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Detection and Counting of Embryonic Stem Cells in Fluorescence Microscopy Images by a Fully Automatic Method

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Abstract: In this paper, an automatic cell counting method under microscopy is proposed. The cell counting process can be performed in two ways: The manual counting in which a specialist counts the cells with naked eye, and the automatic counting that utilizes the computer-based techniques. In manual counting, there are several techniques for dying the cells to turn them visible with naked eye. However, if the concentration is more than normal the cells can overlap. Overlap and incorrect adjusted microscopy parameters are the main factors that cause inaccurate counting results. Furthermore, in manual counting inter-observer variability is high. Even though the same cell image is taken into account by the different specialist, different counting results can be obtained. Because of the above mentioned problems, the cell counting process must be performed automatically.

The proposed automatic stem cell counting process is based on image processing techniques that appropriate the frame of method. At first, stem cell sections were obtained under the fluorescence microscopy. In the following pre-processing step Gaussian filtering and background extraction are performed. Before applying watershed algorithm histogram of the image is partitioned in to four parts and the best combination is determined to obtain the most exact counting results. The aim of using watershed algorithm is to make the boundaries and maximum points of the cells more clear. Finally, spherical contours corresponding to the stem cells are counted.

The effectiveness of the proposed method is evaluated by performing numerous computer simulations. It is shown that the proposed method gives promising results and can eliminate the subjectivity originated from the manual counting. The method is tested on a database contains two image groups at different noise levels validated by the specialists.

Keywords: *cell counting, cell detection, pre-processing, embryonic stem cell, watershed algorithm.*

1. Introduction

In recent years, cell researchers are interested in counting, sorting and tracking the cells. The specialists at clinics and hospitals use cell counting in determining the health condition of a patient. Cell counting is a valuable process used for different investigations. For example, in molecular biology and biochemistry, finding the concentration of cells is important during some experiments. This value is essential for calculating the volume of a particular chemical substance or reagent that should be applied to the concentration of cells in order to obtain reliable results. Cell counting is also used in measuring cell viability. This is the calculation of the number of dead and living cells in a cell culture and useful for determining the life expectancy of a cancerous cell, the effectiveness of insecticides and pesticides or the environmental harmfulness of poisons and toxins.

Stem cells have a special importance in clinical researches and they provide a promising cell source especially for developing new diagnosis and treatment techniques. Stem cells can turn into many different cell types in the body during early life and growth. They can serve as a sort of internal repair system, dividing essentially without limit to replenish other cells as long as the person is still alive. Stem cell research is still one of the most challenging areas in biology.

Embryonic stem cells (ESC) are denominated as “super cells” with their self-renewing elements and properties of generating all endoderm, ectoderm and mesoderm layers. ESC are derived from human pre-implantation embryos. Their differentiation process can be managed by culturing them in non-adherent plates. Counting ESC is an important task that has numerous applications afterwards [1, 2].

To detect early diabetic retinopathy [17]; To determine appropriate lithium dosage [18, 19]; To calculate volumes of brain tissue from magnetic resonance imaging (MRI) [20]; To analyze functional MRI data [21]; to help the physicians to decide fast and efficiently about the dose of the medicine to treat the 200 dialysis patients [22]; To characterize stroke subtypes and coexisting causes of ischemic stroke [23]; To improve decision-making in radiation therapy [24]; To regulate of nicardipine infusion for hypertension control during anesthesia [25]; To determine flexor-tendon repair techniques [26]; To detect breast cancer [27, 28], or prostate cancer [28, 41]; lung cancer [29], To assist the diagnosis of central nervous systems tumors (astrocytic tumors) [30]; To discriminate benign skin lesions from malignant melanomas [31]; To visualize nerve fibers in the human brain [32]; To represent quantitative estimates of drug use [33]; To study the auditory P50 component in schizophrenia [34].

Many other areas of application, to mention a few, are (a) to study fuzzy epidemics [35], (b) to make decisions in nursing [36], (c) to overcome electro acupuncture accommodation [37].

We can observe exponential growth in the number of articles in medicine field comprise fuzzy technology. The preliminary data we have for 2003 and further [38, 11] supports this tendency.

Most of the medical knowledge available to a physician will always be fuzzy. When a person is given a medical examination, a wide variety of parameters, called symptoms in medical language, can be ascertained and measured. Due to the complexity of the human body, it is not possible to give a realistic

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limit for the number of established criteria. Because medical diagnostic investigations are very complex, it would be very difficult to cover this connection to describe this situation using crisp logical operations. When a doctor starts treatment of a patient he uses his personal experience, knowledge from books, and mental ability. The doctor notes the patient's signs and symptoms, combines these with the patient's medical history, physical examination and laboratory findings, and then diagnoses the disease(s) [39, 40].

So, the goal of the fuzzy intelligent system is to imitate behaviour of a doctor and give him consultation. In this paper, we showed some of our applications in different areas of medicine.

2. Pre-Processing Step

In this section, pre-processing step including background extraction and filtering is explained. Because of the distortions in cell images, this step is necessary before counting the cells. For this study, embryoid bodies' images are given by Geisa Martins Faustino. Database contains 92 different cell images. Acquisition of cell images is described in reference [2]. In Figure 1, captured images distorted by low noise and high noise are illustrated as an example.

The necessity of applying pre-processing step arises from the following reasons. At first, it is important to adjust the focus of microscope properly. Wrong adjusted microscopy parameters cause wrong counting results. At this case manual counting with naked eye is becomes impossible. Second, to make cells more visible in microscopy images there are various dying techniques. The concentration level of dying is crucial. For example, if the concentration is high cell overlap occurs. Overlap is one of the main reasons of erroneous counting results. At last, the presence of fluorescence image noise may also cause the inaccurate counting.

In conclusion, the pre-processing step is needed to make cell images more clear and convenient for counting. Now, we describe the procedures, respectively.

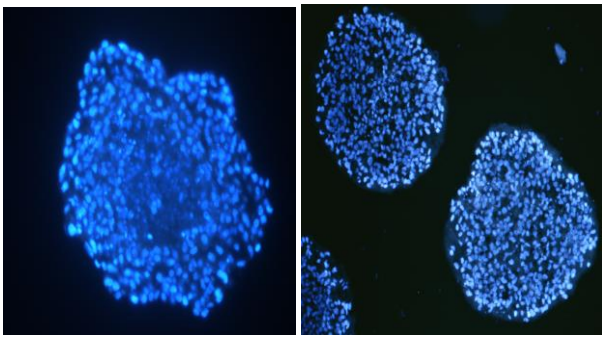

Figure.1. Captured image (a) Low noise example, (b) High noise example

2.1. Filtering

When the cell images are taken with fluorescence microscope, the luminance of pixel in the center of cell is lighter than the boundaries of the cell. By exploiting this property, cell counting with maximum brightness analysis becomes possible. In this study, the Gaussian blur filter is used to smooth the surface and put forward the maximum points of cell images. In Figure 2, surface of a cell image before and after Gaussian filtering is illustrated.

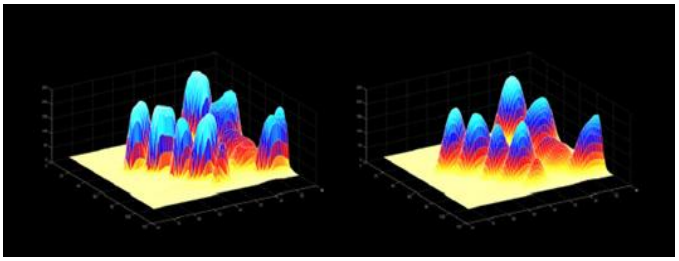

Figure 2. Surface of a cell image (a) before and (b) after Gaussian filtering.

From the figure, we can say that using Gaussian filter makes the maximum points of the image more explicit than normal.

2.2. Background Extraction

Segmentation is one of the influential process in digital image analysis. It is known that, the performance of the segmentation turns the scale of whole counting procedure [14, 15]. Thresholding is a commonly used simple technique to perform background segmentation. In this study, segmentation is implemented with thresholding. Threshold value is determined with mean μ and standard deviation δ of the cell images. We set the threshold value to $t = \mu + x.\delta$ according to the trials [17]. The pixel intensities under t is set to zero for R, G and B channels. The results are given in Figure 3. As clearly seen from the figure, for green channel, overlapping is less than other channels and cell boundaries are more explicit. Pre-processing step for green channel is illustrated in Figure 4. Furthermore, a background segmentation example under low and high-level noise is shown in Figure 5 and 6, respectively.

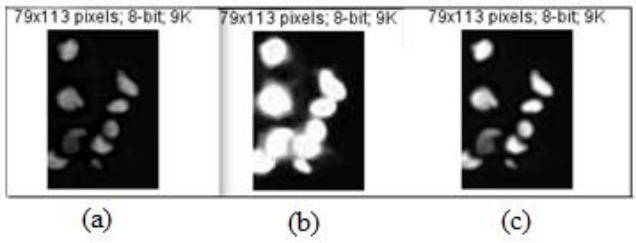

Figure 3. Background segmentation of an input image for a) blue, b) red and c) green channels.

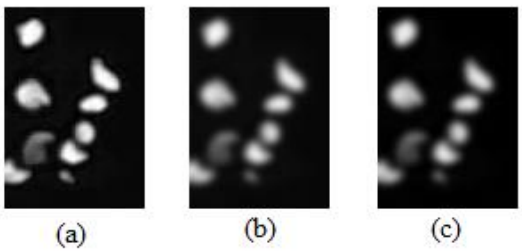

Figure 4. Pre-processing step for green channel: a) Original green channel image b) Gaussian filtered green channel image, and c) result of background extraction.

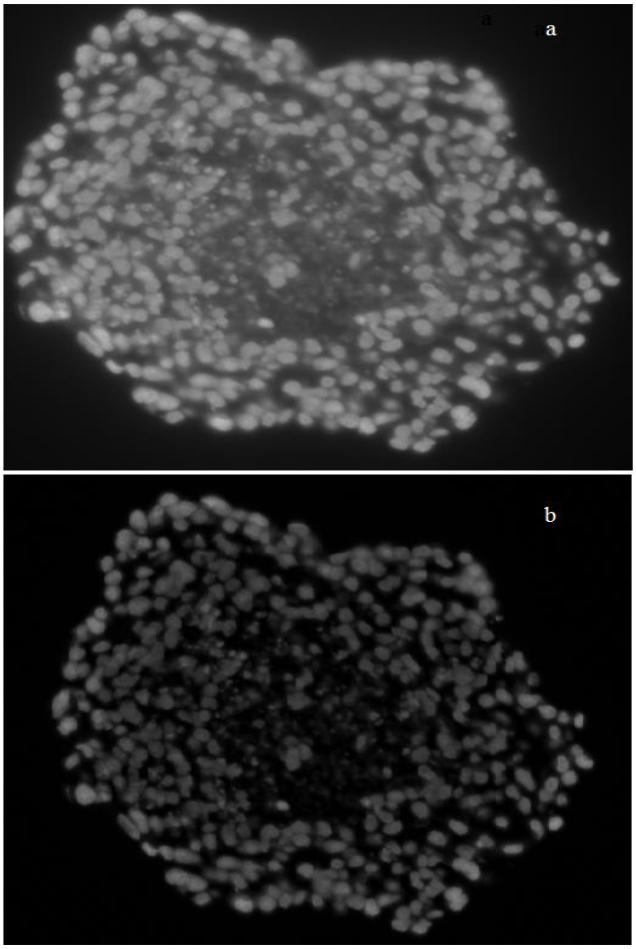

Figure 5. a) Original image fluorescence image contaminated with low-level noise b) Image after background extraction.

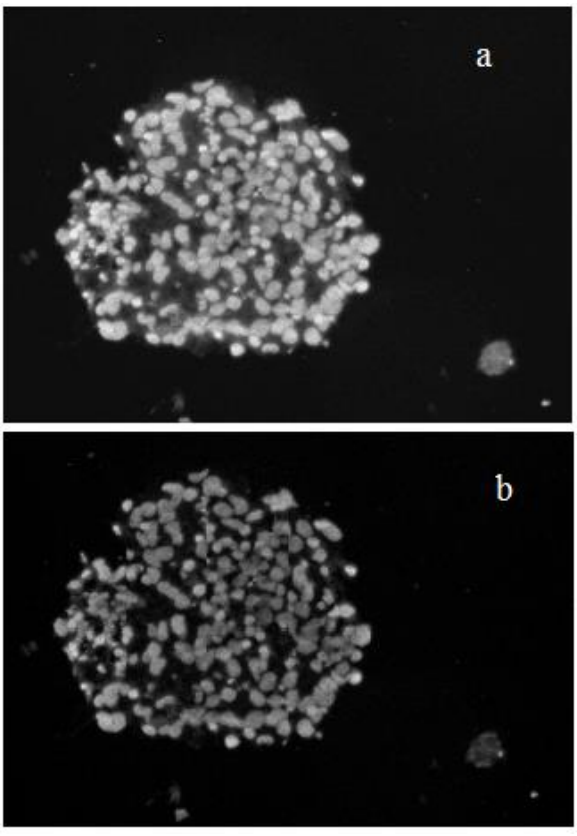

Figure 6. a) Original image fluorescence image contaminated with high-level noise b) Image after background extraction.

3. Watershed Algorithm for Counting

3.1. Applications for Determination of Disease Risk

Before using watershed algorithm, histogram partitioning is applied to the cell images. The aim of histogram partitioning is to identify connected cells. At first, we obtain the histogram of pre-processed cell image. As discussed before, it is calculated for green channel. The histogram of the green channel is split into four equal interval of size 64. Because of the luminance difference between the center and edges of the cells, histogram partitioning provides useful information for counting. The partitions of green channel histogram are demonstrated in Figure 7. Then, each interval is checked from top-to-bottom and left-to-right two times and detected connected components are labelled. The components that have similar luminance take higher labels [16, 17]. In the second study structure of FES is mix (hybrid). Fuzzy part of the system is as follows: Input values are age, cholesterol and blood pressure. Output value is risk. When the fuzzy part was constituted, patient's gender and being smoker were considered and four different groups have been formed regarding to these two criteria. These groups are; man-no smoking, man-smoking, women-no smoking and women-smoking. Thus, for each group, 36 fuzzy rules were generated [42].

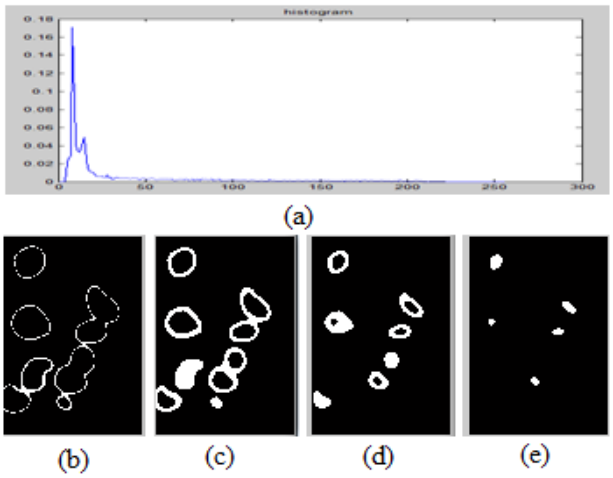

Figure 7. Histogram partitioning: a) Histogram of Figure 4.c. b) [0, 63], c) [64,127] d) [128,191], and e) [192,255] are the bitmaps representation of intervals.

Now, we can apply watershed algorithm which is commonly used algorithm for segmentation of blood cells. In watershed algorithm, a monochrome image is considered to be an altitude surface in which high-amplitude pixels correspond to ridge points and low-amplitude pixels correspond to valley points. If we flood this surface from its minima and if we prevent the merging of the water, we can split the image into two sets referred as catchment and watershed [18]. As required, watershed algorithm is always gives closed contours and has low computation cost in comparison with other segmentation methods. The main drawback of this algorithm is over-segmentation. In this study, the over- segmentation problem is overcome by applying pre-processing step and subtracting the first partition of the histogram before watershed algorithm. In other words, for our algorithm we use the sum of second, third and fourth parts of the histogram to detect all cell boundaries. In Figure 8, pre-processed ESC image and watershed applied ESC image are illustrated. As can be seen from the figure, by performing watershed algorithm overlapped regions are separated from each other. Through, more accurate counting results will be obtained.

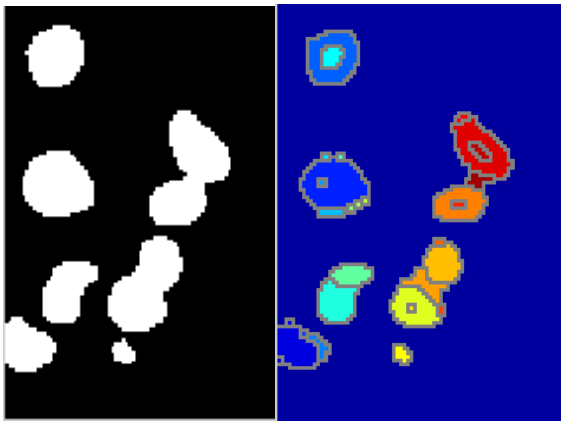

Figure 8. a) Pre-processed input image, b)watershed applied input image.

4. Experimental Results

In this section, we give and discuss the simulation results performed on the database obtained by communicating with the author Geisa Martins Faustino as given in reference [2]. In the database there are two groups containing 69 and 23 ESC images, respectively. For the second group the noise level is stronger than that of the first group. Gaussian radius values for Group 1 and 2 are 2 and 3, respectively. To determine the threshold value as given in Section II, x is chosen as 0.3, μ is the mean value and δ is the standard deviation of green channel input image.

In Figure 9 and 10, watershed applied low-noise (from Group 1) and high-noise (from Group 2) ESC images are illustrated. As it is clear from the figures, counting with naked eye will be exhausting and time consuming. Furthermore, counting results will not be reliable in these cases. Thus, the proposed automatic counting algorithm implemented for different images which are chosen randomly from Groups 1 and 2. Experimental results are given in Table 1 in comparison with the author's before work given in [17] and with reference [2]. Because of the lack of fluorescence microscopy and experts, we are not able to measure the accuracy of the proposed method for all practical cases. However, to show the good performance of our method we compare our results with accepted studies.

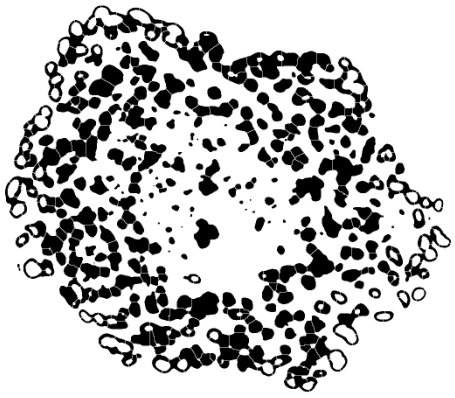

Figure 9. Watershed algorithm is applied to the low noise image.

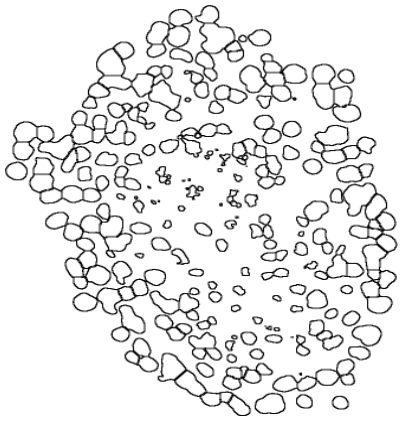

Figure 10. Watershed algorithm is applied to the high noise image.

Table 1. The counting results of the proposed method in comparison with reference [2] and [17].

Image No	Counting results in ref. [2]	Counting results in ref. [17]	Proposed method
1	509	615	620
12	559	659	665
16	404	520	525
36	536	694	699
46	551	692	698
66	330	407	408
72	1224	1629	1631
71	255	231	230
65	273	256	255
96	563	562	558

5. Conclusions

In this study, an automatic ESC counting method based on watershed algorithm is proposed. The main steps of the proposed method are explained in the corresponding sections of the study. In the first step that referred as pre-processing step, Gaussian filtering and background extraction are applied to the ESC images. Then, before performing watershed algorithm image histogram is partitioned into four parts and the best combination is determined for watershed algorithm to obtain the reliable counting results. By means of these processes, the over-segmentation problem of the watershed algorithm is also eliminated. At last, spherical contours corresponding to the stem cells are counted. Experimental results are given in comparison with the author's before study and with one of the accepted popular paper given in reference [2]. According to the results, proposed algorithm provides consistent and promising results for automatic cell counting task. Thus, the proposed method can be used for different blood cell counting processes to provide useful result for specialist. In future, this issue will retain its popularity.

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