Interactive/Automated Method to Count Bacterial Colonies

João Ribeiro [1]
Ramiro Martins [1,2]
Fernando C. Monteiro [1]

[1] **IPB - Polytechnic Institute of Bragança, Portugal**

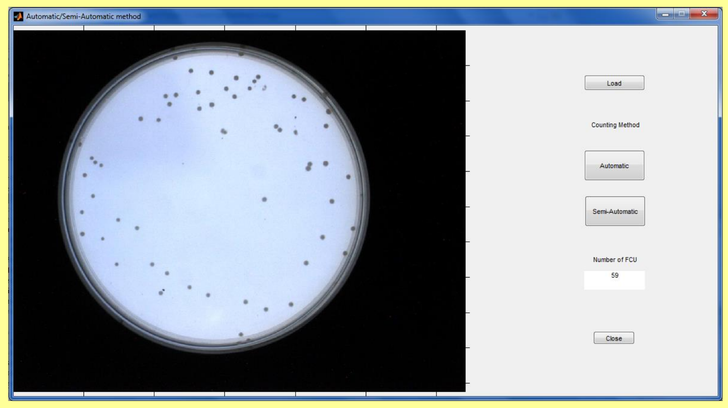
[2] **Laboratory of Separation and Reaction Engineering**

Introduction

The growth and maintenance of bacteria on agar plates (Petri dishes) has long been a common practice in microbiology.

The number of colonies in a culture is usually counted manually to calculate the concentration of bacteria, however, this process is time-consuming, tedious and error prone.

We propose an interactive counting system and a fully automated counting system capable of control these problems.



ABSTRACT

This study proposes an interactive counting system and a fully automated counting system using image processing methods which are capable to reduce the manpower and time required for counting colonies while taking account colonies both around the central area and boundary areas of a Petri dish.

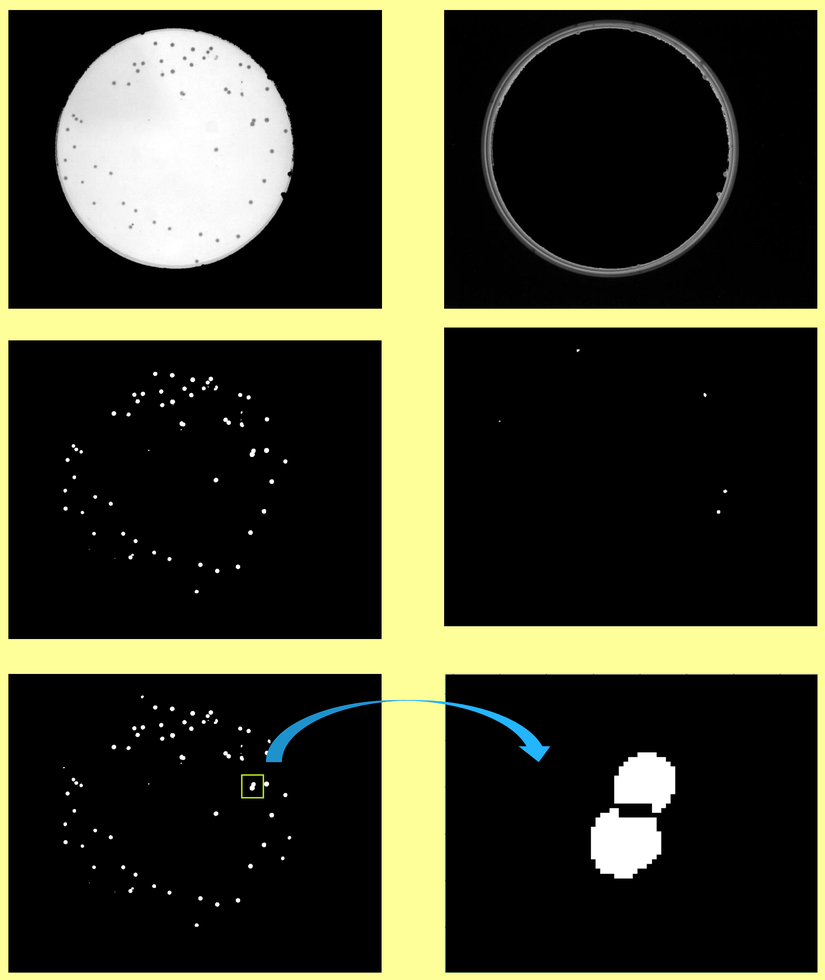
Materials and Methods

In the pre processing step, a mean filter was applied to remove noise and artifacts from the image and a mask was calculated with the intent to multiply it to the original image in order to obtain a image containing only the Petri dish area. The next step was threshold binarization to separate the central area from the rim area.

To segment the colonies from the central area and rim area the bottom-hat transform was applied, followed a binarization. In the rim area, to distinguish colonies from the border of the Petri dish, were used the eccentricity, areas, major and minor axis length .

The next step was identify if the colonies as units or clusters, through the mean area and the eccentricity. If an object had a big area or a eccentricity bigger than 0.5 it was considered as cluster.

The final step was defined by the user initial. The automated count uses a watershed operation to separate the clustered colonies and then count them, and the interactive method uses the click of the user on the image.

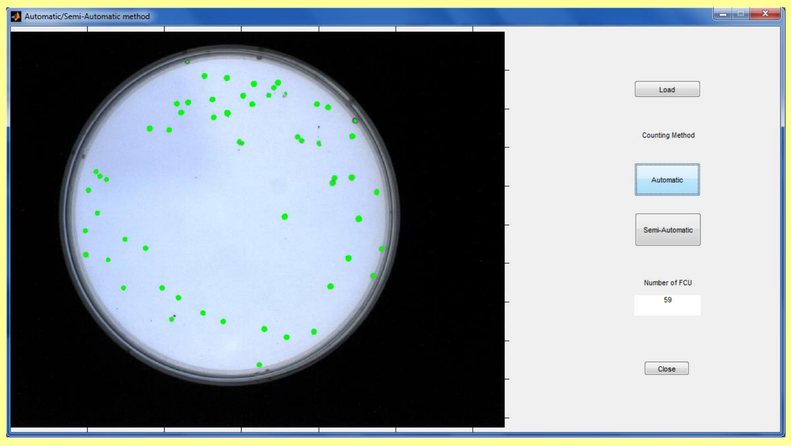


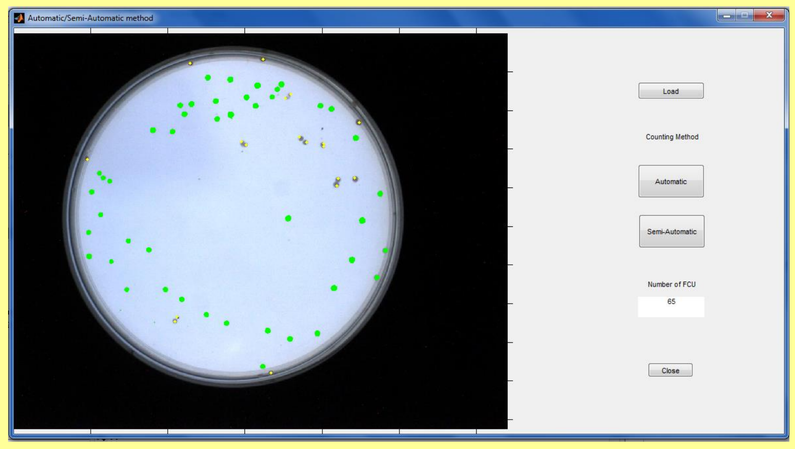
CONCLUSIONS

A Automated bacterial count system is proposed based on bottom-hat transform and on a watershed operation. This shows acceptable results comparing with the other methods. The main difficulty of this system is count some colonies, that presents the same color, on the border of the Petri dish,

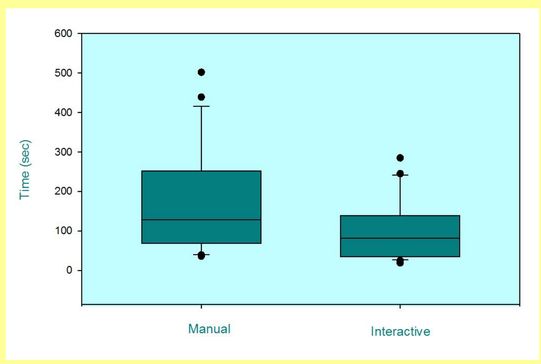
A new approach, the Interactive count system, is suggested based on the interactivity between user and system . This method compensate the failures of the automated systems, presenting results similar and with the same quality of the human count, with an important characteristic, the reduce of time that takes to count an image.

Experimental Results





Method	Measure	
Manual	Precision	0,9879
	Recall	0,988
	F-measure	0,9876
	APE(%)	2,4881
Automatic method	Precision	0,9848
	Recall	0,8698
	F-measure	0,9188
	APE(%)	14,926
Interactive	Precision	0,9808
	Recall	0,9937
	F-measure	0,9869
	APE(%)	2,679
NICE	Precision	0,9607
	Recall	0,878
	F-measure	0,9055
	APE(%)	18,881
Open CFU	Precision	0,9915
	Recall	0,918
	F-measure	0,9514
	APE(%)	9,0881



Method	Time reduction (%)
Interactive	40,10
Automated	94,14

It is possible to observe the different results of automated and interactive counting on the two figures above. The automatic counting fails on the colonies in the border of the Petri dish, although, the interactive method compensate these failures.

In the first table, the 21 images were analyzed and evaluated. The Precision values are similar for the all methods. From Recall, F-measure and APE values, we can see that the proposed automated method and the NICE show the worst results, being the proposed interactive method the best method comparing with the manual counting.

The box graph shows the statistics for the time (in seconds) for each method (manual and interactive).

The second table, shows the reduction (%) of the time that the interactive and the automated methods have, comparing with the manual counting.

REFERENCES

- Geissman Q., "OpenCFU, a new free and open-source software to count cell colonies and other circular objects", PLoS ONE, vol.8(2), p. e54072, 2013.
- Clarke M L., Burton R. L., Hill A. N., Litorja M. & Muhlemann K., "Low-Cost, high-throughput, automated counting of bacterial colonies", Cytometry A, vol.77(8), pp. 790-797, 2010.
- Chiang P. J., Tseng M J., He Z. S., Li C. H., "Automated counting bacterial colonies by image analysis", Journal of Microbiological Methods, vol. 108, pp. 74-82, 2015 Jensen, Simulation of advanced ultrasound systems

CORRESPONDING AUTHOR
Fernando C. Monteiro
E-mail: monteiro@ipb.pt
Tel. (+351) 273 303 012
www.estig.ipb.pt

EJI 2015
III Encontro de Jovens Investigadores do IPB
Bragança - Portugal