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Cell Profiler software: An easy screening tool for DNA damage estimation in fish erythrocytes from comet assay image

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ABSTRACT

DNA fragmentation by single strand breaks (SSBs) or double strand breaks (DSBs) is major concern in genotoxicity research. DNA damages can be easily known through comet assay or single cell gel electrophoresis (SCGE). Since decades, scoring through software for DNA damages in images have been developed by researchers. These softwares depend upon manual scoring on individual comet in a particular interface. The evaluation under software may have biasness and error during scoring by each researcher and few softwares are unable to access easily because many of these are commercial products. However, CellProfiler (CP) image analysis software (Version 2.1.0) is free, easy operation, faster and automated screening by computer itself. An attempt was made to detect DNA damages mainly comet scoring through CP software as whole comet, comet head and comet tail from image of previously studied single cell gel electrophoresis (SCGE) in the peripheral erythrocytes of fish exposed to benzene as experimental image. The results particularly on length and area of whole comet, head and tail were obtained after automated analysis in the CP software. The image processing study was done of the objects present in fluorescence microscopy image to know maximum DNA damage at each cell level for the fish erythrocytes. It was concluded that the present study of image based screening for DNA damages as details of comet and its head and tail evaluation by shape and area in the fish erythrocytes can be a suitable tool for genotoxicity prediction along with risk assessment at DNA level. The shape descriptor as Zernike moments order 0 to 9 can also be suitable parameters to know accuracy of the shape of comet and its head and tail in the image. Finally, high-

throughput automated screening of comet test can help in disease diagnosis and repair mechanisms as well as environmental monitoring of genotoxin(s) within short period of time.

***Keywords*:** Peripheral fish erythrocytes; Comet image analysis; DNA damages scoring; Comet morphology measurement; Image analysis software; CellProfiler software

1. INTRODUCTION

DNA damage for single strand break (SSB) can easily be identified through comet assay (Ostling and Johanson, 1984; Singh et al., 1988; Olive et al., 1990; Talapatra et al., 2004; Banerjee et al., 2008). DNA damage and also repair mechanism study through comet assay is a reliable study in genotoxicity or anti-genotoxicity (Collins and Horvathova, 2001; Collin, 2004; Azqueta et al., 2014). The comet assay is also termed as single cell gel electrophoresis (SCGE) because DNA damage is detected in single cell type of an organism. Researchers have established that the DNA damage occurs due to exposure of physical (irradiation by UV, electromagnetic wave, heat etc.), chemical (inorganic and organic compounds) and biological agents (bacteria, fungus, allelochemicals etc.) in any type of single cell of organisms like invertebrates to mammals, and also human (Ostling and Johanson, 1984; Bolognesi et al., 2004; Talapatra et al., 2004; Banerjee et al., 2008; Petriccione and Ciniglia, 2012, Osipov et al., 2014; Carvalho et al., 2015; Roy et al., 2016) and also repair mechanisms by antioxidants in natural product (Charles et al., 2012; 2014).

Generally comet soring softwares viz. OpenComet, CometScore Pro, CometQ, Comet Assay IV, Komet 7-GLP, CASP Ver. 1.2.2, etc. are available to measure all parameters in each comet such as cell area, coefficient of variance, distribution moment, extent measurement, inertia, mean, mode, optical intensity, skew, standard deviation; in each comet head such as coefficient of variance, distribution moment, DNA content, extent measurement, inertia, mean, mode, optical intensity, skew, standard deviation and for each comet tail viz. length or height, tail moment, olive tail moment, coefficient of variance, distribution moment, DNA content, extent measurement, extent moment, inertia, mean, mode, optical intensity, skew, standard deviation, which reviewed and documented by Kumaravel and Jha, (2006) while comet area, comet intensity, comet length, comet DNA, head area, head intensity, head length, head DNA, head DNA percentage, tail area, tail intensity, tail length, tail DNA, tail DNA percentage, tail moment and olive tail moment described in OpenComet (Gyori et al., 2014) and in other softwares, the parameters viz. DNA head, DNA tail, percent tail DNA, percent head DNA, tail moment, olive tail moment etc. have also emphasized (Konca et al., 2003; Sreelatha et al., 2015; Ganapathy et al., 2016).

Moreover, CellProfiler (CP), Version 2.1.0 is an image analysing, fast screening, non-commercial software, can measure 100 nos. of images within short duration for any cell types such as yeast colony, cell lines, mammalian cells, etc. stained with DNA binding dyes of fluorescent (Carpenter et al., 2006; Lamprecht et al., 2007; Kamentsky et al., 2011; Bray et al., 2015) and non-fluorescent features of nucleus with DNA binding dye in the peripheral fish erythrocytes (Talapatra et al., 2016). Like OpenComet image analysis software, CP is also machine learning algorithm based software to save time and cost, user-friendly and prevent individual eye estimation error (Gyori et al., 2014; Carpenter et al., 2006; Kamentsky et al., 2011; Bray et al., 2015; Talapatra et al., 2016).

The present study was attempted to detect DNA damages from an image of previously studied comet assay or SCGE in the peripheral erythrocytes of fish by using CP image analysis software because several features cannot be identified only under microscope when visualize stained cells or DNA fragments (SSB) onto slides.

2. MATERIALS AND METHODS

2. 1. Selection of image as input in software

The image of ethidium bromide (EtBr) stained DNA damaged peripheral erythrocytes (previously studied comet assay by Talapatra et al., 2004) were processed by using CellProfiler or CP (Version 2.1.0) software. This software was downloaded from the website as http://www.cellprofiler.org/download.shtml.

The input data were incorporated in the present software with some modifications and also with the help of CP manual published through the mentioned link (http://www.cellprofiler.org/linked_files/Documentation/ cp2.1.1_manual_6c2d896.pdf) and published example of comet pipelines (Example FluorescentCometAssay.cppipe and ExampleSilverStainCometAssay.cppipe) for detail description for users (Carpenter et al., 2006; Gonzáleza et al., 2012). In the present study, inbuilt pipeline for fluorescent comet assay was also supported to incorporate several inputs. The CP software interface is depicted on the basis of selected input and analysis modules of pipelines with some modifications for the present study (Fig. 1).

The image was taken from previous research work on benzene induced genotoxicity in peripheral erythrocytes of fish (Talapatra et al., 2004). Herein, the image was selected damaged nucleoids (DNA fragmented nucleoids showed comet like structures). The image was incorporated and analysed in the software as per established input and analysis modules. The original image of benzene induced peripheral fish erythrocytes as experiment was exhibited in Fig 2.

2. 2. Measurement of objects in the image

The measurement of objects as fragmented DNAs appeared like comets were studied through CP software. This is an easy method to quantify individual object by numbers, shape, area, intensity etc. along with measurement of whole comet, comet head, comet tail by an automated algorithm processing in the present software as per method followed by CP manual and comet assay pipelines such as ExampleFluorescentCometAssay.cppipe and ExampleSilverStainCometAssay.cppipe with some modifications (Gonzáleza et al., 2012).

The present study revealed that the measurement of objects in an image was considered as input. For individual parameter viz. calculated of correct illumination, applied of correct illumination, identified primary objects, masking of image, measured objects size and shapes and overlay outlines.

The size and area of data were obtained through several images and various computerized simulation processes and saved as .csv file. Finally, all the data were considered for the automated screening of studied image to detect length and area of whole comet, comet head and comet tail.

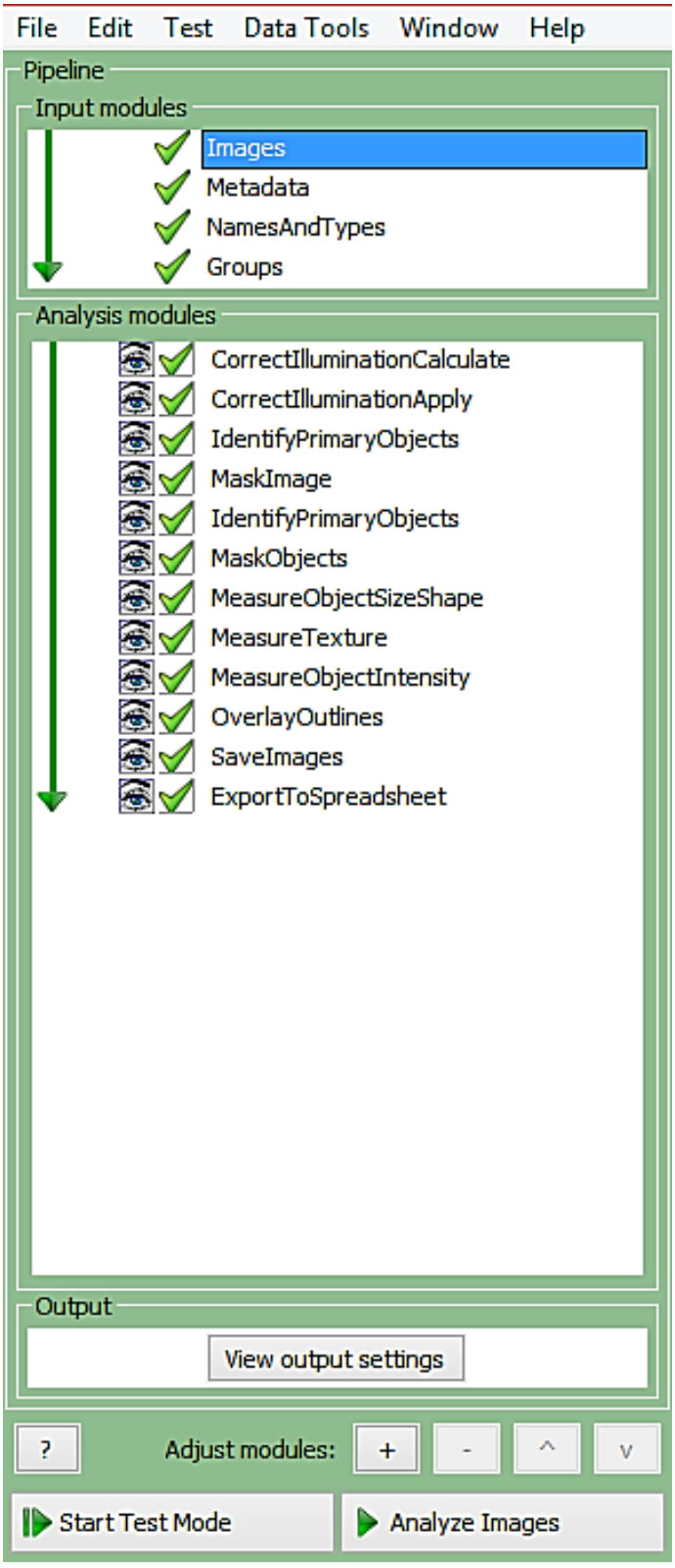

Fig. 1. CP interface of different modules selected for comet assay image

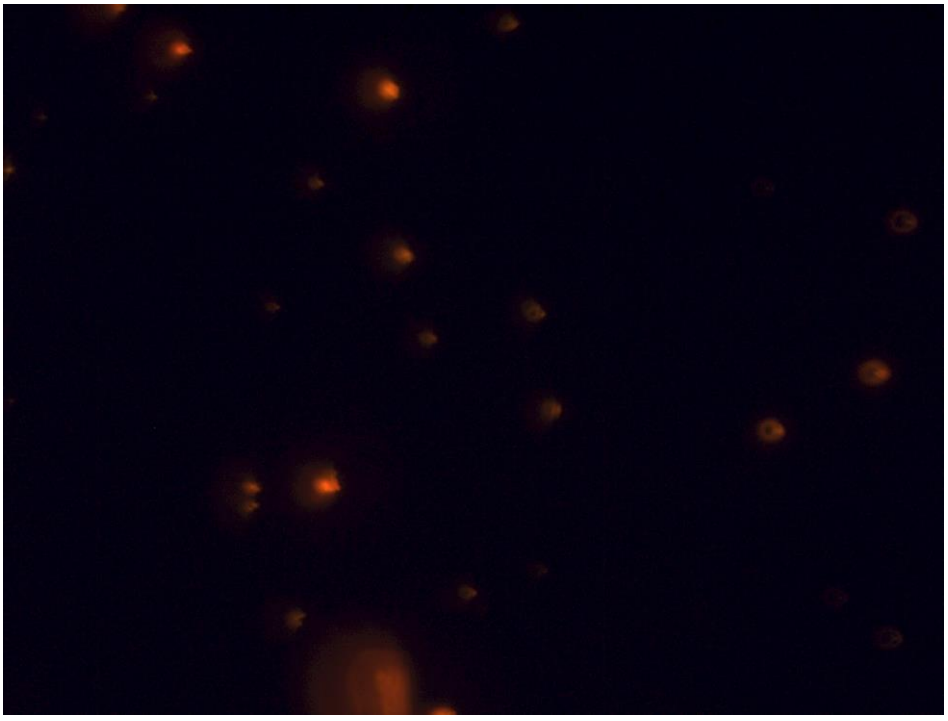

Fig. 2. Original image of comet assay (damaged DNA) in fish erythrocytes.

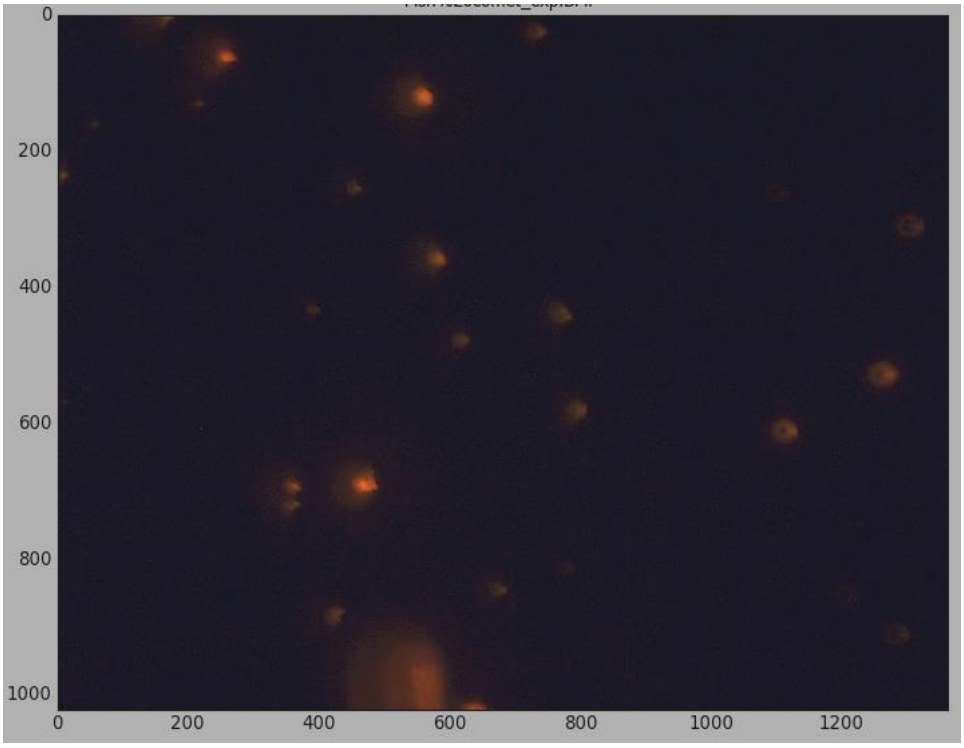

Fig. 3. Image (damaged DNA) of fish erythrocytes and output through CP.

2. 3. Image processing and data gathering for rich information in comets

According to Carpenter et al. (2006), the detail analysis was done by compartmentalize morphology of comets, comets head and comets tail as objects in the image. The features were studied primarily related to object shape of whole comet, head of comet and tail of comet, descriptor based Zernike moments 0 order to 9 order based on total numbers of pixels. The features were compared for whole comet, head of comet and tail of comet for the studied image.

3. RESULTS AND DISCUSSION

The image of EtBr stained comet formed DNA fragments (damaged) as experimental peripheral erythrocytes (Fig. 2) was incorporated in the CP software along with setting of all the selected necessary parameters from pipeline modules as input with some modifications (Fig. 1) and output data were obtained by several image types and .csv files. In the first automated screening, the output image was obtained through CP with a measured x and y position after incorporated as input data (Fig. 3).

The CP software was itself calculated and corrected the illumination in the image and the output as applied illumination calculation and correction images are depicted in Fig. 4 and 5. Generally illumination calculation as well as correction features are an important part of image analysis to create proper quality and sharpen the objects of the studied image and also to maintain uniformities to obtain proper intensity within a particular software (www.cellprofiler.org/examples.shtml). According to Jones et al. (2006), (2009) and Carpenter et al. (2006), illumination calculation and correction parameters in fluorescence image remove noise and found accurate intensity, which cannot visible through human eyes only under microscopic observation.

In case of studied image, the primary object data identification, it was observed that each image has segmentation for each features (Fig. 6, 7 and 8). The whole comet, comet head and comet tail as objects were separately identified by outliner marking. It is interesting to note that CP can be identified clumped objects and their distinct parts through automated image processing algorithm. In this step, segmentation of objects has proceeded and accurate measurement can only be possible for cell types when screening is processed through CP (Carpenter et al., 2006). Herein, we found the measurement of objects in relation to whole comet, head of the comet and tail of the comet and there are 14 comets were identified along with length, area and perimeters, which indicated the rate of DNA fragmentation. The masking and outliner of objects in the image were separately done through CP module pipelines protocol (Fig 9 and 10).

The data of major and minor axis length, maximum radius and maximum Feret diameter (measurement of starting point to ending point distance) for whole comet, comet head and comet tail were obtained for area of each object in image, which determined DNA fragmentation was major and formed long tail had a close resemblance with whole comet because head showed very small compared to tail with a close similarity in previous study (Talapatra et al., 2004).

All the data were exhibited through histogram in Fig. 11 while other two parameters such as area and perimeter were also obtained separately for each object as area of whole comet, comet head and comet tail and it was observed that long tail area of comet detected

maximum fragmentation of strand in DNA in the peripheral erythrocytes of fish when exposed to benzene, which migrated towards anode during minigel electrophoresis had an evidence of previous study (Talapatra et al., 2004). All the data were depicted through histogram in Fig. 12.

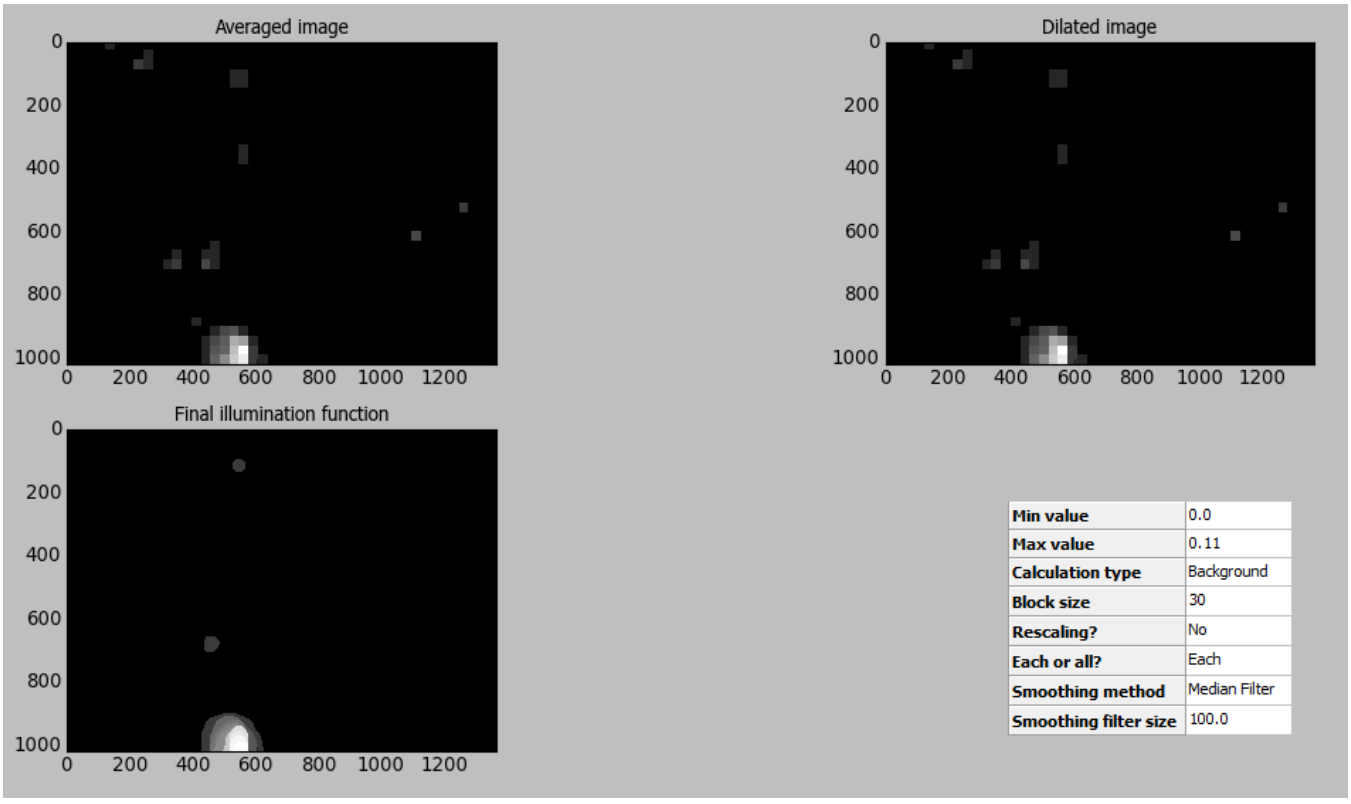

Fig. 4. Output for image correct Illumination calculation.

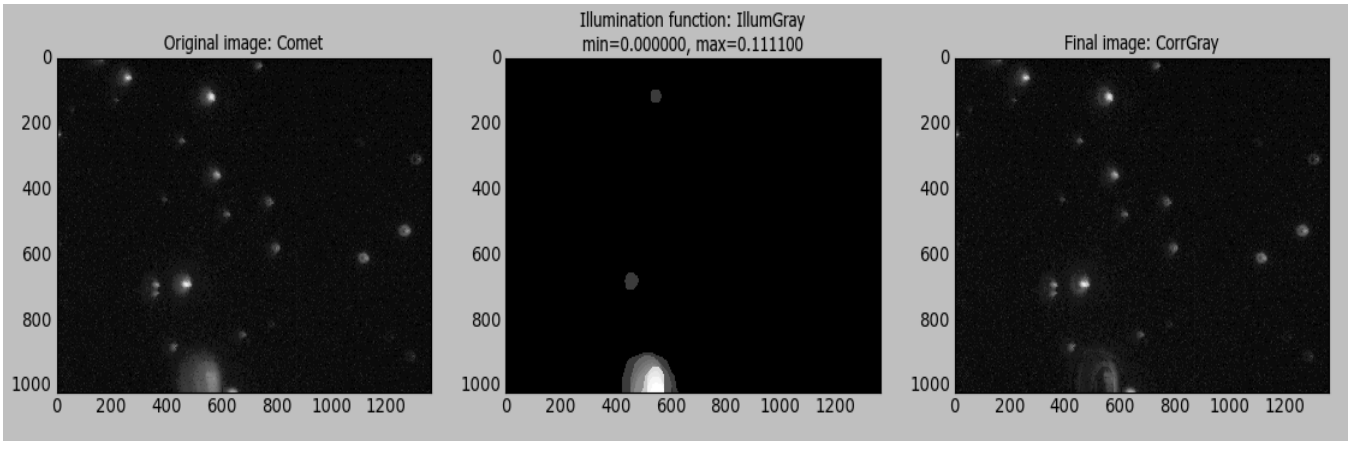

Fig. 5. Output for image correct illumination apply.

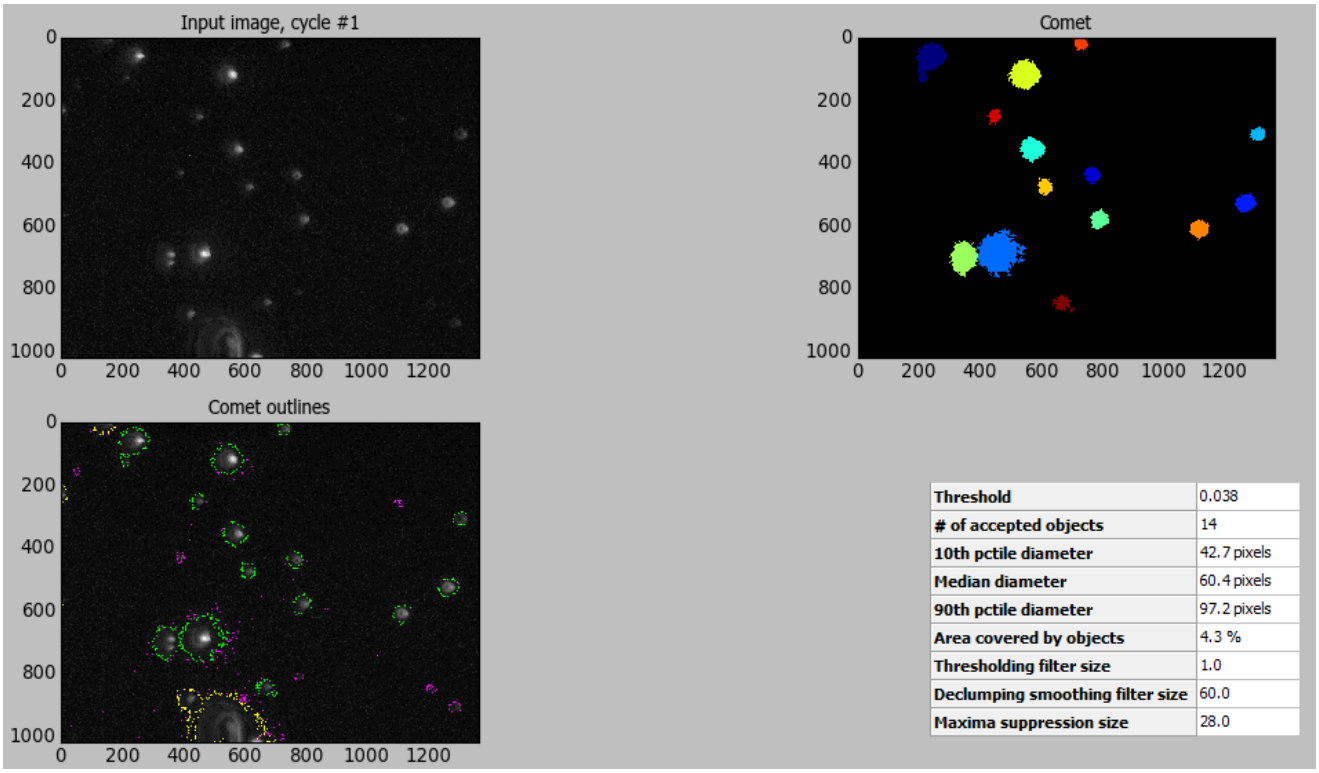

Fig. 6. Output for image identified primary objects as whole comet

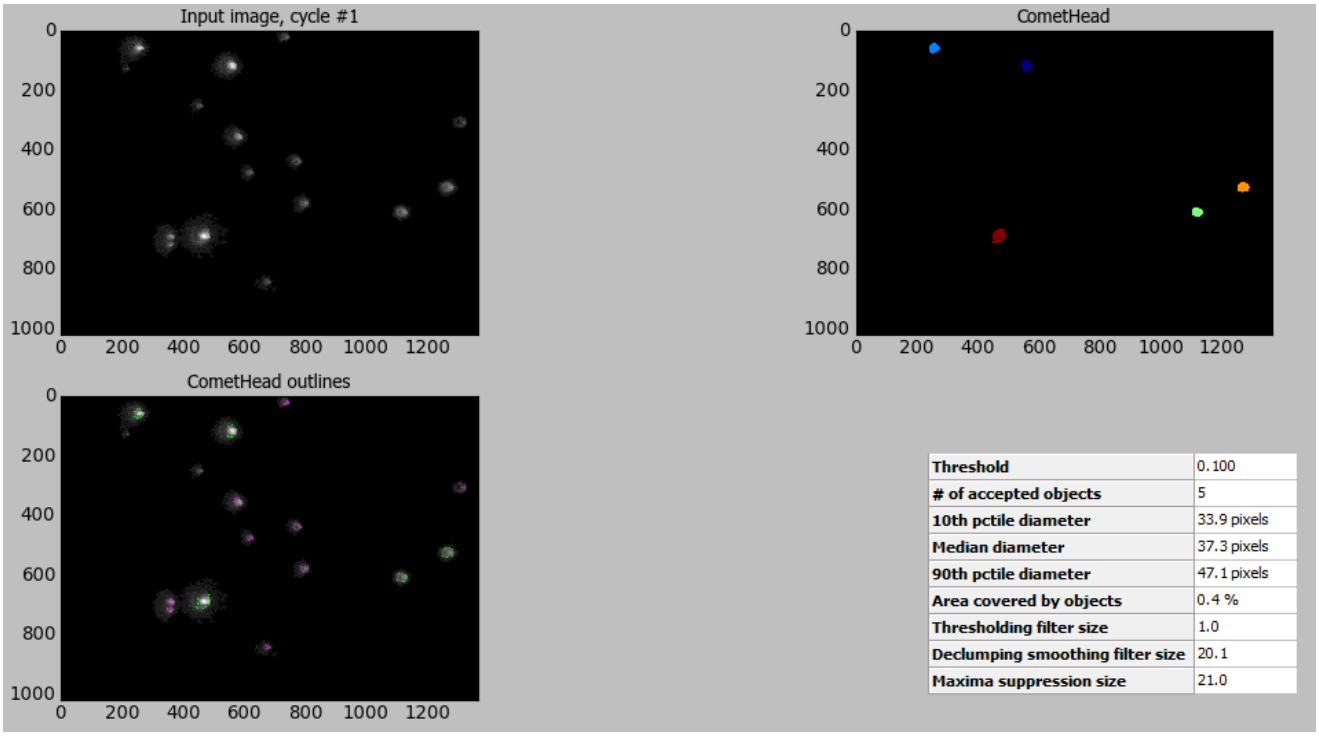

Fig. 7. Output for image identified primary objects as comet head

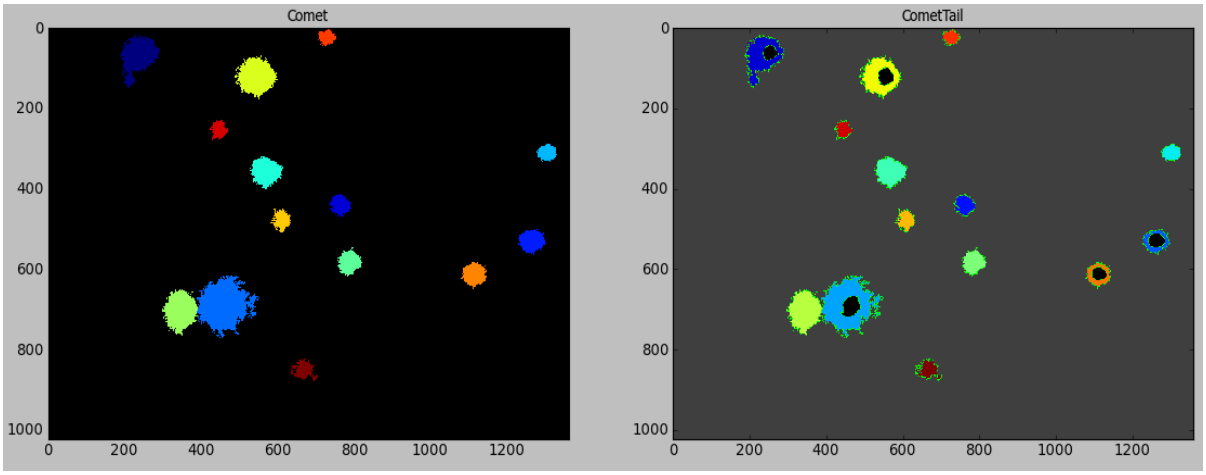

Fig. 8. Output for image identified primary objects as comet tail

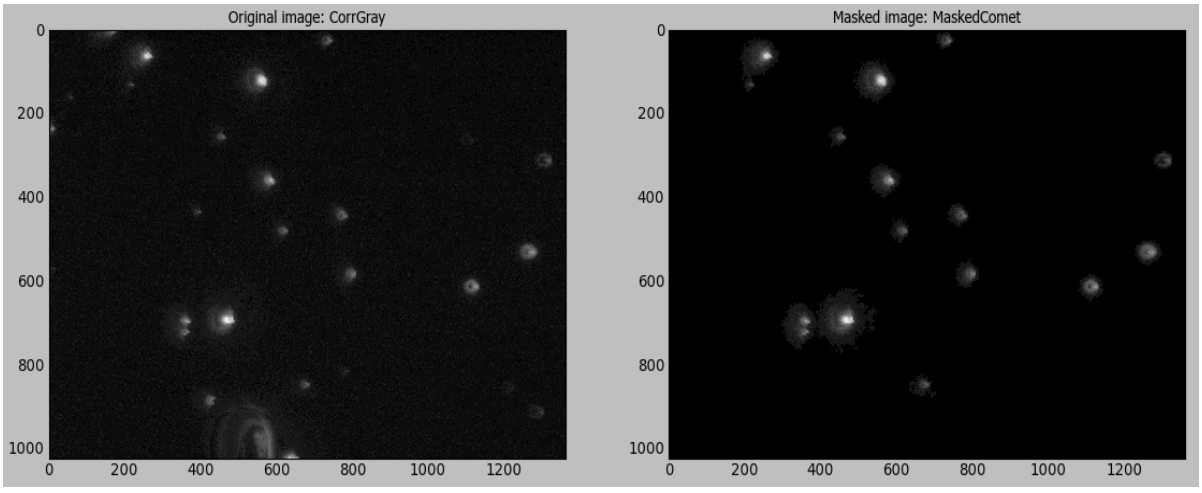

Fig. 9. Output for masked objects in image (masked comet)

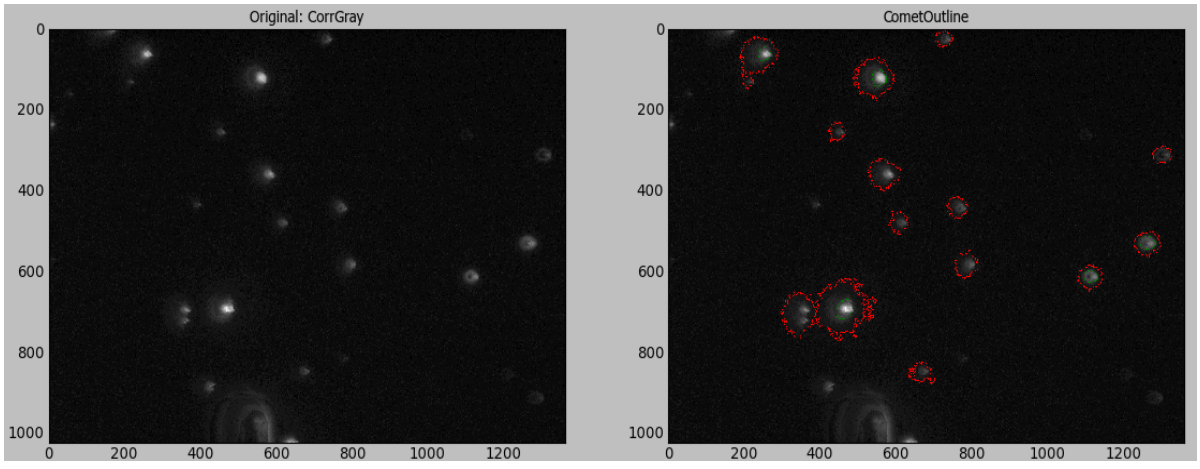

Fig. 10. Output for overlaid objects outlines in image (comet outlines)

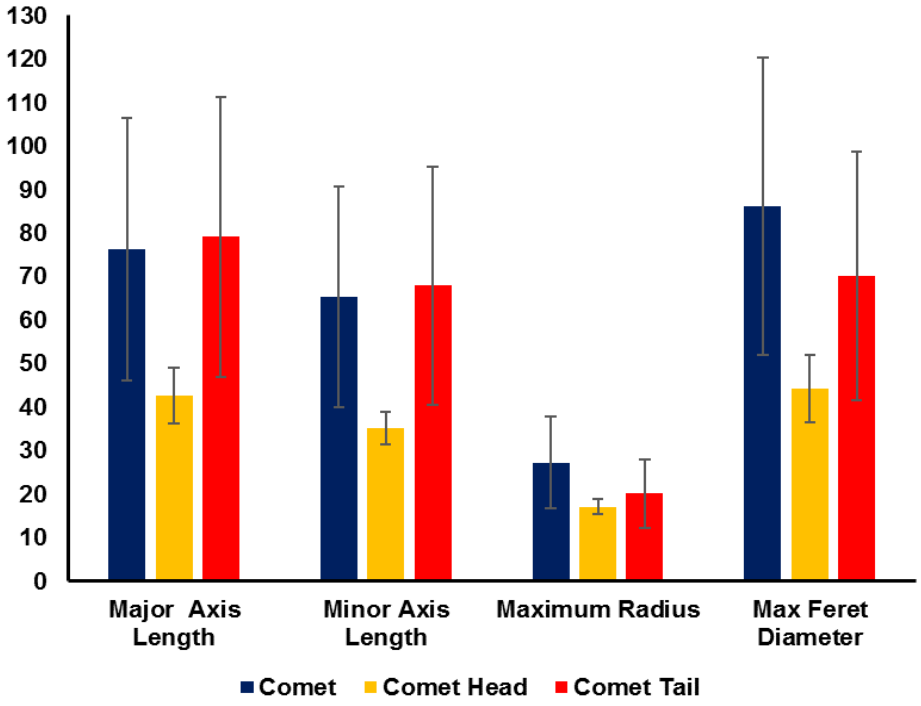

Fig. 11. Histogram of object size data (arbitrary unit)
[n = 14 for comet and comet tail; n = 5 for comet head; Mean ± S.D]

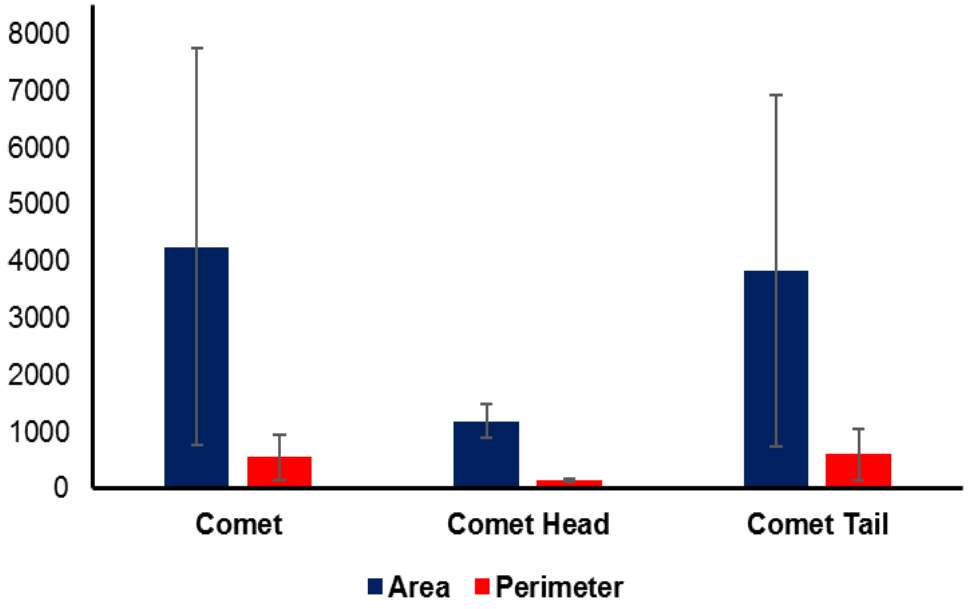

Fig. 12. Histogram of object area data (arbitrary unit)
[n = 14 for comet and comet tail; n = 5 for comet head; Mean ± S.D]

The TriTek CometScore™ (http://tritekcorp.com) software have been used to score data in previous experimental DNA damage study in fish erythrocytes in relation to tail moment value of comet (Talapatra et al., 2004) and found similarities in data for tail length and area of comet as object screening for comet assay image in the present automated screening through CellProfiler. Singh et al. (1988) mentioned in alkaline comet assay that tail length is an important parameter in which the extent of DNA fragmentation can easily be known in each cell, which supports the present tail length obtained in each comet through CP software.

In Fig. 13, the histogram is showing whole comet, comet head and comet tail as object in the image separately for Zernike moments of order 0 to order 9. There was found also close resemblance in between whole comet and comet tail due to higher fragmentation of single strand in DNA and confirmed that benzene is a genotoxic to fish erythrocytes for the studied image (Talapatra et al., 2004). However, Zernike moment is a potent shape descriptor to detect accuracy of each object shape in an image. It was well known in various reports that this screening parameter is an ideal for automated high-throughput applications when little data is provided in algorithm (Khotanzad and Hong, 1990 Zhang and Lu, 2002; Suk et al., 2009; Vorobyov, 2011).

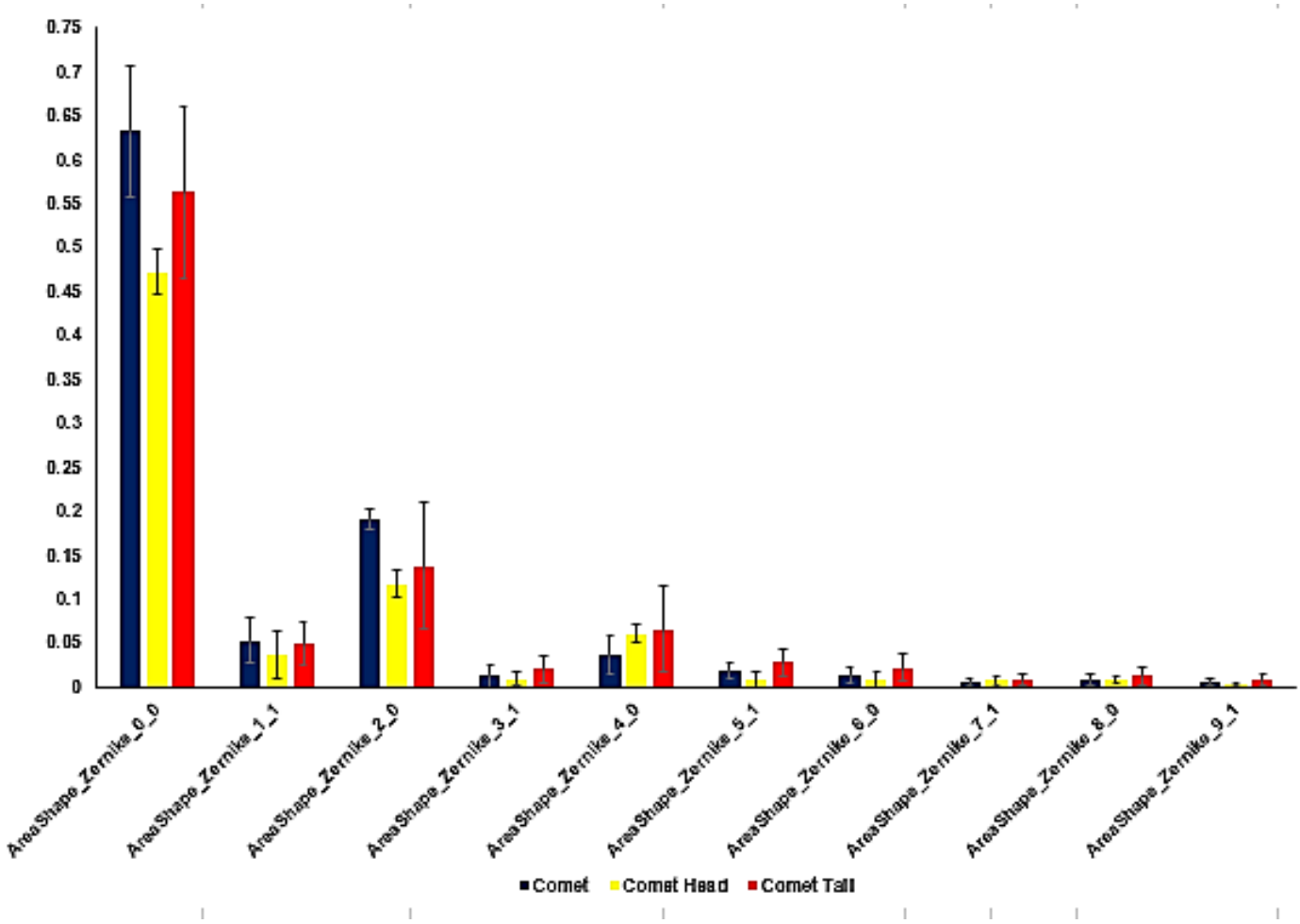

Fig. 13. Histogram of objects Zernike moment data (arbitrary unit)
[n = 14 for comet and comet tail; n = 5 for comet head; Mean ± S.D]

The present findings were determined an easy screening suitable techniques, which may reduce the time for manual screening, remove the eye estimation error and few data, which unable to obtain under manual screening of DNA damage in detail in the comet assay image. The researchers have already been justified that CP software are authentic image based screening software and the generation of data from yeast colony to mammalian cells can be obtained within a short duration (Carpenter et al., 2006; Kamentsky et al., 2011; Bray et al., 2015; Talaptra et al., 2016). It is hypothesized from the present study, CP software able to screen automatically within short duration of about 90 seconds time in an efficient manner and without manual intervention. The present work is based on single image but it is believed that higher numbers of images can be screened with less time to help in clinical research to develops drugs and environmental biomonitoring to know the susceptibility of organisms by genotoxins within polluted area.

4. CONCLUSIONS

It was concluded from the present results of comet assay image, which had an approach to screen images of EtBr stained comets after DNA damage in the peripheral erythrocytes of fish to detect total comet numbers, whole comet, head of the comet and tail of the comet in relation to measurement of length, size, area along with Zernike moment for shape descriptor to detect accuracy of object identification by using CP software, an image processing software developed by Carpenter et al. (2006) and other researchers. However, the comet automated screening to know DNA damage in the erythrocytes of fish with the help of CP software was not studied before but few study with silver stained image for comet assay in CP was documented by Carpenter et al. (2006); Bray et al. (2015) and other developers of CellProfiler Team and developed the comet assay pipeline for the CP software (Example Fluorescent Comet Assay. cppipe and Example Silver Stain Comet Assay.cppipe). It was also found a comparison study with CASP software and CP software and researchers observed an agreement with similar results (Gonzáleza et al., 2012). This study helps to suitable automated high-throughput screening for variety of toxicological and genotoxicological impact by several physical, chemical and biological agents to damage DNA and form comet like structure, which only observed under florescent microscope when stained with fluorescence DNA binding dye or silver stained. But under fluorescence or bright-field microscopy, all information in details unable to observe. Therefore, this automated screening tool helps in biological research to detect rich information in an image when automatically segmented through CP software. In other words, automated screening of comet assay can help in biomedical research as well as environmental monitoring within short period of time.

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