International Journal of Pharmaceutical Research and Applications
Volume 6, Issue 3 May - June 2021, pp: 965-967 www.ijprajournal.com ISSN: 2249-7781

ImageJ for counting of labeled bacteria from smartphone-microscope images

Ibrahim Mahmoud Al-Osta1,*,Marwa Saleh Diab1 and Sundus Abdu Salam Al-Shreef1

1. *Elmergib university, Faculty of pharmacy, Department of pharmacology and clinical pharmacy.*

Date Of Submission: 01-06-2021 Date Of Acceptance: 14-06-2021

ABSTRACT

Objective:The manual counting of gram stained bacteria examined under a microscope becomes difficult when a large number of bacterial cells exist in a microscopic field. The present study was aimed to ease this problem by applying ImageJ software to counting of gram stained bacteria.

Method: This experiment was conducted on Elmergib university, faculty of pharmacy laboratories (Al-Khoms city-Libya).In this study, a microscopic image of a gram stained bacterial cells captured using a student's smartphone, treated and the bacterial cells were then easily and automatically counted using ImageJ.

Results:According to ImageJ reading, the total number of bacterial particles appeared in the field of a microscopic image were 332 cells.

Conclusion: Direct staining and visualization of organisms for counting can benefit greatly from the use of ImageJ software. This method is less expensive, less contamination and less laborious than other methods and is more rapid and reproducible than counting using manual microscopy methods.

Key word: ImageJ, bacterial cells, automated cell counting.

I. INTRODUCTION

Cell counting is a general quality control analysis in research. Manual bacterial cell counting is simple but very time-consuming. Because of the human factor it is as well very subjective and can be variable. Alternatively, hence with the advancements in medical and biological sciences, imaging has become an increasingly important discipline. There are various image processing softwares available but are usually not flexible and do not allow complex manipulations on images. One of the softwares that might ease this disciplineis ImageJ[1, 2]. . ImageJ is a very popular public domain Java image processing and analysis program that was developed at the National Institutes of Health and the Laboratory for Optical and Computational Instrumentation (LOCI, University of Wisconsin) by Wayne Rasband[1, 2]. Its source code is freely available, so that users have complete freedom to run, copy, study, distribute, change and improve the software[3].In this study, ImageJ program was applied to images that obtained from gram stained bacterial smear. The manual enumeration of gram stainedbacteria examined under a microscope becomes difficult when a large number of particles exist in a microscopic field. The small size of these organisms usually makes manual counting difficult as numbers of organisms increase. Here we have applied ImageJ to counting of gram stained bacteria automatically.

II. METHODS

1-Bacterial staining

Bacterial sample was taken from a volunteer's mouth and amonolayer of the sample was spread on glass cover slips and then gram stained. In brief[4], bacterial gram staining involves three processes: staining with a water-soluble dye (crystal violet), decolorization with alcohol, and counterstaining, usually with safranin[5]. Due to peptidoglycan layer thickness differences in the cell membrane between Gram positive and Gram negative bacteria, Gram positive bacteria that have a thicker peptidoglycan layer retain crystal violet stain during the decolorization process[6], in the final staining process, while Gram negative bacteria lose the crystal violet stain and are instead stained by the safranin [7],Both Gram-positive bacteria and Gram-negative bacteria pick up the counterstain. The counterstain, however, is unseen on Gram-positive bacteria because of the darker crystal violet stain[5].

2-Digital image capture

International Journal of Pharmaceutical Research and Applications
Volume 6, Issue 3 May - June 2021, pp: 965-967 www.ijprajournal.com ISSN: 2249-7781

Images of gram stained bacteria were captured by a smartphone (Samsung X7). the images were then transferred to the pc and treated using ImageJ. The method for enumeration of gram stain using ImageJ required the image file to be converted from RGB color to 8-bit grayscale.

3-ImageJ automated counting

Automated counting of the bacterial particles uses threshold algorithms to discriminate the features of interest from background. To set the counting threshold following opening the selected image, the following commandswere used: Image > Adjust > Threshold > select algorithm to be applied > Apply. The image was converted to a binary image by selecting Process > Binary > Make binary. Bacterial particles were counted using the commands: Analyze > Analyze Particles, with the upper and lower limits for the particle size set at 0–infinity, selected to show outlines and checked box to summarize the results. Each counted particle was outlined and numbered in a new window.

III. RESULTS AND DISCUSSION

The collected image was treated and the bacterial cells were counted using ImageJ. The total number of bacterial particles was 332 cells (figure. 1). This method is less expensive, less possible contamination and less laborious than other methods and is more rapid and reproducible than counting using manual microscopy methods.

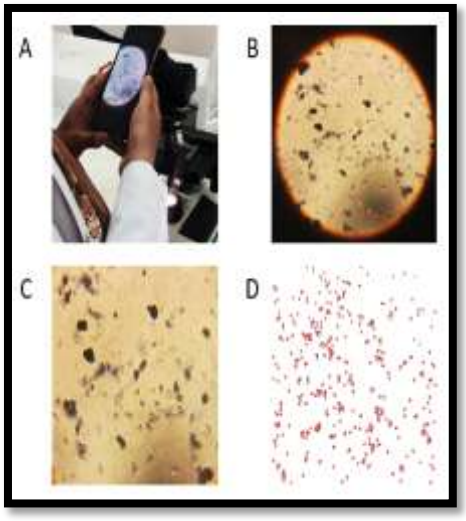

Figure 1.Bacterial cell counting using ImageJ.

A. Microscopic image of the bacterial cells captured using a student's smartphone. B. a collected image by the smartphone. C. zoom in from B. D. Image C is treated using ImageJ and bacterial cells was counted using ImageJ.

IV. CONCLUSION

ImageJ comprises many image analysis capabilities, including functions for calculating area, measuring distances and counting. Direct staining and visualization of organisms for counting can benefit greatly from the use of ImageJ software.It can measure distances and angles[3]. It can create density histograms and line profile plots[1]. It supports standard image processing functions such as contrast manipulation, sharpening, smoothing, edge detection and median filtering[3]. Because of the human factor manual bacterial cell counting is very time-consuming, very subjective and can be variable[8].Alternatively,Automated bacterial cells counting using ImageJ has the advantage that it has a lower error rate per sample and does not suffer from the subjectivity inherent to manual cell counting. Moreover, automation benefits from a high reproducibility compared to manual counting.With current setups of ImageJ, there are more detailed analysis reports available, including graphical display of the cells counted. Furthermore with cloud data storage, the operator can reanalyze data later[1]. This may provide new results otherwise overlooked.Therefore, we –and others-[8]suggest the application of the ImageJ program as an alternative method to manual quantification of bacterial cells.

REFERENCES

[1]. Collins, T.J., ImageJ for microscopy. BioTechniques, 2007. 43(1S): p. S25-S30.
[2]. Schneider, C.A., W.S. Rasband, and K.W. Eliceiri, NIH Image to ImageJ: 25 years of image analysis. Nature methods, 2012. 9(7): p. 671-675.
[3]. Dougherty, G. and P. Cambridge University, Digital image processing for medical applications. 2014, Cambridge: Cambridge University Press.
[4]. Leboffe, M.J. and B.E. Pierce, Microbiology : laboratory theory and application : essentials. 2019.
[5]. Coico, R., Gram staining. Curr Protoc Microbiol, 2005. Appendix 3: p. Appendix 3C.
[6]. Beveridge, T.J. and J.A. Davies, Cellular responses of Bacillus subtilis and Escherichia coli to the Gram stain. Journal of bacteriology, 1983. 156(2): p. 846-858.

[7]. Sandle, T., Pharmaceutical microbiology : essentials for quality assurance and quality control. 2016.
[8]. Stolze, N., et al., Automated image analysis with ImageJ of yeast colony forming units from cannabis flowers. Journal of microbiological methods, 2019. 164: p. 105681-105681.