An Automatic Cell Counting Method for Optical Images

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Abstract---In the research of photodynamic therapy (PDT), we need to know the percent of cells that survive PDT therapy to evaluate the effectiveness of treatment conditions. Manually counting the cell number from digital images is time-consuming and not very objective. In this paper, an automatic cell counting method based on image segmentation technique is introduced and some results have been provided to compare with the human counting results.

Keyword: image processing, cell counting, watershed, and segmentation.

INTRODUCTION

To determine lethality of treatment conditions in photodynamic therapy (PDT), we need to calculate the fraction of surviving cells from digital images. Manually obtaining the number of surviving cells is time-consuming and not very objective. Our objective is to develop and accurate, fast and automatic method for counting the cells from the images.

A typical image of attached cells has the following characteristics that form the basis of our algorithm: 1) Noise and artifacts; 2) Various cell shapes and Cell intensity overlapping; 3) Inner points within a cell with a lower intensity; and 4) Cell object sticking together. Several image segmentation techniques are investigated such as, contour-based, region-based and mixed contour-region-based methods [1], histogram-based and minimum-error-threshold [2], and watershed algorithm [1]. For cell images, none of the above methods can be used directly, so we developed the following algorithm to segment the cell images and count the cell objects.

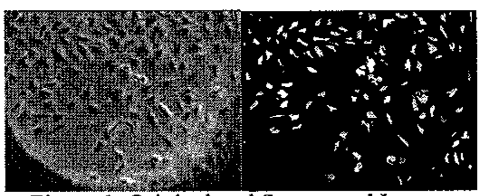

Figure 1. Original and Segmented Images

METHOD

The cell images are 288x384 pixels and 256 grayscale. The process can be decomposed into four main steps.

Step1: Apply the 3×3 spatial adaptive filter [1] to the image to minimize the effect of noise.

Step2: Locate all pixels that have the lowest grayscale level in a 5×5 neighborhood. For each such pixel, determine if the gradient of its grayscale value along every direction (8 directions for all 360°) is also above a specified threshold.

Step 3: Flooding process. Once we have found the potential minima, for each minimum point, we proceed to the progressive flooding of its neighboring points by using a first-in-first-out (FIFO) data structure. First from its innermost neighborhood, gradually extend to far neighborhood. The FIFO initially contains all the pixels in the order of their intensity value, the flooding process stops when the FIFO is empty.

Step 4: Refining the labeled image by post-processing. First we calculate each region's center, if the center is not in this region, the region will be discarded. Second we also eliminate those regions if the total number of points in the regions is beyond a reasonable range of cell size.

RESULTS

In order to test the quality of this method, we have decided to compare the result of the algorithm and those from human counter. All those image files are from the Bioengineering Department, University of Toledo. These images have been counted manually by two different students (KB and AS).

Trial	1	2	3	4	5	6	7	8
HC1	74	67	178	164	113	97	35	194
HC2	76	72	188	162	127	96	34	200
AC	73	78	166	172	117	102	34	184

Table 1. Comparison of counts obtained by three human counters (HC1, HC2) and the automated cell counter (AC) for 8 different cell populations.

On average, the manual method takes 5 minutes to count the cells in one image while the algorithm takes approximately 4 seconds. Comparing, the cell counts finds that our automatic method is very close the counts determined manually (error within ±7%). The strength of this approach comes when up to 10,000 cells need to be counted to determine lethality.

DISCUSSION

The image quality is affected by many factors. The exposure time, the filter, phase contrast, and frequency can affect the final cell image quality and thus affect the accuracy of this method. To refine the final result, mathematical morphology can be used to further enhance the accuracy. In addition, by using fuzzy logic instead of a crisp threshold in the determination of the gradient the result can be even more accurate.

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