Automatic Embryonic Detection in Microscopy Images

Veska Georgieva[1]

Abstract – **In this paper is proposed an automatic embryonic stem cell detection and determination of their size and location in microscopy images. The presented approach includes pre-processing stage for obtaining better information for the number and state of the investigated cells. It includes noise reduction based on homomorphic filter and correction of illumination of the fluoroscopic microscopy images. Then an automatic method to detection and location of the embryo is applied. It is based on Hough Transform to approximate the embryo as a circle. Experimental results showed that the proposed method can detect the position of the eligible embryo accurately. The obtained result can be used to extract criteria for embryo transfer purpose.**

Keywords – **Automatic cell detection, microscopy images, homomorphic filter, illumination correction, Hough transform.**

I. INTRODUCTION

Embryonic stem cells are found in various parts of the human body at every stage of development from embryo to adult and are classified according to their potential to develop into other cell types. Using different cell markers, specialists are able to determine, by manual counting, the total number of cells, how many specialized itself into a specific mature cell and how many cells died [1].

There are methods [2, 3] to identify and quantify sections of cells cultured in suspension. However, these methods are expensive and require a trained technical specialist. Another disadvantage is that the spatial information is lost, because the cells must be separated. This information is important because the specialists are able to observe some phenomena, such as the differentiated cells are located in the colony's extremity while the specialized stem cells are located at the colony's center [4].

Although the main problem was the overlapping of the cells in the images, it was also found that the size (magnification) and the brightness also varied from one image to another [5]. Noise components, blurring and illuminations artefacts are found in the most of images.

In this paper is proposed an approach for automatic embryonic stem cell detection and determination of their size and location in microscopy images. This approach can also be applied in other groups of objects, as long as the object surface is both smooth and concave with illumination source.

The remainder of this paper is organized as follows: section 2 describes the basic steps in the proposed algorithm; section 3 presents the experimental results to show the effectiveness of the method and; section 4 presents our conclusion and some future works.

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II. BASIC STAGES IN ALGORITHM FOR IMAGE PROCESSING

In the paper is proposed an effective algorithm for automatic embryonic stem cell detection and determination of their size and location in microscopy images. It consists of following basic stages:

- Noise reduction
- Correction of illumination
- Edge Detection via the Canny Operator
- Circle Detection via the Circular Hough Transform

The block diagram of the algorithm is presented in Fig.1.

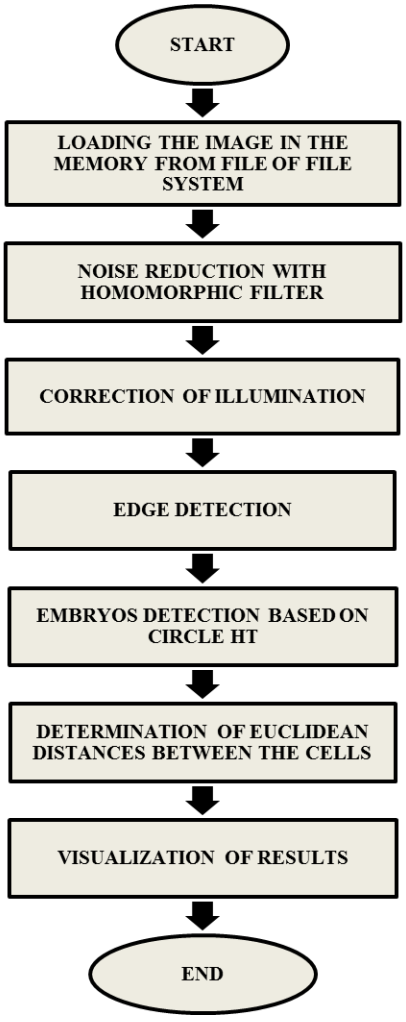

Fig. 1. Block diagram of the basic algorithm

Image pre-processing is performed in order to reduce or eliminate noise and enhance the visual quality.

Homomorphic filtering (HF) can eliminate non-uniformity luminance distribution of image, and keep its original state. The standard homomorphic filtering uses illumination-reflectance model in its operation. This model consider the image is been characterized by two primary components. The first component is the amount of source illumination incident on the scene being viewed $i(x,y)$. The second component is the reflectance component of the objects on the scene $r(x,y)$. The image $f(x,y)$ is then defined as [6-8]:

$$f(x,y) = i(x,y)r(x,y) \tag{1}$$

In this model, the intensity of $i(x,y)$ changes slower than $r(x,y)$. Therefore, $i(x,y)$ is considered to have more low frequency components than $r(x,y)$. Using this fact, homomorphic filtering technique aims to reduce the significance of $i(x,y)$ by reducing the low frequency components of the image. However, before the transformation is taking place, logarithm function has been used to change the multiplication operation of $r(x,y)$ with $i(x,y)$ in Eq.(1) into addition operation.

$$z(x,y) = \ln f(x,y) = \ln i(x,y) + \ln r(x,y) \tag{2}$$

Illumination correction is based on background subtraction. This type of correction assumes the scene is composed of a homogeneous background and relatively small objects brighter or darker than the background. There are two major types of background subtraction techniques depending on whether the illumination model of the images can be given as additional images or not: prospective and retrospective correction. Our approach uses retrospective correction for color images. The image is converted in this case into the HSL color space and then the correction to the lightness channel is applied. The background is estimated by mathematical morphology opening or closing. The estimated background is then subtracted from the original image. The total sequence of operations corresponds to a top-hat of the image. Top-hat removes high frequencies (considered as reflectance) and keeps low frequencies (considered as illumination). Bottom - hat is used for clear background and top-hat is used for dark background. If the background is clear, the corrected image g(x,y) is obtained using:

$$g(x,y) = T_B[f(x,y)] + mean\{closing[f(x,y)]\} \tag{3}$$

$$g(x,y) = [f(x,y)] - closing[f(x,y)] + mean\{closing[f(x,y)]\} \tag{4}$$

where mean [closing (f(x,y)] is the mean value of the closed image and T_B is bottom – hat transform of the image. The bottom-hat returns an image, containing the "objects" or "elements" that: are "smaller" than the structuring element, and are darker than their surroundings. The size, or width, of the elements that are extracted by the top-hat transforms can be controlled by the choice of the structuring element. The bigger the latter, the larger the elements extracted.

For detection of the embryos cells is proposed to perform as next edge detection via the Canny Operator. It is optimal with regards to the following criteria [9]:

1. Detection: The probability of detecting real edge points should be maximized while the probability of falsely detecting non-edge points should be minimized. This corresponds to maximizing the signal-to-noise ratio (SNR).
2. Localization: The detected edges should be as close as possible to the real edges.
3. Number of responses: One real edge should not result in more than one detected edge (one can argue that this is implicitly included in the first requirement).

The Circular Hough Transform is useful for detecting circles of known radius as well to detect circles of various radii. This method is based on creating an accumulator matrix of size of the original image to be processed. The local maxima in accumulator space are obtained by voting procedure. Parameter space is defined by the parametric representation used to describe circles in the picture plane, which is given by Eq.5[10]:

$$r^2 = (x - x_0)^2 + (y - y_0)^2 \tag{5}$$

It implies that the accumulator space is three-dimensional (for three unknown parameters x_0, y_0 and r) and defines a locus of points (x, y) centered on an origin (x_0, y_0) with radius r. Points corresponding to x_0, y_0 and r, which has more votes, are considered to be a circle with center (x_0, y_0) and radius r.

The Euclidean distances between the cells are found in addition. They are necessary by obtaining of better information about cross location of the different cells in regard to their micro-moving. This step can be applied by observation of the cells in the time.

III. EXPERIMENTAL RESULTS

The experiments are made in MATLAB 7.14 environment by using IMAGE PROCESSING TOOLBOX. For the investigations are used 20 fluoroscopic microscopy images with size 235x170 pixels. In Fig. 2 is presented the original image and in Fig. 3 is shown its enhancement modification after homomorphic filtration.

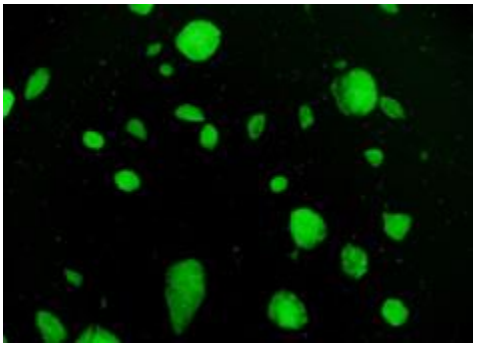

Fig. 2. Original microscopy image

The graphical presentation of background surface and effect from correction of illumination are shown in Fig.4 and Fig.5 respectively. The eligible embryo cells are colored in purple.

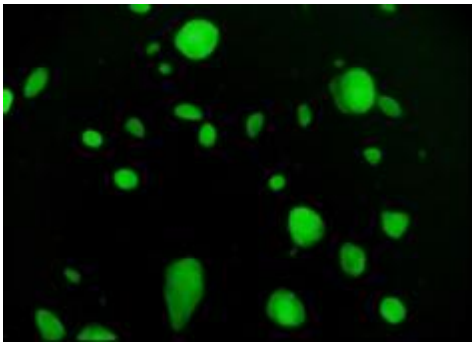

Fig. 3. Microscopy image after homomorphic filtration

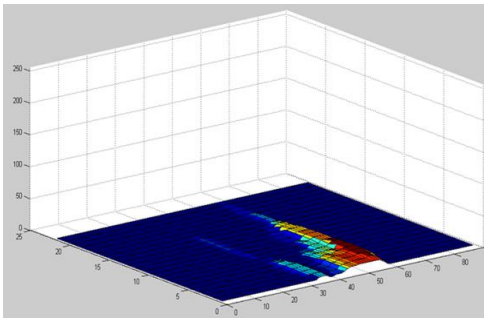

Fig. 4. Graphical presentation of background surface

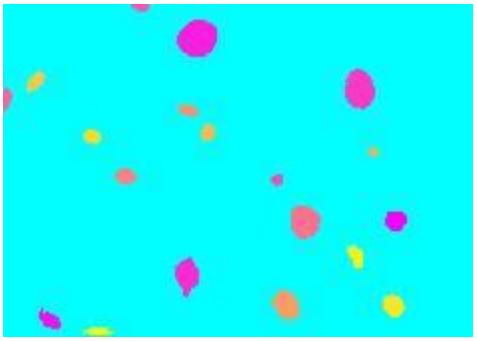

Fig. 5. Microscopy image after illumination correction

The embryo cells are detected and located after application of Canny edge operator and Circle Hough transform. The obtained result is shown in Fig.6.

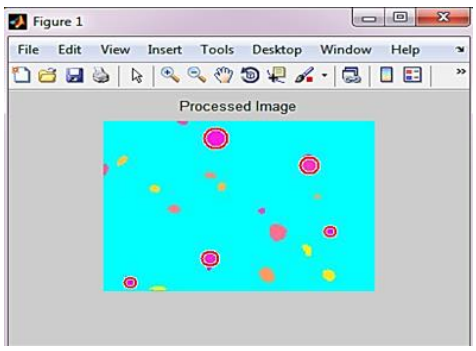

Fig. 6. Detected and located embryos cells

In Table 1 are given the obtained results for the radii and coordinates of the detected cells on the base of Circle Hough transform. The results are evaluated by calculating the measures precision, recall and F-measure [1].The measures were calculated for each image and, then, averaged over all images. The obtained results are given in Table 2.

TABLE I
EXPERIMENTAL RESULTS FOR DETECTED CELLS

Number of cells	X co-ordinate	Y co-ordinate	Radius R
1	97.79	18.0	10
2	178.55	45.14	8
3	92.94	138.47	7
4	196.70	11.29	5
5	24.18	161.69	5

TABLE II
EXPERIMENTAL RESULTS FOR DETECTED CELLS IN %

Precision	Recall	F-measure
97.67	95.23	96.43

The Euclidean distances between the cells are calculated as next. This information is very important for cross location of the different cells in regard to their micro-moving. The connections between the cells are presented in Fig.7 and the calculated Euclidean distances are given in Table 3.

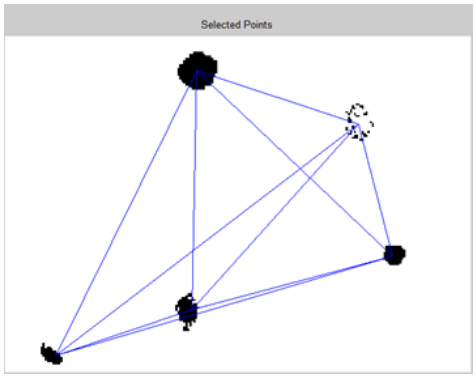

Fig. 7. Connections between the cells

TABLE III
EXPERIMENTAL RESULTS FOR EUCLIDEAN DISTANCES BETWEEN DETECTED CELLS

Number of cells	1	2	3	4	5
1	0	86.33	136.51	121.01	160.55
2	86.33	0	69.12	126.06	192.60
3	136.51	69.12	0	104.54	177.20
4	121.01	126.06	104.54	0	72.73
5	160.55	192.60	177.20	72.73	0

IV. CONCLUSION

In this paper is proposed an effective approach for automatic embryonic stem cell detection and determination of

their size and location in microscopy images. It consists noise reduction based on homomorphic filter and correction of illumination of the fluoroscopic microscopy images. Then an automatic method to detection and location of the embryo is applied. It is based on Hough Transform to approximate the embryo as a circle. Experimental results showed that the proposed method can detect the position of the eligible embryo accurately with average precision, recall and F-measure of 97.67%, 95.23% and 96.43%, respectively, which is satisfactory. Moreover, with the automatic detection, we can eliminate the subjectivity because, unlike in manual detection, is guaranteed that the same criteria are always used to detect cells. In addition, the method could be used in others applications.

Because of the image quality, which depends on a microscope type, some cells can be detected very difficult. These images have a strong noise and the cells are very small to be identified. Better results can be obtained by implementation of more effective methods for filtration. Our future works will be focused in applying of wavelet transformation for more effectiveness of filtration in cases with strong noise.

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