Cell Segmentation Algorithm Using Double Thresholding with Morphology-Based Techniques

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Abstract—**Cell segmentation has attracted increasing interests since it is helpful for medical research and automatic diagnosis. In this paper, we proposed a method that can well segment cells by applying two stages of thresholds, a global one and an adaptive one, with morphology operations to overcome overlapping problems of cells.**

I. Introduction

The problem of cell/nucleus detection and segmentation play a key role in medical image analysis. Cell segmentation is to mark the boundaries of cells and is important for calculating volumes, constructing 3-D models, and cell tracking. However, cell images usually have limited resolution and poor contrast. This may cause the difficulties in splitting two overlapping cells apart while preserving the cells in the low contrast area. In this paper, we try to tackle this problem using double thresholding technique together with morphological operations. With the proposed algorithm, cells can be well extracted even in the darker region and the areas of extracted cells are more uniform.

II. Related Work

The existing algorithms used for cell segmentation apply the techniques of intensity thresholding, feature detection, morphology operation, watershed transform, and deformable models [1-5]. Intensity thresholding is the most efficient approach to this problem since cells usually have higher intensity compared to the background. Although using only the thresholding method does not seem to obtain sophiscated results especially when the goal is to found the boundaries explicitly, it is still attractive because its computation time is very less, especially when the size of the cell image is very large.

Feature detection is usually conducted by filtering the image with specific filters to locate edges, corners, or other patterns, which provides useful information in terms of cell location and boundary. Commonly used filters include Gaussian and Laplacian-of-Gaussian filters. They are suitable for blob detection but may not be able to handle overlapping cells.

Mathematical morphology operations, such as erosion and dilation, are also useful techniques for segmentation. We first choose a structuring element, such as disk or square, and use it as a template to probe the image, in order to examine specific geometrical structures. By properly combining those operations, we can process the image, such as splitting loosely connected regions, filling in holes, or detecting seeds for potential cell positions. Applying pure morphological operations relies on perfect parameter settings and is unable to produce generally good results on different datasets.
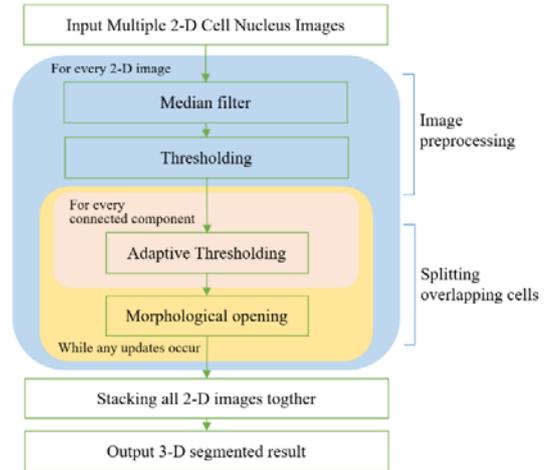

Fig. 1 Flowchart of the proposed algorithm.

The watershed algorithm starts with some given seed points in the image, which can be chosen with combinations of morphological operations, and expand from every seed point to create a region by adding connected pixels. It is powerful in terms of splitting cells, but is subject to over-segmenting problem.

Deformable model requires a predefined energy function composed of information between the image and the contour. By minimizing the energy function, the contour will evolve to object boundaries. The result of this approach highly depends on the energy function. A poorly designed function may lead to incorrect boundaries.

III. Proposed Method

Our method consists of three major steps: image preprocessing, splitting overlapping cells, and stacking 2-D images together. The first step includes noise reduction and thresholding. The second step, we perform adaptive thresholding to each connected component followed by a binary morphology opening. The last step aims to combining every 2-D segmented results into one 3-D segmented image. The flowchart of the proposed method is shown in Fig. 1.

Step 1. Image Pre-processing

We first use a filter to reduce noise on the image. Filter type can be chosen depending on different types of images. Here we choose a n-by-m median filter, where m, n are adjustable parameters. To map original image into a binary matrix that represents possible cell location, we apply a preset global threshold to the grayscale image to find brighter region.
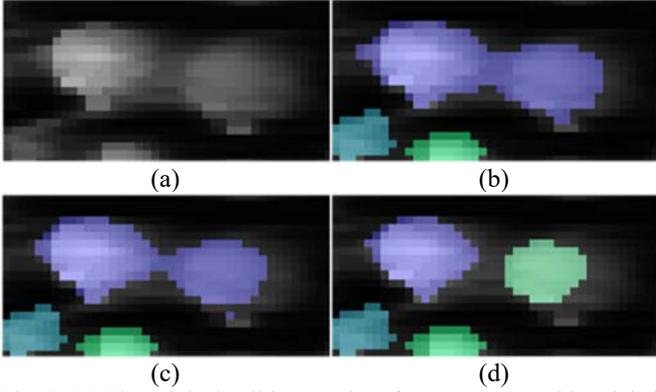
Fig. 2 (a) The original cell image. (b) After pre-processed by global thresholding (first thresholding). (c) After performing adaptive thresholding (second thresholding) for each connective component. (d) After morphology opening.

Step 2. Splitting Overlapping Cells

We then label the connected components in the binary matrix. For each component, we calculate the mean value μ and the standard deviation σ of its intensity, and let

$$\text{Threshold} = \mu - \lambda\sigma \qquad (1)$$

where λ is an adjustable parameter. The pixels with intensity lower than the threshold are set to 0 in the binary map. This step eliminates relatively dark pixels in a component, providing chances to divide two cells when we continue to perform morphological opening. It also prevents missing those cells with poor contrast if λ is properly chosen.

In mathematical morphology, opening is an operation of erosion followed by dilation. Performing opening on the image enables us to divide loosely connected component, as shown in Fig. 2. Here we apply binary morphological opening on the binary map. We recursively run through step 2 until the binary map is not updated anymore. Since this process does not assure convergence, a preset time limit may be needed to prevent infinite loop.

Step 3. Stacking 2-D Images

After every slice of 2-D image is segmented respectively, we can now apply algorithm to connect cells vertically. The algorithm is straightforward. Suppose that we are now trying to connect two layers of 2-D images, and cell A in the upper layer overlaps with cell B in the lower layer. We can set an overlap ratio r, and decide to connect cell A and cell B if

$$\frac{\text{Area}(A \cap B)}{\text{Area}(A)} > r \quad \text{and} \quad \frac{\text{Area}(A \cap B)}{\text{Area}(B)} > r \qquad (2)$$

IV. Experiments

The results of the proposed method compared to four different algorithms are shown in Figs. 3 and 4. It can be seen that, even in the darker region, the cells can be well detected by the proposed method. Moreover, since double thresholding is applied, the cell areas in the darker region are almost the same as those in the bright region, which matches our common sense. Furthermore, the proposed method can successful split most overlapping cells and provides finer cell boundaries.

Moreover, since the proposed algorithm applies the double thresholding mechanism, which is simpler to implement, its computation time is very less.
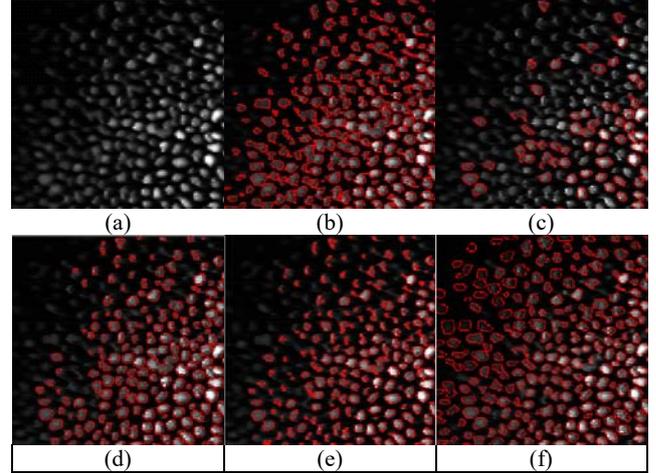

Fig. 3 Cell segmentation results. (a) Input. (b) ATC&BD [3]. (c) CS &HI [4]. (d) MF&MMO [5]. (e)Watershed. (f) Proposed method.
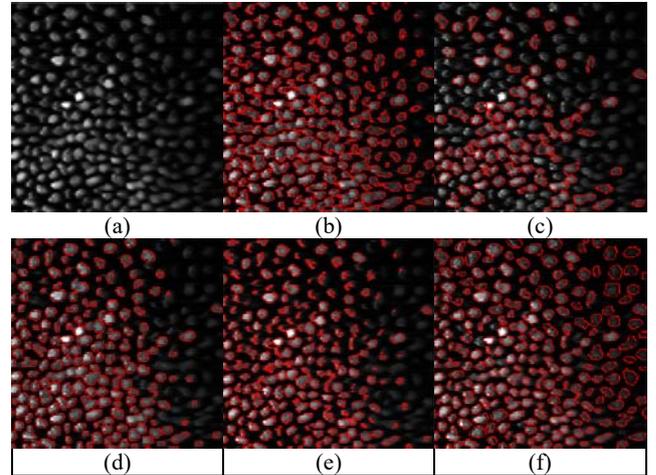

Fig. 4 Cell segmentation results. (a) Input. (b) ATC&BD [3]. (c) CS &HI [4]. (d) MF&MMO [5]. (e)Watershed. (f) Proposed method.

V. Conclusion

The proposed method includes double thresholding together with morphological opening. Both the global threshold and the adaptive threshold are applied so that cells with lower brightness are still be detected. The adaptive threshold is determined by the mean and the standard deviation of each connected component so that overlapping cells either in bright or dark regions can be divided; meanwhile, the boundaries can be refined.

References

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