Automated scoring as numbers and shape measurementof bacterial colony by using Cell Profiler tool

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ABSTRACT

The present study examined gray image of bacterial colony by using CP tool (version 2.1.0) for obtaining numbers and area shape (mean radius). An image analysis for bacterial colony (Gray scale) was processed by using CP tool (version 2.1.0).For the input data,CP program and an example "pipeline" file were also downloaded from this tool and incorporated in the present tool. All the output data were obtained through the incorporated image as per severalcomputationalsimulations and saved as .csv file.In the present study, a total 255 colonies were identified in the image of bacterial colony. Among these colonies, 80 nos. of tiny colonies, 152 nos. of small colonies and 23 nos. of large colonies as per mean radius were scored. It is concluded that scoring and shape measurement of bacterial colony might be performed easily from an image, which is an automated image processing scoring technique.

Keywords: Cell Profiler tool, Image processing, Bacterial colony, Automated analysis

Introduction

Animage analysis software namelyCellProfiler (CP), is a freely available, couldbe capable of handling 100 nos. of images of any cell types from yeast colony to mammalian cells (Carpenter et al., 2006; Bray et al., 2015).

Many experiments in a biology laboratory involve visual inspection, such as examining yeastcoloniesorgrowthpatchesonagarplates,orexamininglivestainedcellsamples bymicroscopy (Carpenter et al., 2006;Bray et al., 2015).

The image analysis can easily be done after acquiringimagesandanalyzedautomaticallyby usingimageanalysis tool as persveral advantages such as less tedious, more objective and quantitative, and, no visual error, proper measurementof shape of objects viz. cells, cytoplasm, nuclei, colonies, etc. with a prper input set up (Carpenter et al., 2006; Bray et al., 2015; Talapatra et al., 2016; 2021).

This unit outlines a protocol for the automated counting and analysis of yeast colonies growing on agar plates; however, the methods described can be adapted to a wide variety of biological “objects” and can be used to measure a wide variety of features for each object studied earlier in CP(Carpenter et al., 2006; Lamprecht et al., 2007; Kamentsky et al. 2011; Bray et al., 2015; Talapatra et al., 2016; 2021).

In an earlier study, Bray et al. (2015) evaluated through a pipeline of colour image of yeast colony while the present study examined gray image of bacterial colony by using CP tool (version 2.1.0).

Materials and Methods

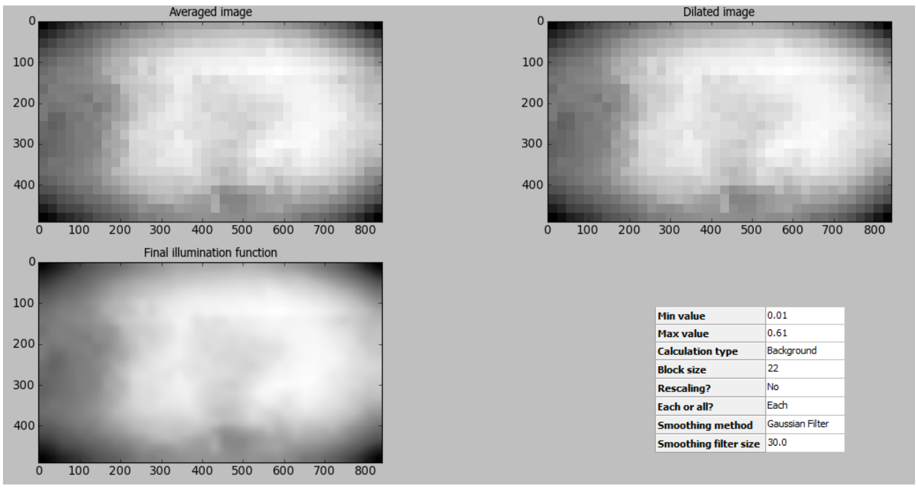
In the present study, an image ofbacterial colony (Gray coloured) was processed by using CP(version 2.1.0)tool (Fig 1).This software was downloaded from the designated website (http://www.cellprofiler.org/download.shtml). For the input data,CP program and an example “pipeline” filewere also downloaded from this tool as per Bray et al. (2015) and incorporated in the present tool. The input interface is depicted in Fig 2. The step of image processing is exhibited in Fig 3A-H. All the output data were obtained through the incorporatedimage as per severalcomputationalsimulations and saved as .csv file.

Fig 1: An input image of bacterial colony used in the present study

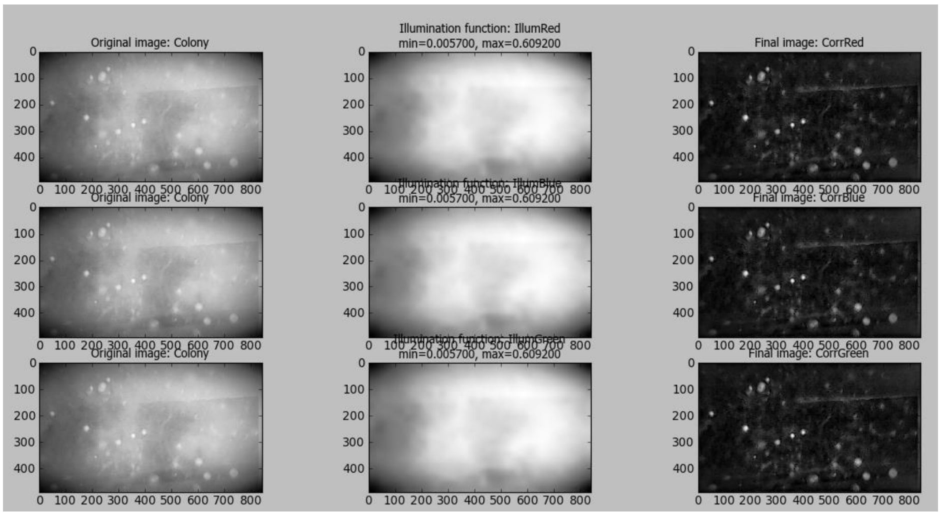
Fig 2: Input interface of CP tool used for present study

A. Original image (Gray coloured) retrieved from CP tool

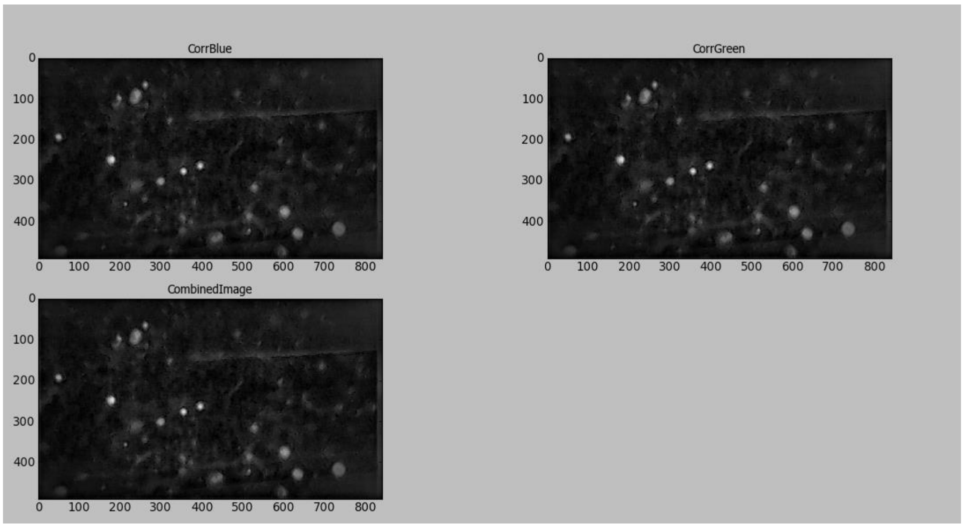
B.



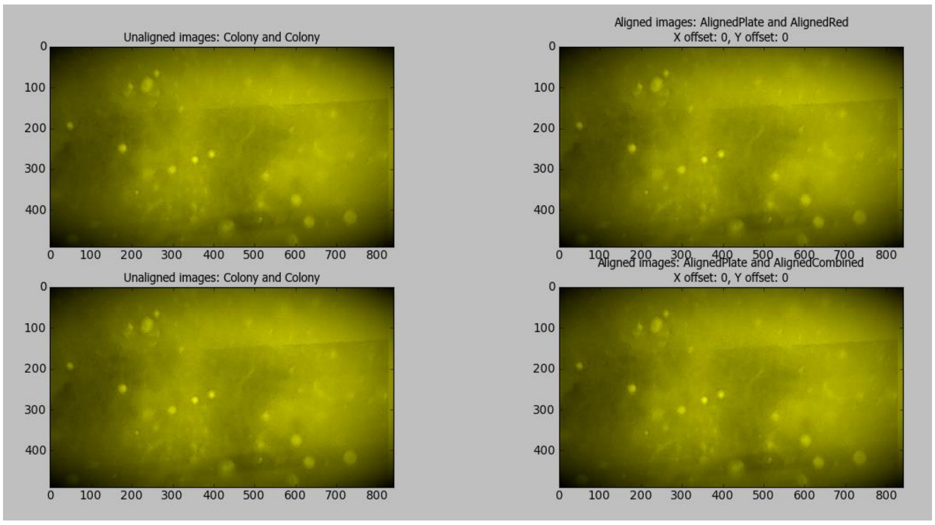
B. Calculation of correct illumination of image in CP tool



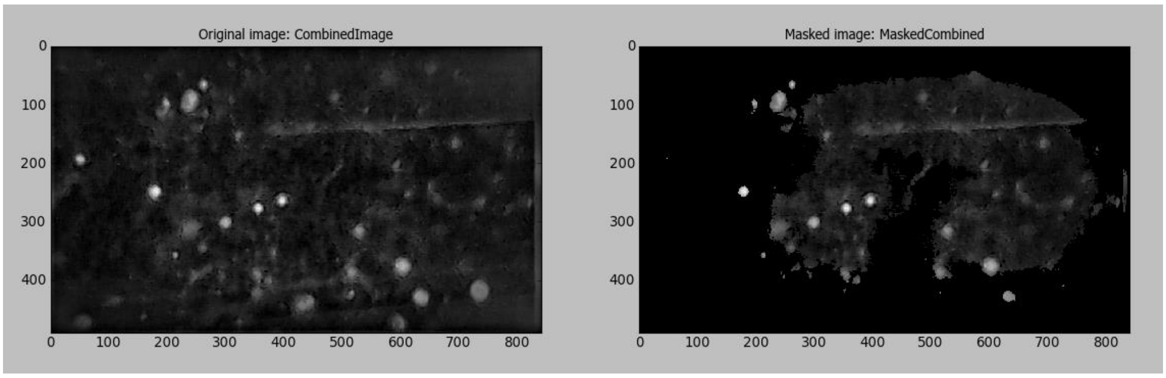
C. Application of correct illumination of image in CP tool



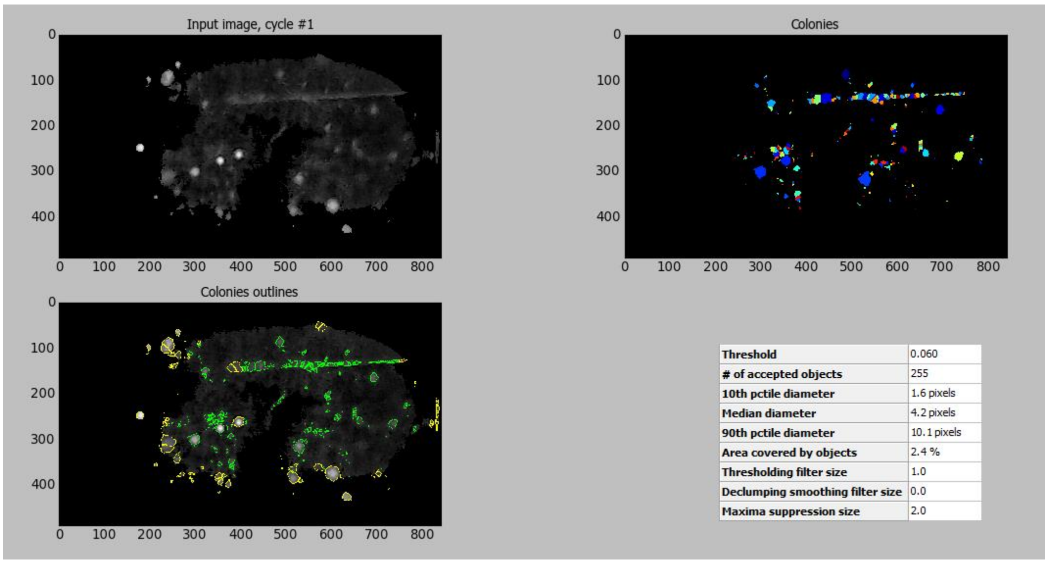
D. Mathematical calculation of image in CP tool



E. Alignment of image in CP tool

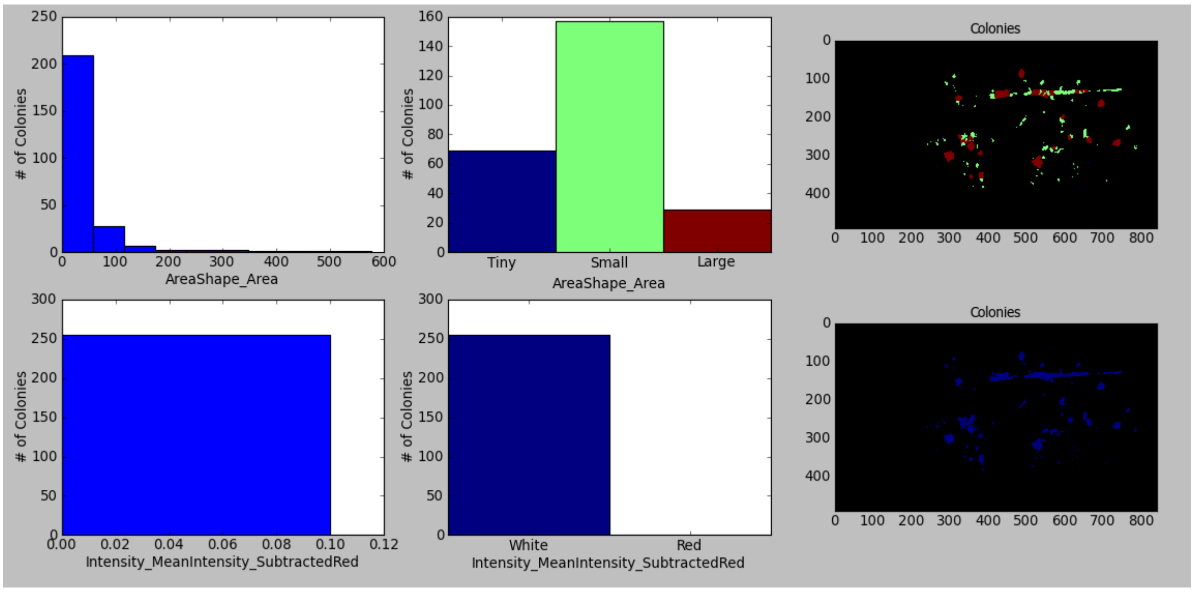


F. Masking of image in CP tool



Threshold	0.060
# of accepted objects	255
10th pctile diameter	1.6 pixels
Median diameter	4.2 pixels
90th pctile diameter	10.1 pixels
Area covered by objects	2.4 %
Thresholding filter size	1.0
Declumping smoothing filter size	0.0
Maxima suppression size	2.0

G. Identification of primary object of image in CP tool



H. Area shape of object of image in CP tool

Fig 3A-H: Different steps of image processing through CP tool

Results and Discussion

In the present study, a total 255 colonies were identified in the image of bacterial colony. Among these colonies, 80 nos. of tiny colonies, 152 nos. of small colonies and 23 nos. of large colonies as per mean radius were scored (Fig 4-6).

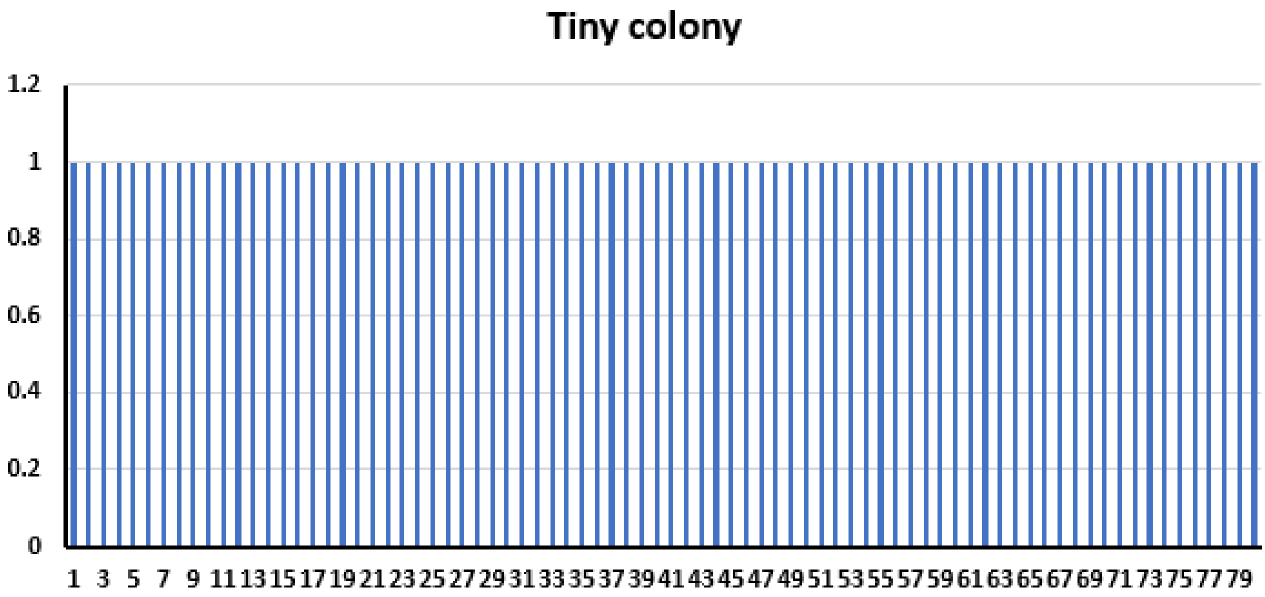


Fig 4: Area (mean radius) of tiny colonies

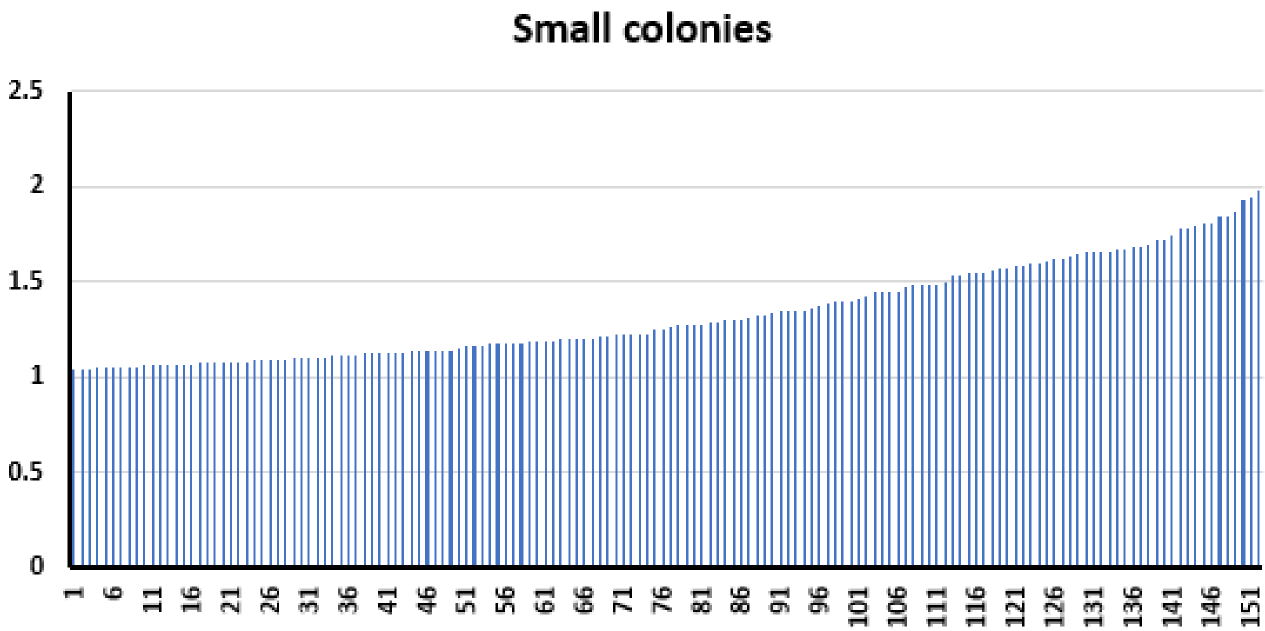


Fig 5: Area (mean radius) of small colonies

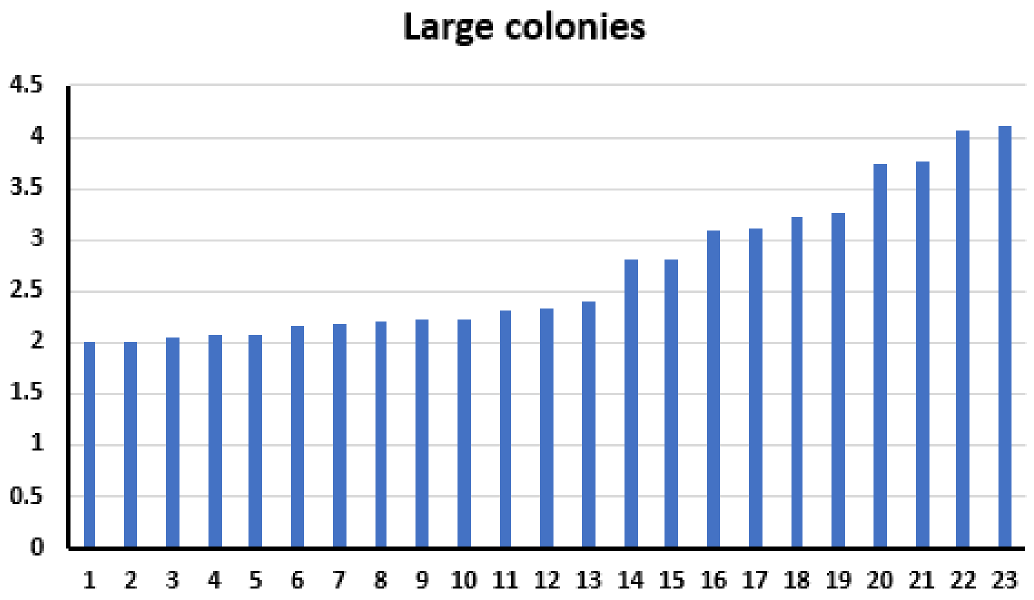


Fig 6: Area (mean radius) of large colonies

In worldwide context, CP tool has already been used by many investigators for identifying a varietyof biological processes in different cell types and organisms, viz. yeast colony,different cell types, etc.(Carpenter et al., 2006; Moffat et al., 2006; Bray et al., 2015; Talapatra et al., 2016; 2021).Lamprecht et al. (2007)documented that CP tool has also modified for the measurement of yeast colonies, yeast growth patches, wounds healing assays and tumours quantification, which is supported the present study.According to Chiang et al. (2015), manual counting of bacterial colonies can be time-consuming and imprecise while automated screening is suitable for obtaining faster results.

Conclusion

In conclusion, present study attempted to screen image (Gray scale) of bacterial colony on the basis of object identification especially numbers and shape (mean radius) by using CP tool, an image-based analysis software. However, the previous study has been studiedon colour image of yeast colony,but we performed with the image of bacterial colony (Gray scale). This study can be a beneficial findinginmicrobiological research to extract the rich information especially numbers and shape of coloniesin an image.

Acknowledgement

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Conflict of interest

Authors declare no conflict of interest.

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