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Journal of Advanced Scientific Research

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ISSN 0976-9595

Research Article

MEASUREMENT OF HAEMOCYTES OF GASTROPODS THROUGH AN AUTOMATED IMAGE SCREENING APPROACH BY USING IMAGE PROCESSING TOOL

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Received: 20-09-2021; Revised: 16-02-2022; Accepted: 05-03-2022; Published: 31-03-2022

 https://doi.org/10.55218/JASR.202213214

ABSTRACT

The present study was screened to determine the number of total haemocytes and measurement (mean radius) of haemocytes, cytoplasm, and nuclei in an image of gastropod haemocytes (Leishman stained) by using Cell Profiler (CP) version 2.1.0; an image analysis tool. The results of primary (nucleus), secondary (haemocyte) and tertiary (cytoplasm) object data, shown that marking of nuclei, haemocytes and cytoplasm by outline and total accepted objects were obtained 23 nos. in the studied image. The area shape of objects especially haemocytes, cytoplasm, and nuclei (arbitrary unit) in an image were also obtained. In conclusion, this study helps to easily screen the total nos. of haemocytes and area (mean radius) measurement of haemocytes, cytoplasm, and nuclei in the image.

Keywords: Haemocyte's quantification, Area measurement, Image analysis, CellProfiler tool, Automatic screening.

1. INTRODUCTION

The numbers and shape of any cell can easily be determined under microscope after using appropriate stain. These differential staining help to know area of cells, cytoplasm and nuclei of the cells after stage and ocular micrometer calculation. A technique for the shape measurement of the erythrocyte was developed by Adams [1] followed by the study of red blood cells [2] Moreover, the study of fish blood cells morpho-metry is determined the pathological features [3-7].

But this measurement may be time consuming, individual eye estimation error, missing of cellular or nuclear features, and require proper expertise to recognize cellular and nuclear features to clarify under microscope. The measurement of size of the cells and nuclei manually is very tedious work and possibilities of error in data analysis exist. In recent days, measurement of shape of cells and nuclei by using high throughput tools are showing less time, no visual error, proper measurement of cells and nuclei, etc. The cell measurement through image processing algorithm-based software has been recommended by many researchers [8-12] but the input set up in the software is an important task [12].

In general, image analysis for cellular features has been documented exact recognition and measurement of different types of cellular features by automatic evaluation of certain phenotypes by using image processing tools as studied by many researchers [7, 9, 12-26].

According to Carpenter et al. [7], the Cell Profiler (CP) tool is well known for easy screening image analysis software available as free, non-commercial tool, can be capable of handling of >100 nos. of images of any cell types like yeast colony to mammalian cells [12]. CP software helps to identify the cellular information within a short duration with statistical valuesas well as the simple cellular morphological features viz. cell numbers, shape, etc. from stained images. Many works have been carried out on fluorescent stained cells, but earlier study attempted Giemsa-stained image analysis from erythrocytes of fish [26] to detect measurement information from non-fluorescent stained cells by using CP.

The present study was attempted to detect the number of cells and measurement of haemocytes, cytoplasm, and nuclei of gastropod haemocytes (Leishman's stained) image by using CP (version 2.1.0), an image analysis tool.

2. MATERIAL AND METHODS

2.1. Selection of software and image as input

An image with Leishman's stained (non-fluorescent) of haemocytes of gastropod was processed by using

CellProfiler or CP (Version 2.1.0). This image analysis software was used as per the developers and users' protocol [7, 26]. In the present study, the CP software input data was selected as per the work of Talapatra et al. [26]. Moreover, an image was selected to quantify the total cells and to easily determine the shape especially the cells, cytoplasm, and nuclei of gastropod haemocytes. The image was incorporated and analysed in the software as per selected input and analysis modules as per earlier study [26]. Fig. 1 describes the different modules used as inputs in the interface of CP software. The original image was taken and given as an input image in the CP software for image analysis is exhibited in Fig. 2.

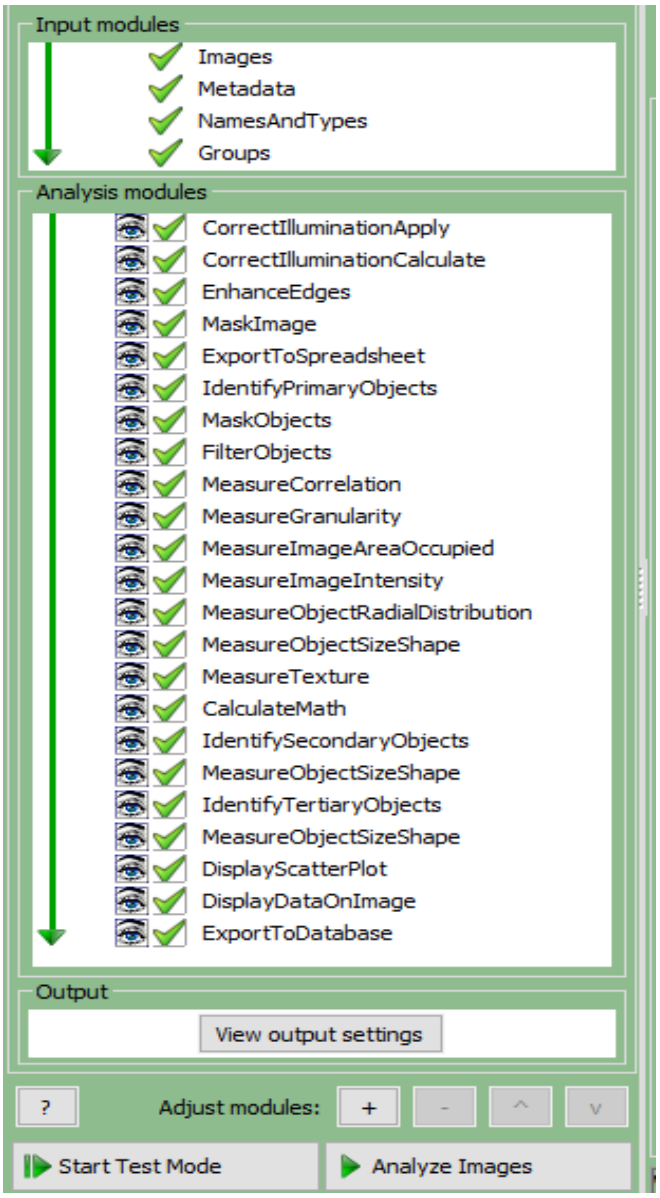

Fig. 1: Modules used as inputs in the interface of CP software

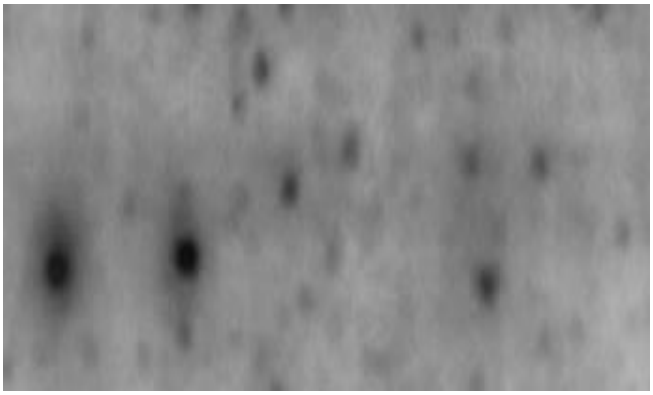

Fig. 2: Original stained image of gastropod haemocytes used as input image

2.2. Evaluation of cellular features

In the studied image, the area, shape, intensity, and texture were evaluated as per selected input data used in earlier study [26] and output data were found in *.csv* extension. The measurement of cellular features such as area, shape, intensity, and texture of objects were done as per protocol described by researchers in earlier studies [6, 11, 13, 26-27]. In the present study, the measurement of many objects in an image was considered as input data. For individual parameter viz. threshold application, correct illumination application, image cropping, enhancing edges, masking, and morphing image, identifying primary, secondary, and tertiary objects, masking objects, intensity checking, size and shape, radial distribution of objects, haemocytes, cytoplasm, and nuclei location centre from X axis were selected in the software and analysed all the data of an image. The output data as *.csv* file was obtained from an image through the computational simulation processes.

2.3. Image processing and data gathering for rich information ofhaemocytes

According to Carpenter et al. [7], the numbers and shape measurement of the haemocytes were done by compartmentalize morphology of haemocytes, object validated by red, green, and blue (RGB) colours, which identified nuclei, cytoplasm and haemocytes boundaries, location of nuclei, cytoplasm, and haemocytes in each image.

3. RESULTS

The Leishman-stained haemocytes of gastropod was used as an input image along with setting up of selected parameters such as correct illumination application and calculation, edge enhancement, masking of image,

primary objects (nuclei) identification, nuclei masking and filtering in image, secondary objects (haemocytes) and tertiary objects (cytoplasm) identification in the image. All these parameters were used as input criteria in the CP software.

The original image was obtained through the CP in measured position after incorporated in the software (Fig. 3). The results indicated that all input parameters were provided appropriate data and images within the CP tool. The output image of illumination correction application for haemocytes is depicted in Fig. 4. For illumination correction calculation, regular type of calculation found by the software and the image was calculated through the process of averaged, dilated, and final illumination of haemocytes (Fig. 5). The enhance edge application was done peripheral boundary of each object (haemocyte) in the original image is exhibited in Fig. 6. The masking was done for each haemocyte in the image through the CP tool and resulted image was obtained and is exhibited in Fig. 7.

From the results of primary (nucleus), secondary (haemocyte) and tertiary (cytoplasm) object data, it was observed that marking of nuclei by outline and total accepted objects were obtained 23 nos. The data were obtained 10th pctile diameter (14.0 pixels) and median diameter (21.1 pixels) while 90th pctile diameter (34.1 pixels) respectively (Fig. 8). The masking and filtering objects for all the haemocytes and masked nuclei were obtained for original image and are depicted separately in Fig. 9 and 10. For secondary object data, the data were obtained 10th pctile diameter (14.6 pixels) and median diameter (24.9 pixels) while 90th pctile diameter (37.9 pixels) respectively (Fig. 11). The tertiary object data was observed that marking of all the nuclei, haemocytes, and cytoplasm by separate outline for the original image (Fig. 12).

The scatter plot diagram revealed that location of nuclei, haemocytes, and cytoplasm from X axis (Fig. 13). In the present result, the density plot observed the smooth distribution of the points as nuclei, haemocytes, and cytoplasm location along the numeric axis. Fig. 14 shows the histogram of area shape of objects especially haemocytes, cytoplasm, and nuclei (arbitrary unit) in an image. Among 23 nos. of haemocytes, the area shape value of each object in which haemocyte, cytoplasm and nucleus were obtained.

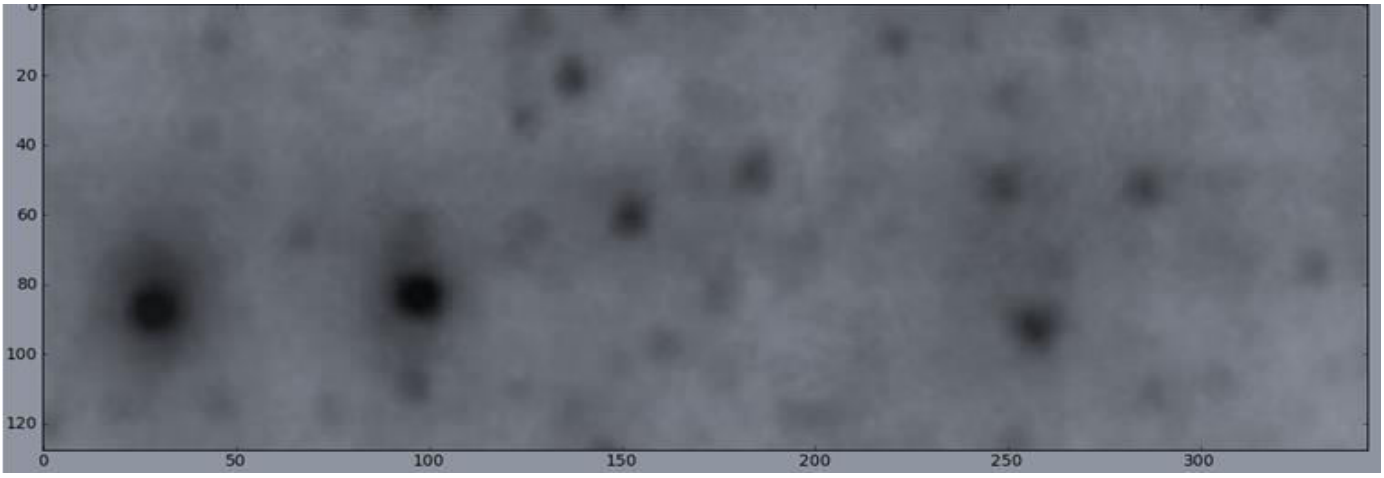

Fig. 3: Photograph of an original image of gastropod haemocytes processed through software

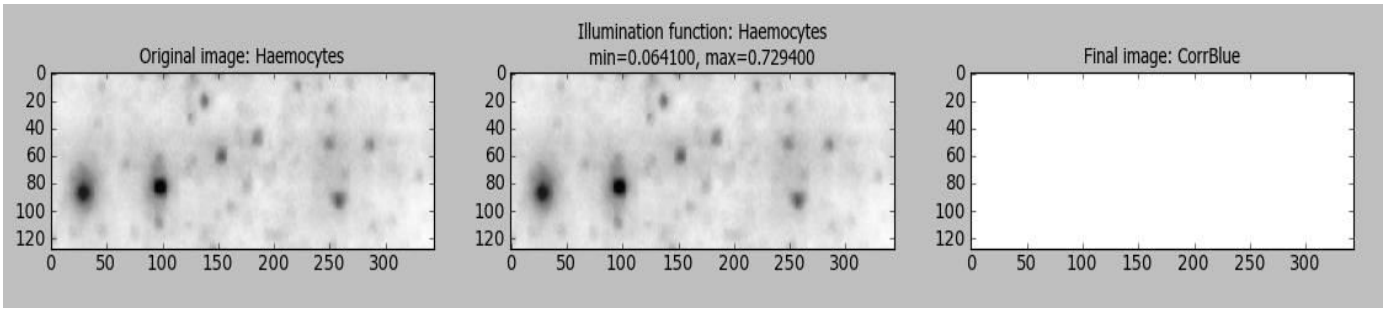

Fig. 4: Photograph of correct illumination application of image of gastropod haemocytes processed through software

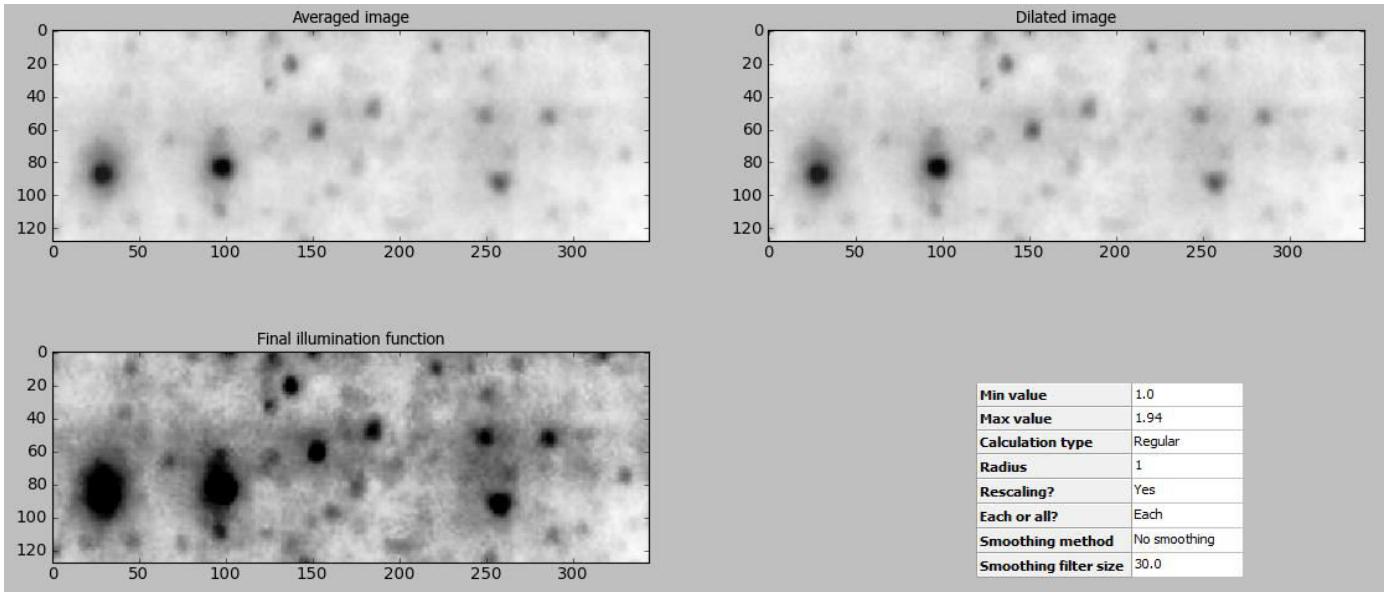

Fig. 5: Photograph of correct illumination calculation of image of gastropod haemocytes through software

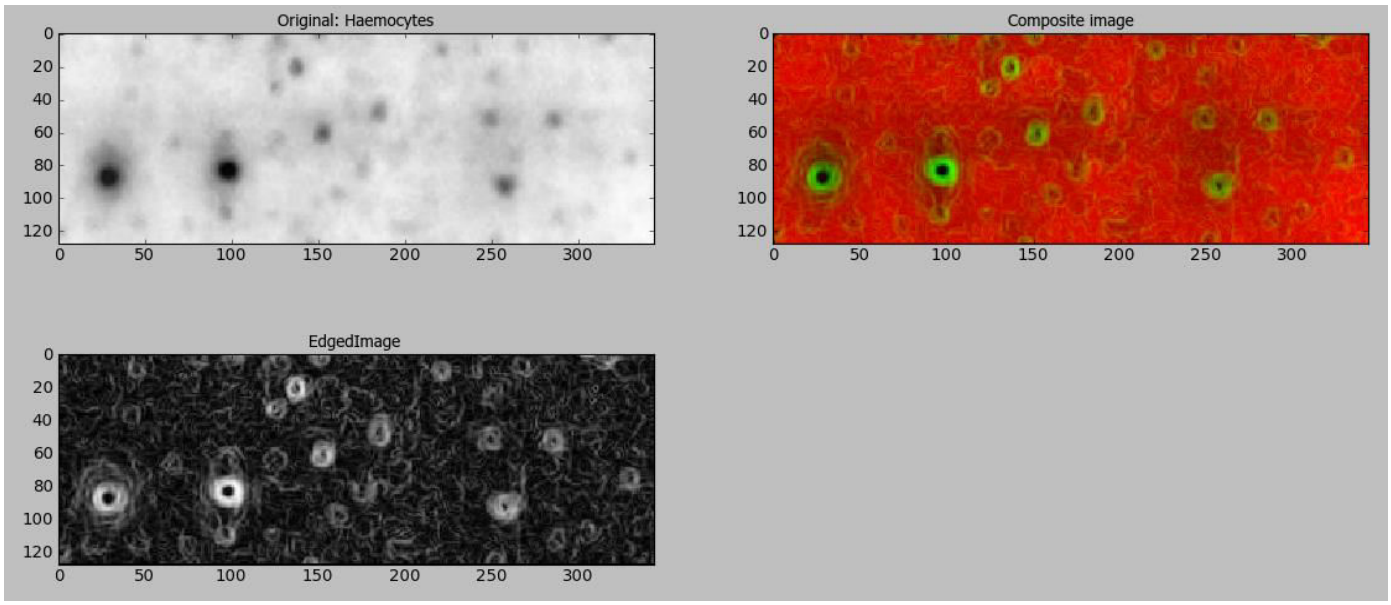

Fig. 6: Photograph of edge enhancement of image of carb haemocytes through software

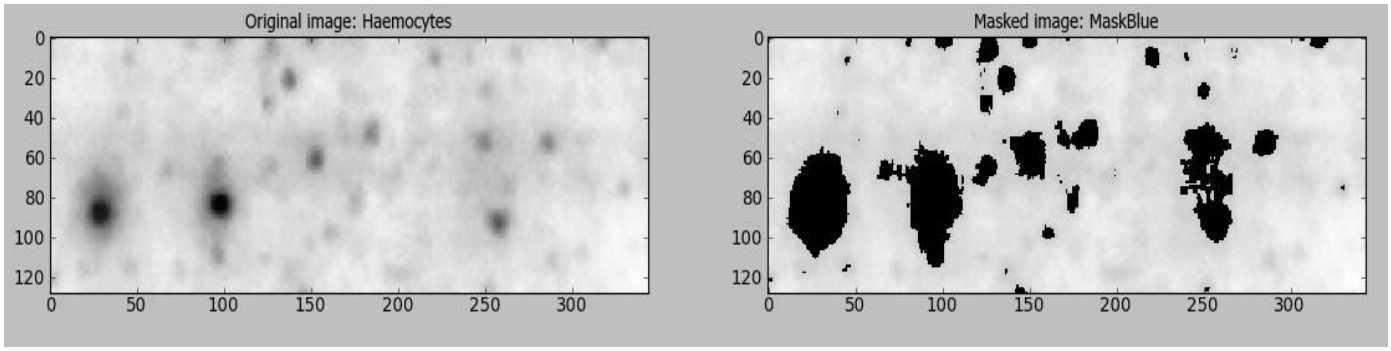

Fig. 7: Photograph of masking of image of gastropod haemocytes through software

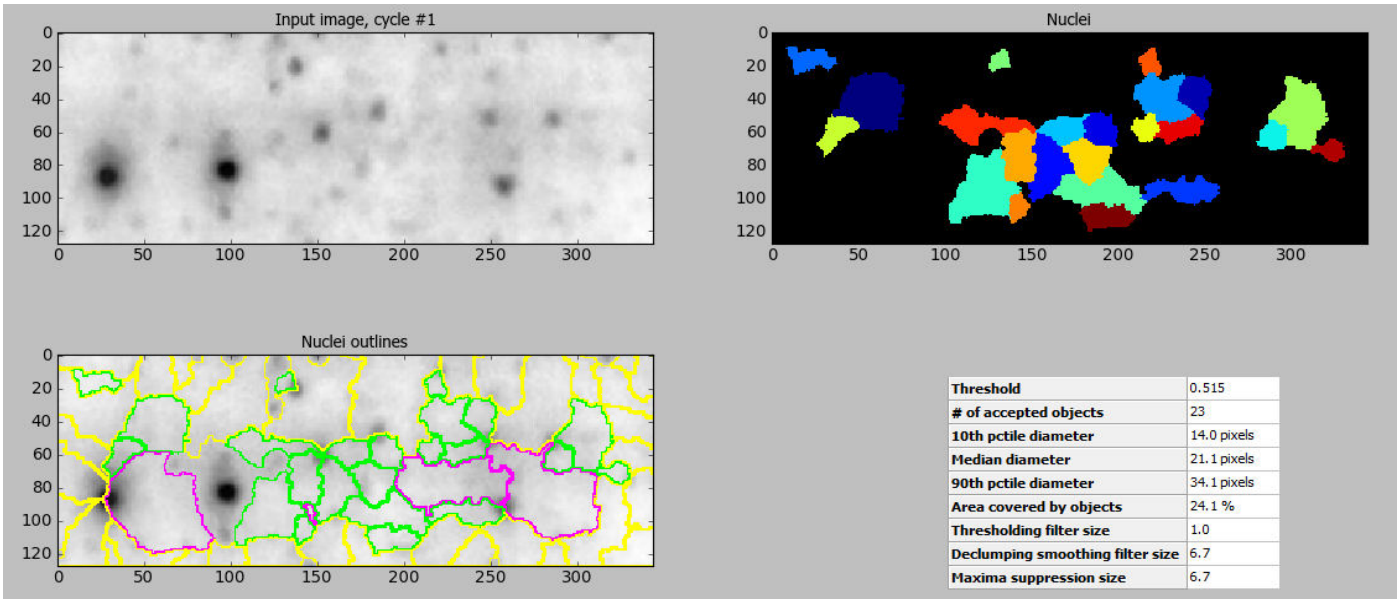

Fig. 8: Photograph of primary objects (nuclei) identification in image of gastropod haemocytes through software

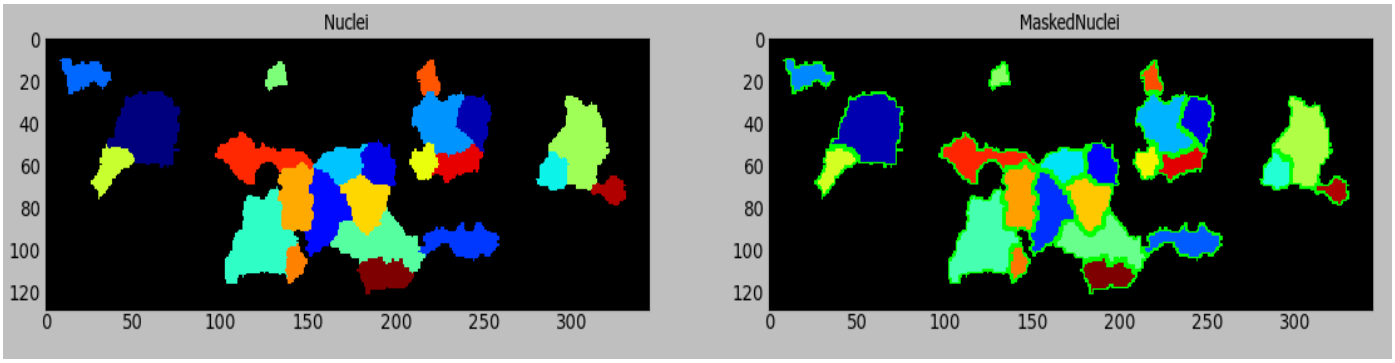

Fig. 9: Photograph of nuclei masking in image of gastropod haemocytes through software

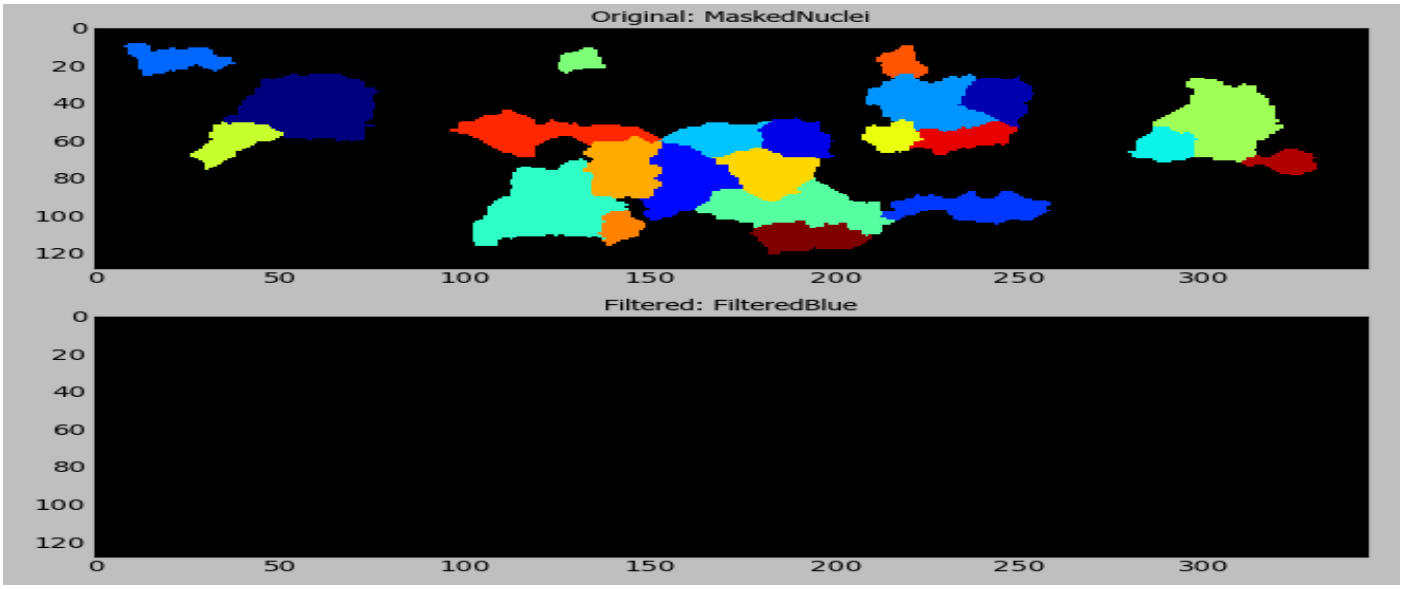

Fig. 10: Photograph of nuclei filtering in image of gastropod haemocytes through software

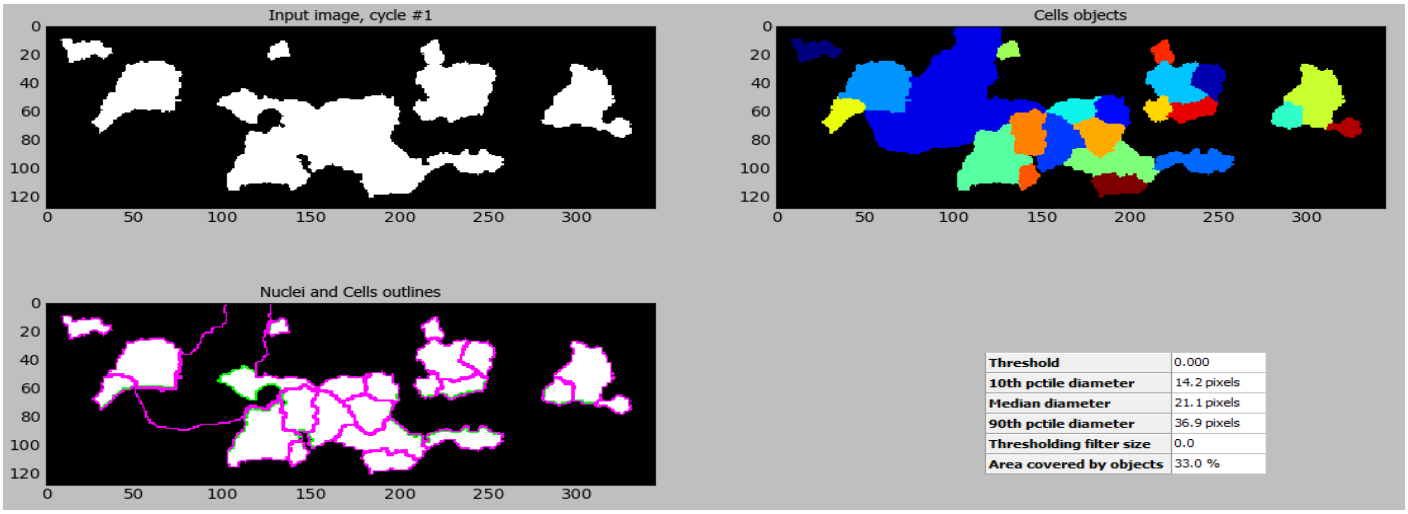

Fig. 11: Photograph of secondary objects (haemocytes) identification in image gastropod haemocytes through software

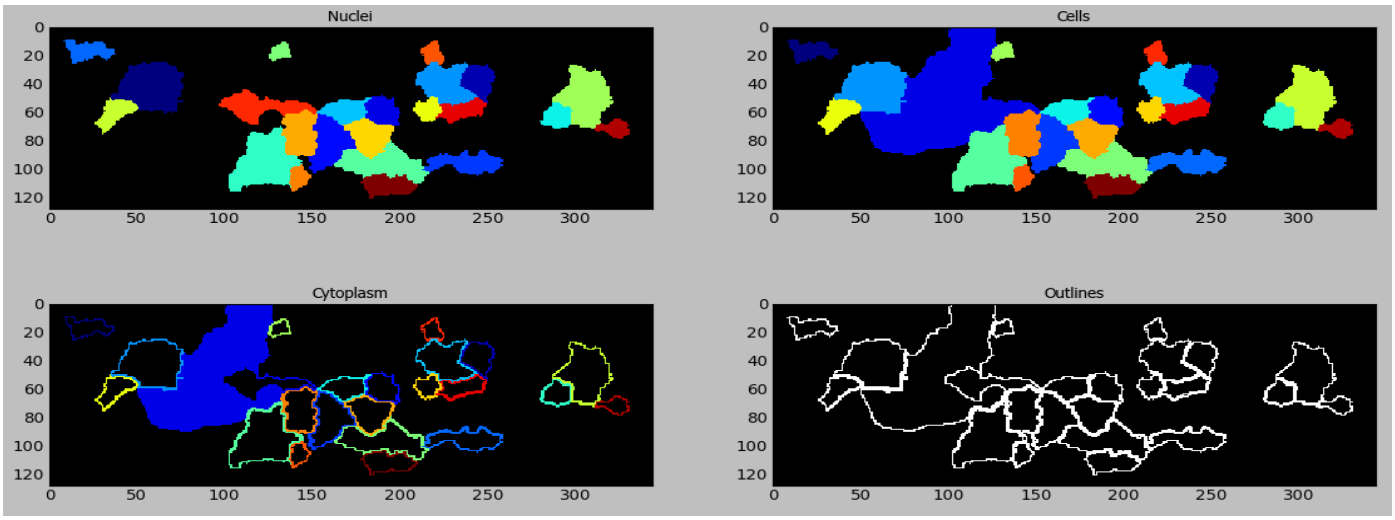

Fig. 12: Photograph of tertiary objects (cytoplasm) identification in image of gastropod haemocytes through software

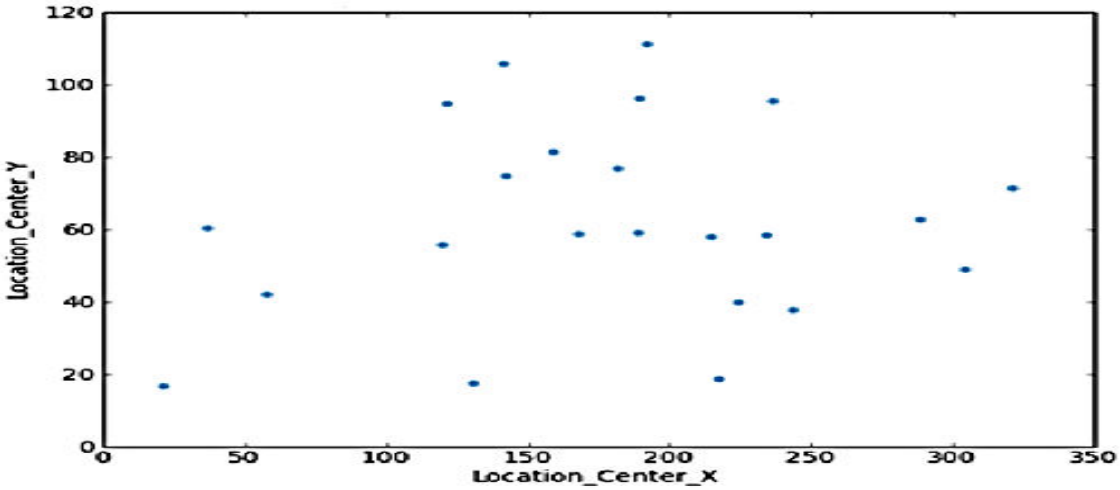

Fig. 13: Scatter plot diagram representation of nuclei, haemocytes, and cytoplasm location through software

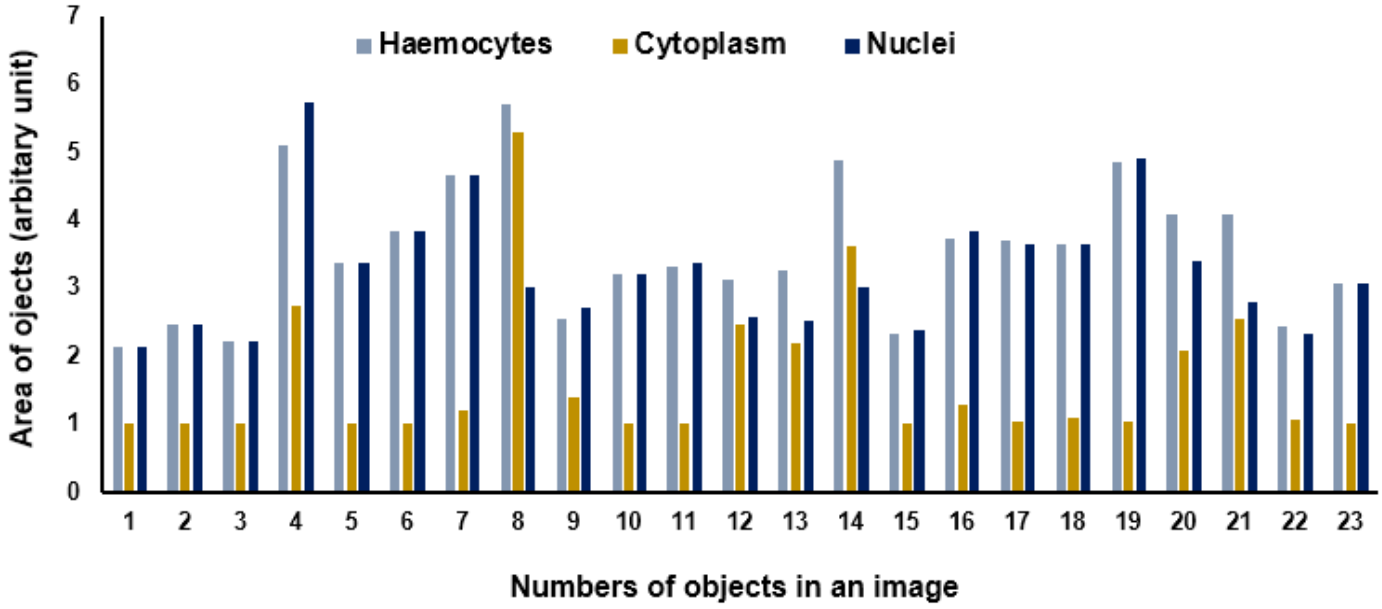

Fig. 14: Histogram of area shape of objects especially haemocytes, cytoplasm, and nuclei (arbitrary unit) in an image

4. DISCUSSION

The present study easily screened objects in the image of Leishman-stained haemocytes of gastropod after automated analysis through CP; an image analysis software. This software can be an alternative of scoring tool for microscopic images in which the quantification of haemocytes as well as measurement of shape of the haemocytes structure through high throughput way [7]. Moreover, the shape of the cells can measure manually under microscope but the measurement of the haemocytes, cytoplasm, and nucleus separately a tedious job and may have possibilities of errors. According to the concept and software manual, the present study evaluated an image containing numerous objects. The cellular and nuclear features can easily be obtained after the analysis of CP software, which is a faster screening tool to know the shape of the haemocytes, cytoplasm, and nuclei in each haemocyte type in numerous haemocytes population.

Internationally, CP has already been approved by several laboratories for image processing research. The researchers have already studied the images of different cell types of microorganism like *Drosophila melanogaster*, yeast colony, and macroorganisms cell types (S2R+ cells, epithelial tissues), various human samples such as TOV21G, prostate gland tissue, stem cells of mesenchymal origin, H1299 lung cancer cell lines, mouse sampleslike cell lines (NIH/3T3), cells of embryos, lung tissue, germ cells and also cell lines (H9c2) of rat model, fish erythrocytes, etc. [7, 12, 17, 26-31]. The present study with Leishman-stained haemocytes of gastropod screened very easily quantification and shape measurement properly by using this software.

5. CONCLUSION

The present study is an approach to screen image of Leishman-stained haemocytes of gastropod to know the number and shape (mean radius) of the haemocytes based on object identification, shape, size by using CP software; an image-based analysis software developed by Carpenter et al. [7]. However, earlier study has been reported majorly in fluorescence-stained images except the study of yeast colony [12] and non-florescent stained cells [26] but studied with few cells. This study can be a suitable tool for biological research for extraction of rich information in image and to easily know the change of objects intensity, shape, size and area of haemocytes, nuclei, and cytoplasm.

6. ACKNOWLEDGEMENT

Authors mention thanks to the developers of CP software.

Conflict of interest

None

Source of funding

None declared

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