Designing an Auxiliary Software to Interpret the Screening of Apoptotic Effect of Drug Compounds by Using AO-EB Test

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A B S T R A C T

In order to solve the problem of counting apoptotic cells stained by AO-EB test, an auxiliary softwar which can count alive , early apoptosis and late apoptosis cells is designed.

1 Introduction

There are two different types of cell death necrosis and apoptosis. Necrosis happens because of an extrinsic factor and after the cell is in necrosis process the embryonic layer of cell will be inflame and then the cell will die .

The other type of cell death is apoptosis which is a programmed death of cells. Apoptosis can happen both naturally like death of cells which make the membrane between embryos' fingers or can happen by adding drug compounds [1].

Apoptos is a Greek word that means autumn , as autumn doesn't have a bad effect on trees , apoptosis process won't hurt the organism unless we use drug compounds in a wrong way [1].

Both negative and positive effects of apoptosis are possible in different disease.

In one and by chemotherapy extra cancerous cells will be in apoptosis process and die by adding drug compounds, on the other hand apoptosis process needs to be stop in some diseases like Parkinson or heart attack[1,2].

As mentioned the effect of apoptosis process matters and patients must use the right drug to be treated. There are different ways to check the effect of drug compounds on cells, one of the most frequent ways is to stain cells by fluorescence colours like DAPI, HOUKHEST, AO-EB and a specialist count them[2,3].

AO-EB colour is a mixture of both AO and EB colours. All cells absorb AO which is vital stain while EtBr is only absorbed by cells with disrupted membrane (cells in early and late apoptosis state). Alive cells which only absorbed AO will have dark green colour, and cells which absorb both stains (apoptotic cells) become light green (early) and orange-red (late)[4,5].

But the question is that what is the need of this software? Today there are two main ways to check and count the number of cells , one is counting cells by high-tech but too expensive machines like cell machines which are not enough to be used for normal people in some developing countries .

The other way is that specialist count cells . They are able to categorize and count dark and light green apoptotic cells stained by AO-EB. Surely in this way some eye – errors might happen and the accuracy will be low. This software almost solve these problems.

2 Method

Finding the spectrum of red, light and dark green was the first step to analyse pictures. Every digital screen is made by pixels and each pixel has three components R(red), G(green), B(blue) and the measurement of each varies from 0 to 255. Three numbers for each component make the colour of pixels. By these numbers the spectrum are found and then defined to the software.

First of all to analyse one picture the software produce three same sizes of black pictures as the original one for each level of apoptosis. Second pixels which are in one of the defined spectrum in the original picture turn to white in one of the produced black pictures. This process repeats three times in three levels of apoptotic cells and at the end there are three black white pictures that have some errors and mistakes and because the software count the white parts they're not ready to be counted.

To solve mistakes that happen in all image processing programs, four morphology were used ,imdilate , imerode , imopen and imclose. There were some options we had to choose for each morphology. The disk diagram was chosen as a result of cells' shape and then we had to choose a number as the size of filters of each morphology. This morphology has effect on the white parts of black white pictures.

Imdilate makes all white parts thicker ,imerode makes them thinner , imopen deletes all white dots (noises) in the black side of the picture and imclose fills black dots in the white part of the picture.

The first problem was the similarity between dark and light green spectrum and as an example there were some dots in dark green cells (alive) that were in light green (early apoptosis) spectrum so imclose was used to fill the blanks .

Second problem was decomposed cells which were red and didn't have to be counted as a cell and also pixels around light green cells were in light green spectrum so we used imdilate for extra parts to become smaller and in the next step , using imopen made them totally disappear.

At the end there were some cells which weren't fixed and they separated in two or more parts so the last step was using imdilate and all white parts became thicker so separated parts were connected .

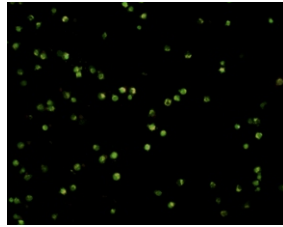After using this morphology, the final picture is complete and also ready to be counted(Figures 1-6).

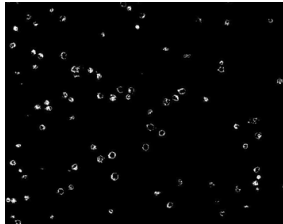**Fig. 1:** Main picture(captured by microscope)

Fig. 2: First step dark green pixels recognition

Fig. 3: Second step imclose morphology

Fig. 4: Third steps imerode morphology

Fig. 5: Fourth step imopen morphology

Fig. 6: Final step imdilate morphology

3 Results

The software starts to analyse pixels from top left of the picture , it analyses pixels one after another in a line. If it is

a black pixel nothing happens but for a white one as it's the first white pixel, it counts it as a cell and turns it into black. Then software by checking eight connected pixels to it, turns the white ones into black without counting them. This process repeats three times for all levels.

This way all cells will be counted and the software shows the final result by the percentages of the number of alive, early apoptosis and late apoptosis cells in a pie chart. In order to check the accuracy of the software ,the result of software were compared with the number of cells counted in some authentic articles (tables 1-4) and (Figures 7-10).

Table 1: The comparison [6] of the results for code No751a

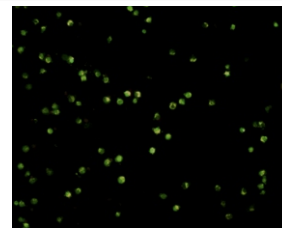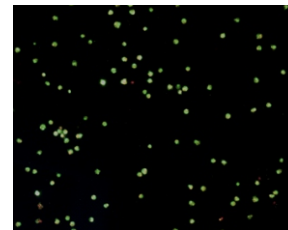| Type of cells	The real affluence	Software's affluence
alive	55%	55.3%
early apoptosis	45%	44.7%
Late apoptosis	0%	0%

Fig. 7: Picture of code No751a

Table 2: The comparison [6] of the results for code No533a

Type of cells	The real affluence	Software's affluence
alive	78%	68%
early apoptosis	18%	28.5%
Late apoptosis	4%	3.5%

Fig. 8: Picture of code No 533a

Table 3: The comparison [6] of the results for code No1143a

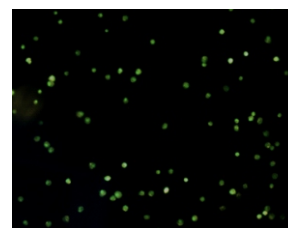| Type of cells	The real affluence	Software's affluence
alive	34%	27%
early apoptosis	62%	73%
Late apoptosis	4%	0%

Fig. 9: Picture of code No1143a

Table 4: The comparison [6] of the results for code No22 43a

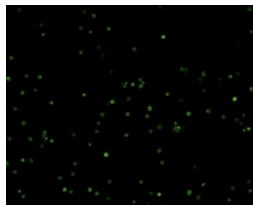| Type of cells	The real affluence	Software's affluence
alive	65%	10%
early apoptosis	28%	90%
Late apoptosis	7%	0%

Fig. 10: Picture of code No 2243a

4 Conclusion

In this new case a different colour range is found, so a new mode can be defined for DAPI staining. In this mode the majority of the pixels are in blue colour range but the detection progress is not far different. First all pixels of the image are classified in two categories, Black and Blue. Then morphological filters are used on the result to remove unwanted cells. This is done by dilating all of the cells. Finally a counting phase will be performed. Since this designated program showed good result in interpretation of AO-EB test result, this program is developed for analysing. The results of staining in determining apoptotic state of treated cells, are the same as DAPI staining which can show apoptotic characteristic of apoptotic cells such as chromatin condensation and DNA fragmentation (Figures 11 and 12) [7,8].
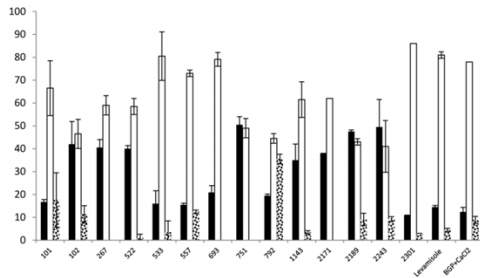

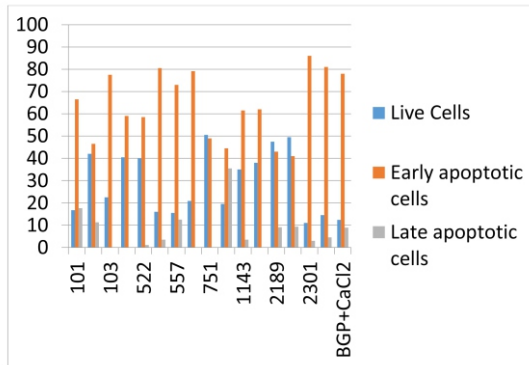**Fig. 11:** UTMC codes of the strains

Fig. 12: Comparing different codes

References

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