Morphology and phenotype of peripheral erythrocytes of fish: A rapid screening of images by using software

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ABSTRACT. Many information of biological study as stained cells analysis under microscope cannot be obtained rich information like detail morphology, shape, size, proper intensity etc. but image analysis software can easily be detected all these parameters within short duration. The cells types can be yeast cells to mammalian cells. An attempt has been made to detect cellular abnormalities from an image of metronidazole (MTZ) treated compared to control images of peripheral erythrocytes of fish by using non-commercial, open-source, CellProfiler (CP) image analysis software (Ver. 2.1.0). The comparative results were obtained after analysis the software. In conclusion, this image based screening of Giemsa stained fish erythrocytes can be a suitable tool in biological research for primary toxicity prediction at DNA level alongwith cellular phenotypes. Moreover, still suggestions are needed in relation to accuracy of present analysis for Giemsa stained fish erythrocytes because previous works have been carried out images of cells with fluorescence dye.

1. INTRODUCTION

The cells analysis with different stains under microscope is a technique where different cellular morphology can be identified. It has been well established by several researchers from decade [1-4]. But this analysis may be time consuming by large scale screening, visual error by individual eye estimation, sometimes missing of cellular features as subtle change undistinguishable by eye visually and also requires proper expertise to recognize cellular features. To minimize time, visual error, proper identification of cellular features etc. cell analysis with algorithm based software has been recommended by researchers [5-11] however, the proper set up may be tedious to incorporate the input data in the software [11].

Generally cell image analysis has been showed accurate identification and measurement of all types of cellular features by automatic analysis of certain phenotypes through software, previously studied by many researchers [6, 8, 11, 12-24]. It was also known that machine learning methods, which have been selected and combined multiple features for automated cell classification and used to detect many cellular phenotypes [12, 13, 15, 17, 18-25].

According to Carpenter et al. [6], the easy screening image analysis software, CellProfiler (CP) is freely available (non-commercial) image analysis software, can capable of handling 100 nos. of

images of any cell types like yeast colony to mammalian cells [11]. CP software helps to detect the biological information quickly with statistical power and the simple cellular morphology viz. cell count, shape, size, etc. alongwith protein levels in individual cell as well as complex morphological parameters like cell/organelle shape or subcellular patterns of DNA or protein from stained images. Many works have been carried out on fluorescent stained cells but no one has been attempted before Giemsa stained image analysis from erythrocytes of fish to detect rich information from stained cells by using CP.

The present study attempts to detect cells morphology and phenotype from Giemsa stained images of micronucleated erythrocytes compared with normal erythrocytes by using CP image analysis software because several features cannot be identified only under microscope when visualize stained cells.

2. MATERIALS AND METHODS

2.1 Selection of software and images as input

Images of Giemsa stained normal and micronucleated peripheral erythrocytes were processed by using CellProfiler or CP (Version 2.1.0), this software was downloaded from the website as <http://www.cellprofiler.org/download.shtml>. The input data were incorporated in the present software by trial and error method with the help of CP manual published through the mentioned link (http://www.cellprofiler.org/linked_files/Documentation/cp2.1.1_manual_6c2d896.pdf) for detail description for users [6]. The CP software interface is depicted on the basis of selected input and analysis modules for the present study (Fig. 1).

The two images were taken from previous research work on metronidazole (MTZ) induced genotoxicity in peripheral erythrocytes of fish [26]. In the present study, the images were selected two types of cells and nuclei deformities as micronucleated and normal erythrocytes of fish. Both images were incorporated and analysed in the software separately as per selected input and analysis modules. These images were obtained through CP are depicted in Fig 2 and 2a.

2.2 Measurement of cellular features

In each image, the area, shape, intensity, and texture was measured as per appropriate input and data were found in .csv extension. It was well established that CP can measure a variety and number of features for individual identified cell or compartmentalize at subcellular level included area, shape, intensity and texture of objects. These are common features, which described by researchers [6, 11, 13, 27] but the complex measurements like zernike shape features (shape descriptors of objects in image), Haralick and Gabor texture features have also been studied before by other researchers, which are found in detail in the CP manual [12, 28-31].

In the present study, the measurement of both images were considered separately as input. For individual parameter viz. apply threshold, correct illumination apply, crop image, enhance edges, mask and morph image, identify primary, secondary and tertiary objects, mask objects, intensity, size and shape, radial distribution of objects, cells, cytoplasm and nuclei location centre from X axis were selected in the software and analysed all the data (Fig. 1). The data were obtained through images and various computerized simulation processes and saved as .csv file.

2.3 Image processing and data gathering for rich information in cells

According to Carpenter et al. [6], the cellular analysis were done by compartmentalize morphology of cells, object validities by green, yellow and red colours, which identified nuclei, cytoplasm and cells boundaries, location comparison of nuclei, cytoplasm and cells in each image, total intensities were compared for nuclei, cytoplasm and cells. The features were studied primarily related to object shape of nuclei, cytoplasm and cells, descriptor based zernike moments 0 order to 9 order and total nos. of pixels. The features were compared for nuclei, cytoplasm and cells for each image separately.

3. RESULTS

Images of Giemsa stained normal (control) and micronucleated (experiment) peripheral erythrocytes were inserted in the CP software separately along with setting of all the selected necessary parameters as input and analysed data (Fig. 1). The images were obtained through CP in measured position after incorporated in the software (Fig. 2 and 2a). In case of threshold application, it was observed the threshold value of 0.08 and 0.19 for control and experiment images respectively and the images after thresholding are depicted in Fig. 3 and 3a. The output as illumination correction after apply is depicted in Fig. 4 and 4a for control and experiment respectively. The image crop was applied for both the control and experiment images and the results are depicted in Fig. 5 and 5a. The enhance edge application as peripheral boundary of individual object for the both images were obtained and the results are depicted in Fig. 5 and 5a. The results were obtained after masking and morphing the images for control and experiment and are depicted in Fig. 7 and 7a and 8 and 8a respectively.

In case of control and experiment primary object data, it was observed that marking of nuclei by outline, the data were obtained 10th pctl diameter (14.3 and 13.4 pixels) while 90th pctl diameter (34.1 and 32.3 pixels) respectively. The results for both types of images as output are depicted in Fig. 9 and 9a. In case of control and experiment secondary object data, it was observed that marking of all the cells and nuclei boundary in individual image, the data were obtained 10th pctl diameter (14.9 and 13.9 pixels) while 90th pctl diameter (36.5 and 36.1 pixels) respectively. The results for both types of images as output are depicted in Fig. 10 and 10a. In case of control and experiment tertiary object data, it was observed that marking of all the nuclei, cells and cytoplasm by separate outline for individual image. The results for both types of images as output are depicted in Fig. 11 and 11a. The masking objects for all the cells and masked nuclei were obtained for control and experiment images separately and are depicted in Fig. 12 and 12a.

The object intensities were obtained of different parameters viz. integrated intensity, integrated intensity edge, center mass intensity X, center mass intensity Y, maximum intensity X and maximum intensity Y for cells, cytoplasms and nuclei separately for both control and experiment images, which are expressed by histogram (Fig. 13 and 13a). Lower intensity values were observed in experiment when compared to control image for cells, cytoplasms and nuclei. In case of objects shape and size data, it was observed that in experiment image the area, perimeter and minimum and maximum ferret diameter of cells, cytoplasm and nuclei were showed lower value except center X and Y (higher value), when compared to control value, histogram illustrated in Fig. 14 and 14a.

In Fig. 15 and 15a, it was observed for cells, cytoplasms and nuclei of control and experiment images separately for zernike moments of order 0 to order 9. The minimal differences were observed between control and experiment images.

4. DISCUSSION

The present study was emphasized on the basis of visually observed peripheral erythrocytes of fish, one is normal (without treatment as control) image and other is micronucleated cells (treated with MTZ) image to screen morphological and phenotype differences after automated analysis through CP, an image based analysis software. This software can screen easily of 2D images and detect different parameters of cellular phenotype viz. size, shape, intensity, texture etc. through high throughput way [6]. According to their concept and software manual, the present study was dealt with Giemsa stained nuclear abnormalities cells compared with control cells. The data obtained after the analysis of CP software that cannot be possible to study cellular morphology visualization only under microscope.

CP has already been used by several laboratories in the globe and researchers are studying a variety of biological processes in different cell types and organisms, viz. yeast colony, *Drosophila melanogaster* (S2R+ cells, epithelial tissues), various human samples namely TOV21G, prostate gland tissue, stem cells of mesenchymal origin, H1299 lung cancer cell lines, mouse samples such

as NIH/3T3 cell lines, neural precursor cells of embryos, cells from lung tissue, germ cells and also H9c2 cell lines of rat model [6, 11, 16, 32-36].

It was reported by Lamprecht et al. [7] that CP has also modified for the measurement of yeast colonies, yeast growth patches, wounds healing assays and tumours quantification. But in the present study, Giemsa stained fish blood cells may provide cellular features through CP analysis and also informed cellular deformities with special reference to nuclei abnormalities by object intensity, shape and size and pixel size of primary (nuclei) and secondary (cells) object data.

According to Khotanzad and Hong, [37], Suk et al. [38] and [39], zernike moment is a viable shape descriptors to screen accuracy and this parameter could be ideal for applications when little data is provided in algorithm. The present results were showed very minimal difference between control and experiment images, which is expressed in histogram (Fig. 14 and 14a) while other parameters such as object intensity and object shape and size were observed a good differences in experiment when compared to control images (Fig. 12 and 12a and 13 and 13a). The present study with Giemsa stained erythrocytes whether worked properly by using this present software still unclear in many aspects. It is required necessary suggestions by other researchers, those who have been investigated and/or working with fluorescence stained cells.

5. CONCLUSION

In conclusion, present study is an approach to screen images of Giemsa stained peripheral erythrocytes of fish to know differences of MTZ treated micronucleated cells compared to control cells on the basis of object identification, shape, size, zernike moment by using CP software, an image based analysis software developed by Carpenter et al. [6]. However, the study has been reported majorly in fluorescence stained images except the study of yeast colony [11]. This study can be a suitable tool for biological research for extraction of rich information in image for nuclei stained with DNA binding non-fluorescence dye and to know easily cell deformities with the special reference to the change of objects intensity, shape, size and area of cells, nuclei and cytoplasm after the exposure of toxins.

In other words, also necessary suggestions are required to know how far the present work is done accurately for Giemsa stained cells because previous works have been carried out with images of fluorescence dye in different cell types except yeast colony.

Fig. 1 CP interface of different modules selected for control and experiment images

Fig. 2 Original image of control obtaining through CP software

Fig. 2a Original image of experiment obtaining through CP software

Fig. 3 Threshold apply control image output

Fig. 3a Threshold apply experiment image output

Fig. 4 Correct Illumination apply control image output

Fig. 4a Correct Illumination apply experiment image output

Fig. 5 Crop output of control image

Fig. 5a Crop output of experiment image

Fig. 6 Enhance edges output of control image

Fig. 6a Enhance edges output of experiment image

Fig. 7 Mask output of control image

Fig. 7a Mask output of experiment image

Fig. 8 Morph output of control image

Fig. 8a Morph output of experiment image

Fig. 9 Primary object output data for control image

Fig. 9a Primary object output data for experiment image

Fig. 10 Secondary object output data for control image

Fig. 10a Secondary object output data for experiment image

Fig. 11 Tertiary object output data for control image

Fig. 11a Tertiary object output data for experiment image

Fig. 12 Mask objects output for control image

Fig. 12a Mask objects output for experiment image

Fig. 13 Histogram of control object intensity data (arbitrary unit)

Fig 13a Histogram of experiment object intensity data (arbitrary unit)

Fig 14 Histogram of control object shape and size data (arbitrary unit)

Fig 14a Histogram of experiment object shape and size data (arbitrary unit)

Fig. 15 Histogram of control object zernike moment data (arbitrary unit)

Fig. 15a Histogram of experimental object zernike moment data (arbitrary unit)

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