Apoptosis Analysis in Classification Paradigm: A Neural Network based Approach

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Abstract— In the past few years, research on cell death has developed significantly, owing to its importance for various diseases' severity determination and diagnosis. Different varieties of cell death are often identified by morphological criteria, exclusive of a clear reference to specific biochemical mechanisms. Therefore, it is significant to determine accurately the "percentage apoptosis". This work proposed an algorithm that created in ImageJ v. 1.49v commands and macro language functions to classify both dead cells and dying cells. A stained images using Caspase stain of albino rats hippocampus specimens using light microscope are used to evaluate the system performance. The algorithm analyzes color of the obtained objects on the enhanced image after using the median filter and the logarithmic transformation. The main descriptors used for feature extraction are the area, shape, light nucleus, and the brown regions intensity. A threshold value is to be used to distinguish between the dead cells that characterized by dark brown color, while the dying cells have less brown intensity. This study proposed a novel system to perform the classification based on color intensity and contrast changes. After feature extraction stage, the Back propagation neural network (BPNN) classifier was used. The experimental results proved that the proposed automated system achieves 82.92% mean accuracy, where the dying cells and dead cells classification accuracy have the values of 76.76%, and 89.08%; respectively, thus the mean accuracy of the proposed system has the value of 82.92%.

Keywords—Apoptosis; Microscopic imaging; Dead cells classification; Color intensity; Color contrast; Back propagation neural network.

I. INTRODUCTION

Human/ animal cells engage several mechanisms leading to death or demise. The cell demise is one of the key procedures in living creatures. It can be either apoptosis or necrosis. Apoptosis is known as an active, programmed process of independent cellular dismantling that is regulated and controlled [1]. Thus, it has received enormous attention. It is suspected that the proportion between the dead and live cells gives vital data around various diseases. It helps recognizing the disease type and its stage, thus can realize the reaction to the treatment. The relation between diseases and cell deaths comprises a significant step towards understanding the diseases and drug action against them.

Presently, there is confusion in cell death terminology, which attracts the attention toward cells classification. The molecular understanding of the cell death is inadequate to create ultimate classifications based on precise biochemical pathways. The Nomenclature Committee on Cell Death (NCCD) recommended unified criteria for the definition of cell death and of its different morphologies. Thus, it is feasible to classify the cell death scenarios based on morphological criteria as stated originally in [2, 3] and later in [4].

The classification is generally accomplished via detecting the dead, dying cells and lives cells under a microscope. Consequently, it is very imperative to develop an image-based system for the automatic and objective classification and quantification of cell deaths. Therefore, this study proposed an automated system for microscopic stained apoptosis images classification based on both color and contrast intensities to distinguish the completely dead cells from those going to die (dying) cells. The BPNN classifier is used to perform the classification step.

The structure of the remaining sections is as follows: section II includes the related work that followed by the proposed system in section III. Then, the results and their discussion are presented in section IV. Finally, the conclusion in section V.

II. RELATED WORK

Recently, an intensive research focuses on the image analysis for various medical and biological applications and the

development of the automated microscopic cells detection,

imaging approaches have been developed to study and investigate the apoptosis in individual cells.

Based on the morphological characteristics, Galluzzi *et al*. suggested a classification of the dead cells into four types: apoptosis, autophagy, necrosis, and mitotic catastrophe [8]. To detect apoptosis, Huh *et al*. presented a cell area detection approach based on the optical principle of phase-contrast microscopy for adherent cells [9]. This procedure employed the cell morphology changes and image intensity during apoptosis and accomplished 90% accuracy. Cheng *et al*. proposed an automatic extraction of a single cell using an Ultrahigh-resolution optical coherence tomography (UR-OCT) system to distinguish between live and dead cells [14]. This system used both the morphological recognition and the parametric analysis. In amplitude contrast bright field images, the authors classified the macrophages infection state using segmentation and morphological quantification [15].

For cells classification, Hurst *et al*. reported correct classification of up to 78% of test cells by using a neural network with direct input of gray pixel intensities [16]. In [17] the artificial neural network (ANN) methods were used on digitized microscopy fields without pre-ANN feature extraction, using the error back-propagation algorithm. The results proved that using neural network provides speed of segmentation, counting and classification as in [5-13]. Various analysis and consistency. Zheng *et al*. employed different neural network types to determine the viability of direct classification using pixel intensity information on the cells images [18].

Theses related work shows that the morphological changes based on the cells color intensity and contrast is used for living and dead cell detection. In addition, for classification the neural network classifier is recommended. Thus, the current proposed system designs a novel algorithm based color intensity and contrast for apoptosis detection and then classified into dying and dead cells using BPNN as follows.

III. METHODOLOGY AND PROPOSED SYSTEM

This study proposed an automated classification system of the dead and dying albino rats stained cells that captured using light microscope. The tested specimens are taken from rats' hippocampus to obtain colored images. The proposed algorithm is created in ImageJ v. 1.49v [http://imagej.nih.gov] and uses commands and macro language functions for this software. The proposed system consists of two main stages: i) features extraction based color intensity and contrast and ii) classification stage to distinguish the dead cells and dying cells using BPNN [19], as illustrated in Figure 1. As the input is the colored microscopic RGB (red-green-blue) image.

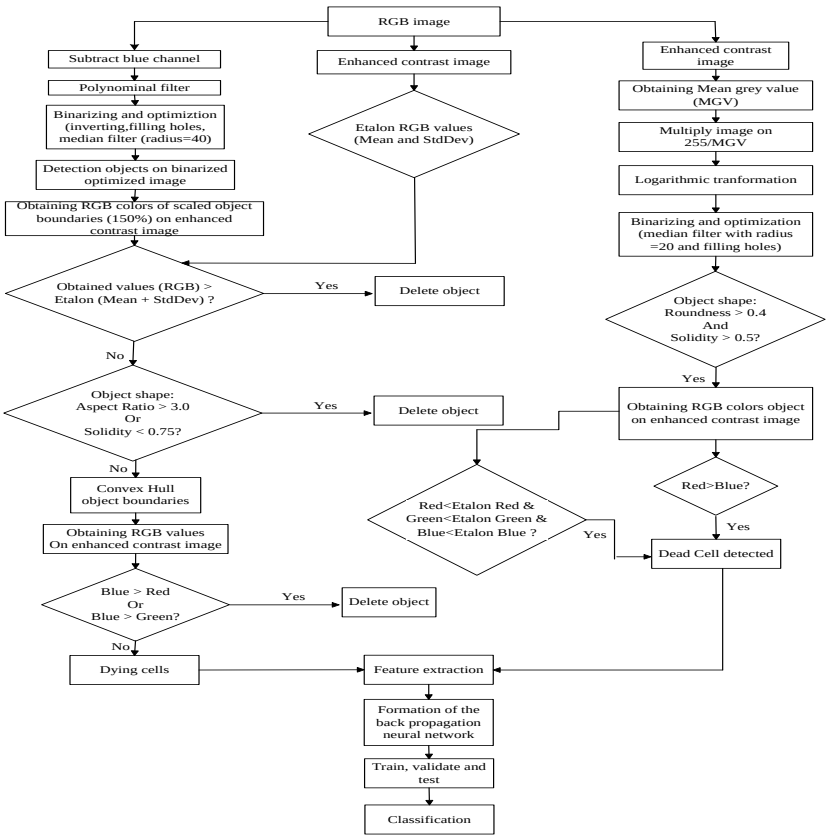

Fig. 1. Proposed algorithm for the automated dead/dying cells classifier

The system is divided into two branches as follows, where each branch used the previously mentioned two stages.

A. Determination of dying cells

As indicates Fig. 2 (A), dying cells can be characterized by area, shape, light nucleus and darker brown region around. Last one also is more than background brown color. These features are the main descriptors in the proposed algorithm for dying cells determination.

Firstly, the original RGB image is enhanced contrast (saturated pixels = 0.35 % as default value in ImageJ), and mean and standard deviation of the darkest region of the red, green, blue channels are measured without including light region (are the nucleus of dying cells). This region is showed with black boundaries in Fig. 2 (B) that are better values.

Now, the algorithm starts identification of dying cells (left branch in Fig.1). From the original RGB image, the blue channel is subtracted and the polynomial filter is used. Then, the image is binarized and inverted as shown in Fig. 2 (C). Since, this image contains noise, thus for image optimization, filling holes and median filter (radius=40) are to be included into in proposed algorithm.

Currently the optimized binarized image contains large objects that indicates dying cell's nucleus and small objects that indicates noise, which was not reduced on previous steps. Simultaneously, the dying cells are also characterized by the area. That is why algorithm analyzes on binarized image only objects with area>10000pixel^2 (this threshold value was obtained experimentally with fact that original images size were (3136x2352) pixels). Then, for each object (with area area>10000pixel^2): the boundaries are restored on enhanced contrast image, scaled into 150% and mean values of red, green and blue are obtained (only for that pixels that located on the scaled boundaries). Obtained values are compared with better values, where if the mean of each color on boundaries is greater than (better mean + better standard deviation), thus this object is deleted because around it there are values of color on the scaled boundaries that indicate more light color. If the obtained mean color values are not greater than (better mean + better standard deviation) mean that the region around the potential nucleus is dark enough and this object can be analyzed in the next steps.

In this step, the algorithm checks the objects shape according to the shape descriptors such as Aspect Ratio (AR) and Solidity (S). If the AR descriptor is greater than 3.0 and S is less than 0.75, thus the object is deleted from the analysis. This rule prevents analyzing oblong objects, because dying cells are also characterized by shape, which is more round. Before the next step, the objects boundaries are convex hull. So, the algorithm gets values of red, green and blue for each object on the enhanced contrast image and if blue is greater than green or red one this object is deleted. It is required to prevent the determination of the "blue" objects as dying cells.

Fig. 2(D) indicates the results of the "cleaning steps", and in comparing to that in Fig. 2 (C). At this moment, it is clear that the binarized image number of objects is less. Only after all this checking of the area, shape and color, the proposed algorithm decides that analyzed object can be dying cells and marks it on original image as indicated in Fig. 2 (E).

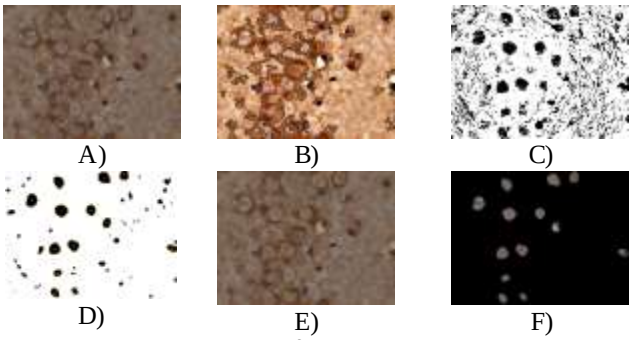

Fig. 2. Determination of the dying hippocampus cells, where: A) the original RGB image, B) the image with enhanced contrast and marked by black color boundaries with high intensively of color, C) the inverted binarized image that obtained after subtraction blue channel from original RGB image and using polynomial fit plug-in for ImageJ, D) the binarized image after using "cleaning algorithm", objects marked by orange correspond dying cells on the original image, E) the dying cells marked by orange color on original image, and F) the binary mask image.

Finally, the features extracted from this first stage are then feed to the second classification stage based on BPNN. The algorithm of the dying cells classification can be given by:

Algorithm 1: *Dying Cells Classification*

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1. Read *the original RGB image*
2. Enhance contrast *(saturated pixels = 0.35%)*
3. Determine *the better values of the (mean and standard deviation) of red, green and blue for darkness regions on enhanced contrast image*
4. Subtract *blue channel from original RGB image*
5. Apply *polynomial filter*
6. Convert *into binary image, use the median filter (with radius 40) and filling holes*
7. Scale *boundary of object (150%)*
8. Determine *values of red, green and blue of scaled boundaries on enhanced contrast image*
9. Compare *values of red, green and blue of scaled boundaries on enhanced contrast image with better values of red, green and blue, which were obtained in step 3.*
10. Analyze *object shape (aspect ratio and solidity)*
11. Convex *hull object boundary*
12. Determine *values of red, green and blue on enhanced contrast image.*
13. *Classification based BPNN*
14. *Determine whether the cell is dying cell or no.*

B. Determination dead cells

Initially, the algorithm increased contrast of original RGB image (saturated pixel = 0.35% as default value in ImageJ) as showed in Fig. 3 (B) and calculate the mean gray value (MGV) of this image. For normalization, multiply the image with the coefficients, as the last one is calculated as 255/MGV. Then, the logarithmic transformation is performed. The output of these two steps is showed in Fig. 3 (C), where these steps are required to reduce all objects with high color intensity. The image is binarized and the objects with low color intensity are presented. Some of these objects are dead cells and others are noise. For noise reduction, the median filter (with radius 10 pixels) and filling holes are applied. Then, the algorithm takes all objects with area >10000pixels^2, and analyzes the objects' shape and selects only the objects with roundness > 0.4 and solidity > 0.5 into the next step. Such threshold values are obtained experimentally for prevention of noise or any detection of dying cells parts with high contrast as dead cells.

Afterward, the algorithm analyzes color of the obtained objects on the enhanced image. The dead cells are characterized by dark brown color. Therefore, all objects with blue higher than red are deleted, while those with mean red value that is higher than blue one are accepted as dead cells. In addition, the mean color values of the potential dead cells on the enhanced color image are compared with better values from previous part (obtained in dying cells detection). If the red color of the object is less than (better mean red – ½ StdDev better red), green is less than (better mean green – StdDev better green) and blue is less than (better mean blue – StdDev better blue) object is accepted as dead cell. Such rule assists to detect objects that are very dark, but red color is some higher (green and blue: mean – StdDev, but for red: mean – ½ StdDev), thus the base color is red for brown. In the previous rules, the "StdDev" stands for the Standard deviation.

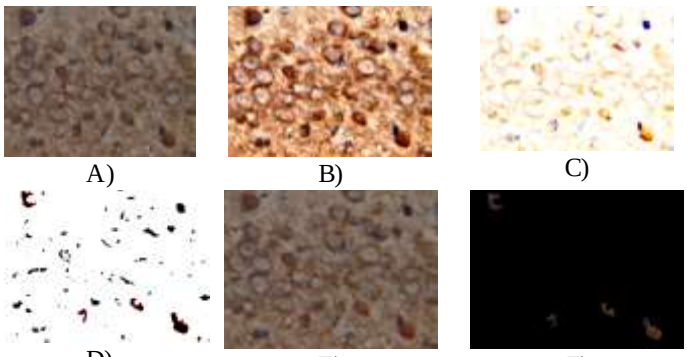

Fig. 3. Determination dead hippocampus cells, where: A) the original RGB image, B) the image with enhanced contrast, C) the image after multiplying on (255/mean grey of enhanced contrast image) and logarithmic transformation, D) the binarized image, objects marked by red color correspond dead cells, E) the dead cells marked by red color on original image, and F) the binary mask image.

Finally, the features extracted from this first stage are then feed to the second classification stage based on BPNN. The algorithm of the dying cells classification can be given by:

Algorithm 2: *Dead Cells Classification*

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1. ***Read*** *the original RGB image*
2. ***Enhance contrast*** *(saturated pixels = 0.35%)*
3. ***Determine*** *better values (mean and standard deviation) of red, green and blue for darkness regions on enchanced contrast image*
4. ***Determine*** *mean grey value, calculate coefficient as 255 / (mean grey value)*
5. ***Multiply*** *image on coefficient*
6. ***Perform*** *logarithmic transformation*
7. ***Convert*** *into binary image, use the median filter (with radius 20) and filling holes*
8. ***Analyze*** *object shape (roundness and solidity)*
9. ***Determine*** *value of red, green and blue on enhanced contrast image*
10. ***Compare*** *value of red and blue one;* ***compare*** *values of red, green and blue with better values determined in step 3.*
11. *Determine whether the cell is dead cell or no.*

IV. RESULTS AND DISCUSSION

In the current study, an albino rat hippocampus dataset images captured using light microscopic images in the Anatomy department laboratory, faculty of medicine, Tanta University, Egypt were used. The tested specimen images were stained with caspase that taken from a pool of 642 albino rats images.

A BPNN with extensive weight sharing in the first hidden layer was deployed and trained on 513 samples using the error back-propagation algorithm, where the number of hidden neurons was determined and the trained system was validated.

In order to evaluate the proposed automated neural based classification system for apoptosis, various standard analysis performance metrics' parameters were used to show how correctly the proposed system determined either the dead or the dying cells. These parameters are: i) the True Positive (TP)- the total area of dead or dying cells that are classified correctly, ii) False Positive (FP)- total area of dead or dying cells that are classified as dead cells, True Negative (TN)- total area of dead cells that are classified as dying cell or vice versus, and False Negative (FN)- total area of dead or dying cells cases that are classified incorrectly. These parameters are used to calculate some metrics such as: accuracy (ACC), sensitivity, specificity (SPC)= TN/(TN+FP), Positive predictive value (PPV)=TP/ (TP+FP), Negative predictive value (NPV)= TN/ (TN+FN). Table I, illustrates the mean of some performance metrics over the 642 dataset images, when using the automated proposed system.

From Table I, it is noted that the proposed system provided better classification performance for the dead cells detection than with the dying cells detection/ classification.

TABLE I. STATISTICAL PERFORMANCE MEASUREMENTS

	Dying Cells	Dead Cells	Mean
Accuracy (ACC)	76.76%	89.08%	82.92%
True Prediction Ratio (TPR)	75.22%	82.22%	78.72%
Specificity (SPC)	76.86%	91.04%	83.95%
Positive predictive value (PPV)	81.72%	69.82%	75.77%
Negative predictive value (NPV)	76.14%	94.83%	85.49%
False Prediction ratio (FPR)	23.14%	8.96%	16.05%
False Negative Ratio (FNR)	24.78%	17.78%	21.28%
False Detection Ratio (FDR)	18.28%	26.61%	22.44%

These results established that, the proposed system achieved good mean accuracy of value 82.92% with 83.95% Specificity. While, all false ratios provided values that were less than 25%. As, high specificity and high sensitivity indicate an ideal test scenario.

The confusion matrices in Fig. 4 illustrated the results obtained when using 642 samples with 80% training, 10% for verification and 10% for validation.

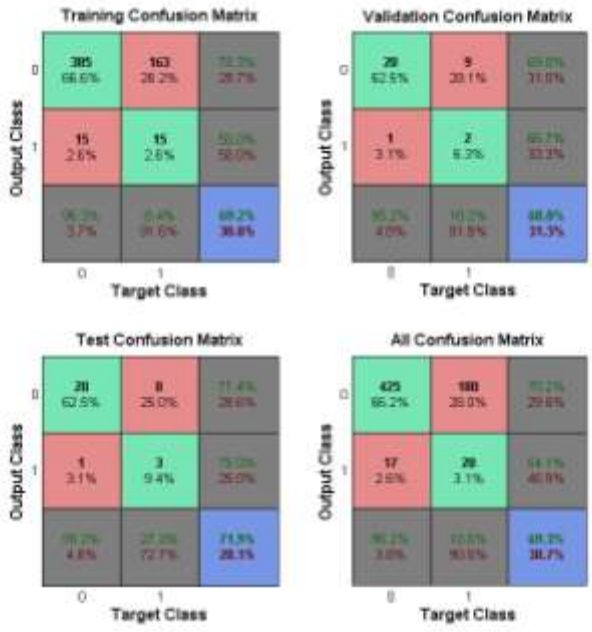

Fig. 4. Confusion Matrices

To prove the performance of the proposed system a Receiver operating characteristic (ROC) curve to analyze the classifier's performance is demonstrated in Fig.5. The ROC graph represents the false positive rate on the X axis and the true positive rate on the Y axis for the training, test, validation and all BPNN steps. The point (0,1) signifies the perfect classifier. It performs accurate classification for all the positive cases and negative cases correctly. The (0,1) point denotes that the false positive rate is 0 (none) and the true positive rate is 1 (all). The (0,0) point stands for a classifier that predicts all the cases to be negative, whereas the point (1,1) corresponds to a classifier that predicts each and every case to be positive. Point (1,0) represents that it is incorrect for all the classifications.

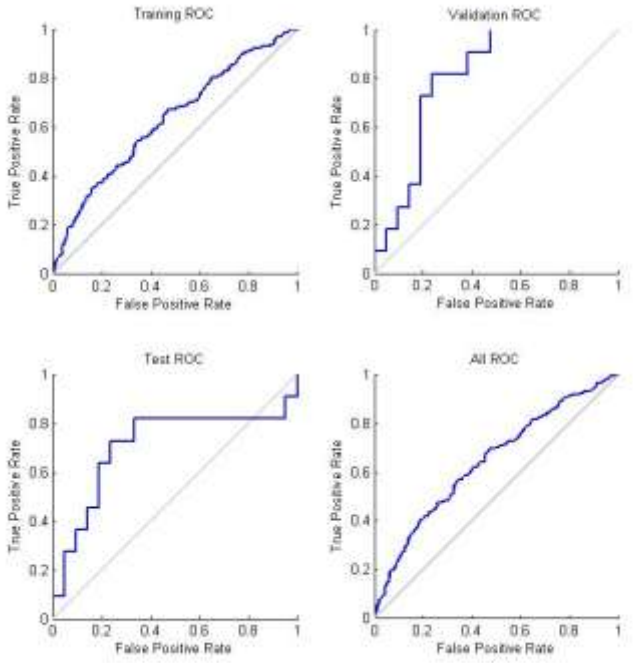

Fig. 5. ROC curves

The overall ROC indicated the good performance of the proposed classification method.

Accordingly, these experimental results clarify the good performance of the proposed automated apoptosis detection using image analysis methods and then classification into dead or dying cells based on BPNN with mean accuracy of 82.92%.

V. CONCLUSION

Cell samples are an ordinary tool in biological and medical research domains for assessments of the cell variability. Cell images are captured with the use of digital camera under the microscope. This allows the computer analysis for inspection and evaluation of the cells that is useful for diseases diagnosis, follow up, and in the drug effect.

The cells status and count can be determined accurately via identification and classification of each cell on the image with classification algorithms. This is considered difficult

image processing task, where different features and objects can be miss detected and classified as cells. Therefore, the more practicable task is the regions identification that measure the area occupied be each class of cells.

Consequently, the main contribution of the proposed system is to use the image analysis based on the color intensity and contrast to detect the Apoptosis cells and then using the BPNN to classify into either dying or dead cells. This automated system is outperforming the manual system in accuracy as it achieved 82.92% mean accuracy value.

An integrated automated system is recommended to detect, count and classify the Apoptosis cases and then calculates the percentage of the living, dying and dead cells. In addition, compare between the pixel intensity and the color intensity and contrast for features extraction.

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