

# An Automatic Embryonic Stem Cell Counting Method

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### Abstract

Embryonic stem cells are undifferentiated cells that are able to differentiate into all derivatives of the three germ layers called as ectoderm, endoderm and mesoderm. They can develop into the more than 200 cell types of the adult body. Tracking the proliferation of embryonic stem cells is a tedious task which often is undertaken manually. So, for this purpose automatic counting methods are strongly desirable.

In this study, we propose automatic embryonic stem cell detection and a counting method. Stem cell sections were obtained under the fluorescence microscopy. In the pre-processing stage color image is split into channels. Then a Gaussian filter is applied to green channel of the cell image to eliminate the noise and highlight the maximum points of the cell. Furthermore background segmentation is applied by thresholding. Histogram partitioning is performed to detect the connected component of the pre-processed cell image. Finally maximum point analysis is investigated to count the cells automatically. The effectiveness of the proposed method is evaluated by using Matlab. 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 of 92 images that were validated by the specialists.

Key words: stem cell, embryonic stem cell, image processing, cell counting, cell detection,

### **1.Introduction**

Stem cells (mesencymal stem cells, cancer stem cells, embryonic stem cells etc.) are very important in clinical researchers, provides a promising cell source especially for developing new diagnosis and treatment techniques Embryonic stem cells (ESC) have a huge potential in field of tissue engineering and regenerative medicine which are able to differentiate into all derivatives of the germ layers and also specialized adult cells such as neurons, osteoblasts, hepatocytes, cardiomyocytes at the human body [1,2].

Cell researchers are interested in counting, sorting and tracking the cells in the last decades. The stem cells, especially for investigating drug therapy capacity, the specialists use different cell markers for counting manually. This is a tedious and time consuming task. Moreover, high contrast, cultured cells, microscopy parameters and various cell size and morphology makes



Figure 1. Captured cell image examples.

manual counting difficult. As a result, number of cells attested by specialist varies according to their personal interpretation. Thus, automatic cell counting algorithms come into prominence.

There are several cell counting methods. Semi-automatic or automatic methods involving thresholding, watershed, nearest neighborhood graphs have been proposed for cell segmentation mainly used for cell counting algorithms [3, 4, 5, 6, 7, and 8]. In [9,10] a segmentation and tracking algorithms for neural stem cells is proposed. These approaches are based on advanced segmentation methods in digital image processing and pattern recognition which use prior information about the cell position. A deconvolution method in the form of an optimized ellipse fitting algorithm is investigated in [11] to locate the HSCs (Hematopoietic Stem Cell). The authors proposed a statistical thresholding method for cell tracking in [12] and probabilistic model based cell tracking in [13].

In this study, automatic embryonic stem cell detecting and counting method is proposed stem cell sections were obtained under fluorescence microscopy. Embryonic bodies obtained from ESC in vitro are investigated. In the pre-processing stage color image is split into three channels (R, G, and B). Then a Gaussian filter is applied to green channel, that gives best counting results, to eliminate the noise and highlight the maximum point of the cells. Finally maximum point analysis performed to count the cells automatically.

The paper is organized as follows: the pre-processing step is explained and image characterization is given in Section (2). In Section (3) histogram partitioning and its results are given. Section (4) discusses the maximum point analysis to count the cells automatically. Simulation results are given in Section (5), and Section (6) concludes the paper.

#### 2. Pre-Processing

In this section, the filtering and background segmentation with thresholding is explained before performing cell counting. At first, the cell database and problems encountered in manually counting is described. In this work, used embryoid bodies images were provided by Geisa Martins Faustino. The image characterization is given in reference [2] with details. Figure 1 shows some examples of the captured images.



Figure 2. a) Input cell image, b)The surface of the input image, c) The surface of the input image after filtering.

The focus of the microscopy is quite important to generate the correct images. If the microscopy parameters are incorrect the edges of the cells will be shady and some cells cannot be counted manually. Overlap is another difficulty that causes from the presence of many cells in a single scene. Therefore manual counting becomes slower, inaccurate and demanding task with naked eye. The DAPI (Diamidino phenilindole) dyes cell DNA and turn it visible with naked eye. It can be seen lighter on the center and become darker in the edges. However, if the DNA is more concentrated, two or more lighter points can overlap which causes the difficult and inaccurate cell counting. In addition, the acquisition parameters can cause the different types of noise in fluorescence image. The presence of the noise and above mentioned disadvantages make a challenge to improve an automatic cell counting methods. Thus, pre-processing step is needed to eliminate the undesired components from the input images.

#### 2.1. Filtering

In the fluorescence images the pixels of the cell are lighter in the center of cell and pixel luminance value decreases gradually as it reaches to the cell boundaries. Each pixel considered as a point situated at some altitude according to its grey level by evaluating the luminance information. So, the center of the cell has maximum altitude and can be used for maximum point analysis.

In the light of above discussed information, the Gaussian blur filter is used smooth the surface and emphasizes the maximum points of images. In Figure 2 a, b, and c, the input image, the surface of the input image and the surface after Gaussian filter are shown respectively.

Now we must find the local maximum points of the filtered image surface. For this purpose, histogram partitioning is performed discussed in Section 3. To obtain the better counting results, background segmentation given in the next section can be hold before histogram partitioning.



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



Figure 4. Pre-processing steps for green channel: a) Green channel b) Gaussian filtered green channel, and c) result of background segmentation.

### 2.2. Background Segmentation

Segmentation is one of the most important tasks in image processing. Segmentation accuracy determines the eventual success or failure of computerized analysis procedures [14, 15]. In this subsection background segmentation with thresholding is applied to filtered cell images that have nonuniform background rises from fluorescence. To determine the threshold value, we utilize the spatial information of cell images these are mean  $\mu$  and standard deviation  $\delta$ . Then, the threshold value which puts forward the cell agglomerate from the background chosen as the  $t = \mu + x\delta$ . By setting the pixel intensities below t to zero thresholding is performed which clarifies the maximum points. The thresholding results of each channel (R, G, B) is given in Figure 3. As can be seen from the figure the best results are obtained with green channel. Furthermore, pre-processing steps for green channel is demonstrated in Figure 4.

### **3. Histogram Partition**

After background segmentation, maximum points of the image are emphasized. In histogram partition, our purpose is to identify the connected cells. In other words, difference between the center of cell and the boundary of the cell is utilized by applying histogram partition. At the beginning, we calculate the histogram of background segmented green channel image given in Figure 4c. The histogram is separated into four equal intervals. The size of each interval is 64. In Figure 5, histogram partition of Figure 4c is demonstrated.



Figure 5. Histogram partition of Figure 4c: 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, to identify the connected components in each interval the images are scanned twice, from bottom to-top and left-to-right. The first scan constructs on equivalence relation among the components it finds. The second scan assigns labels to the connected components [16]. Each component receives a label according to the order which it was detected. It is clear that, the higher label is the smaller luminance of the components.

#### 4. Maximum Point Detection

All the parts of the cell must be determined to distinguish the cell boundaries. Circular border plays mainly important role in the algorithm to separate the cells from each other. The main parameter is the distance between the separated cells. In order to obtain best counting results, different intervals of the histogram given in Section 3 are summed in proposed algorithm. There are numerous combinations for this summation. In Figure 6, some of those combinations and their labeling results are given. As can be seen from the figure the most exact results are obtained with summing second and fourth interval.

Let's discuss the Figure 6, because of the discontinuities in the first interval summing first interval with fourth interval will give unrealistic (so much number of cells) counting results. Summing second and fourth interval provides best counting results because of aforementioned luminance difference between the cell center and boundary. By this summation labeling will provide plausible results so that the discontinuities are eliminated and the maximum points become more distinctive. In the following section simulation results are given to investigate the effectiveness of the method.



Figure 6. The combination and their labeling results for histogram partitioning: a) Interval 1 and 4, b) Interval 2, 3 and 4, and c) Interval 2 and 4 are summed.

#### **5. Simulation Results**

In this section we give and discuss the simulation results performed on the database by contact an author Geisa Martins Faustino in Ref [2]. The database contains two groups. There are 69 and 23 images in these groups, respectively. In the second group noise level is stronger than the first group. The proposed method parameters to be used are choosen according to the experiments as follows: The values for Gaussian radius are 2,3 in group 1 and 2 respectively. x=0.3,  $\mu$  is the mean value and  $\delta$  is the standart value of the green channel of the input image. In the first experiment we count the embryonic stem cells in the given images with proposed method to compare our results with manual counting results and automatic counting results in reference [2]. Counting results and images are illustrated in Figures 7 and 8. As can be seen from the figures best counting results are obtained with proposed algorithm. Most of the embryonic cells that are not able to count with naked eye, and automatic counting method in [2] are catched in proposed algorithm.

In the second experiment a table is constructed for ten embryonic stem cell images choosen abbreviately from groups 1 and 2. The authomatic counting results obtained by proposed method and method in reference [2] are compared. Because of the absence of flourescence microscopy and specialists we are not able to compute a measure for these results. But if someone investigates the images in figures can evaluate the counting results clearly.

Image	Automated	Automated	Image	Automated İn[2]	Automated (Proposed)
	İn[2]	(Proposed)			
1	509	615	66	330	407
12	559	659	72	1224	1629
16	404	520	71	255	231
36	536	694	65	273	256
46	551	692	96	563	562

Table1. Embryonic stem cell numbers for proposed method method in reference [2] for groups 1 and 2.



Figure 7. Counting results for a) proposed method, b)manual method, and c) method in reference[2].



Figure 8. Counting results for a) proposed method, b) manual method and, c) method in reference [2].

### 6. Conclusions

In this paper, an automatic embryonic stem cell counting and detecting method based on connected component and maximum point analysis. Embryoid bodies are obtained from embryonic stem cells cultured in vitro. The method consists of a three main steps. The first step is the pre-processing step with Gaussian blur filter and background segmentation followed by histogram partition given as second step. The aim of the histogram partition is to detect the connected components of the images by utilizing the luminance information of the cells. After histogram partition, to obtain the better counting results second and the fourth histogram intervals are combined. At last maximum points of the resulting images are calculated. Simulation results are compared with manual counting results and automatic counting results given in reference [2]. It is shown that proposed method gives promising results and can be expanded for future works.

In future work, our purpose is to combine this method with probabilistic models to obtain correct and objective results. Cell counting and cell detecting method are mainly important for drug and disease research. So this subject will maintain its popularity.

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