9], Lojk et al. developed a software called Cell Counter for semi-

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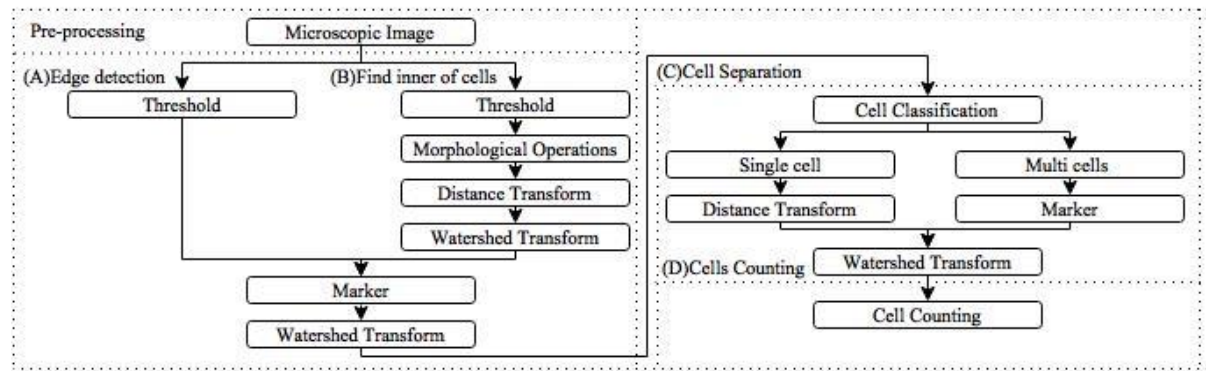
Figure 1 Overview of the proposed method
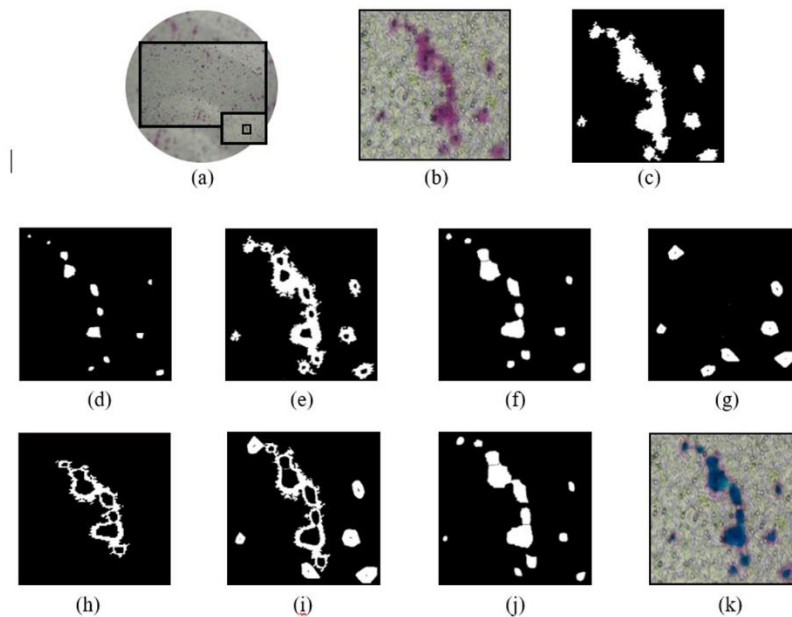
Figure 2 Example images showing the results of each step of the proposed method. (a)Microscopic image, (b) Images zoom, (c) Edge detection, (d) Find the inner cells, (e) Marker, (f) Watershed transform, (g) Single cell and distance transform, (h) Cell group and mask, (i) Multi cell, (j) Watershed transform, (k) Cell counting.

automatic and automatic cell counting of a fluorescent microscopic image. The automatic segmentation process used a watershed algorithm. They also showed an improvement in cell counting combining manual counting with automatic counting.

In this paper we developed an automatic method to determine cell numbers of bile duct cancer cell using segmentation technique based on watershed transform algorithm. The performance is evaluated with the results of cell counting from three blinded experienced researchers in cancer field.

2. Proposed method

Our proposed method of cancer cell counting can be divided into three parts, namely, 1. pre image processing, 2. cell segmentation, and 3. cell counting as shown in Figure 1.

In pre-image processing, several image processing steps were performed including HSV conversion and noise removing. Our technique for segmentation is to initially classify each cell segment into image of single cell or multiple cell of which will be processed with associated methods. The classification step includes with edge detection

and finding inner of cells. The example image after edge detection is shown in Figure 2c. The finding inner of cells step includes threshold, morphological operations, distance transform and watershed transform. The example image after finding inner step is shown in Figure 2d. The images obtained from edge detection and finding inner of cells will be combined by using marker process. The example of image after marker is shown in Figure 2e. Then watershed transform will be applied. Image of multi cells will be separated into single cells while image of single cell with inner cell will be shown correctly as single cell. The maximum and minimum of cells will be obtained. However, single cells without inner cell will disappear in this process.

In separation process, cell size of edge detection image (Figure 2c) will be compared with the obtained maximum and minimum size. If cell size is more than or equal to the maximum size, the cell will be multi cell. Otherwise, the cell will be single cell. And those cells that is smaller than the minimum of single cell, will be considered noise. The disappear of some of the single cells is due to those cells do not have inner cell in step as shown by the single cell on the left of Figure 2c disappears in Figure 2d. The aim of this additional separation is to correct those disappearing cells.

The multi cell will be put in mask from step B to obtain inner image cell shown in Figure 2h. Finally, the process of counting cell in image will be performed. The process based on the continuity of the with boundary line as shown Figure 2k.

3. Results and discussion

A cell is Cholangiocarcinoma (bile duct cancer) cell line, KKU- 213, that was established from Thai patient and obtained from Japanese Collection of Research Bioresources (JCRB) Cell Bank, Osaka, Japan. Each photo was cell counted using the proposed method. Photos of migrated cells were taken under 100X magnification using professional DSLR camera. The obtained photos were in RGB mode showing the stained cells as red color and unstained membrane as white color. The photos were randomly obtained from 5 fields of each membrane and total of 30 fields from 6 membranes were photographed. Each picture's size 10M and picture's resolution was 4272x2848 pixels. The results were compared with the numbers counted by 3 blinded experienced researchers. The counting of migrated cells was achieved using Image J software. The computer spec used was notebook processor i5- 3210m @2. 50GH, RAM 10. 0 GB System type 64- bit, model win7 SP1 Ultimate. Figure 3 and 4 showed that our proposed method can accurately count the cell as 88. 99- 99. 35% when compared to the experts result with the average accuracy of 97. 01%. The average processing time is 4. 28 minutes compare to 20 minutes approximately used by the experts.
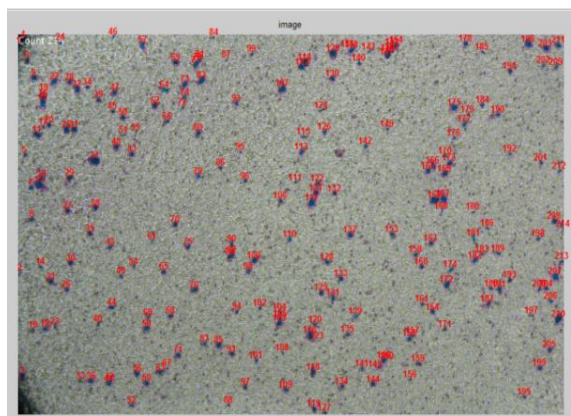
Figure 3 Example of image with the cell counting mark
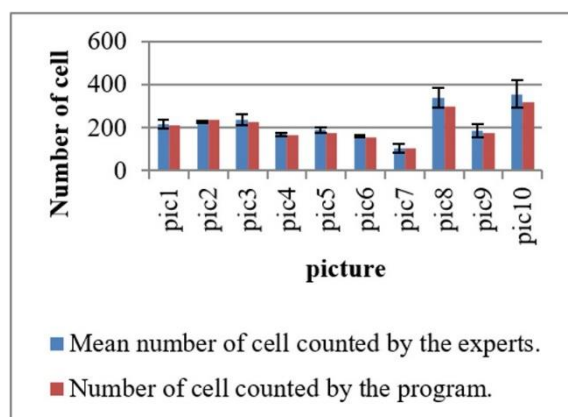
Figure 4 Cell counting results of the experimented images compared with the counting results by the experts

4. Conclusions

We have developed an algorithm based on watershed transform in order to count the number of cancer cells in digital images. The algorithm was evaluated using the microscopic images of migration - invasion assay of bile duct cancer line. The comparison with the results of cell counting from three blinded experienced researchers in corresponding field shows that the algorithm is accurately and runs in acceptable time. This would lead to a development of software that can be used in real experiment in order to aid the Pre- clinical research, so that the study that need cell counting can be completed faster and more efficiently.

5. Acknowledgment

6. References

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