RECPAD 2015

21st Edition of the

Portuguese Conference on Pattern Recognition

University of the Algarve

Instituto Superior de Engenharia

Edited by

João M. F. Rodrigues

Pedro J. S.Cardoso

Roberto Lam

Mauro Figueiredo

Faro, October 30, 2015

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UNIVERSIDADE DO ALGARVE
INSTITUTO SUPERIOR DE ENGENHARIA

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APRP

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Conference Program

PROGRAMME OVERVIEW

29TH OF OCTOBER

20h15 - 20h30	Registration*	EVA Hotel restaurant hall
20h30	Welcome dinner*	EVA Hotel restaurant

* - Limited to the registrations with dinner included (Localization: `http://goo.gl/uA3fCw`)

30TH OF OCTOBER

Venue: Instituto Superior de Engenharia
University of the Algarve, Campus da Penha (`http://goo.gl/GhK8iA`)

09:30 - 10:00	Registration	Room 6
10:00 - 10:15	Welcome session	Anf. José Silvestre
10:15 - 11:00	Poster session I	Hall
11:00 - 11:30	Coffee break	Room 6
11:30 - 12:30	Poster session II	Hall
12:30 - 14:00	Lunch	
14:00 - 15:00	Invited Talk	Anf. José Silvestre
Deep Hierarchies in Human and Computer Vision, by prof. Norbert Krüger, University of Southern Denmark		
15:00 - 16:00	Poster session III	Hall ‖ APRP assembly (Anf. José Silvestre)"
16:00 - 16:30	Coffee break	Room 6
16:30 - 17:00	APRP - Thesis Awards, Best Poster Award and Closing Session	Anf. José Silvestre

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LOCAL TECHNICAL SUPPORT

- Gisela Oliveira

FOREWORD

The 21st edition of the Portuguese Conference on Pattern Recognition, RECPAD 2015, is held at the Universidade of the Algarve (UAlg), Faro, Portugal on the 30th of October, 2015. It is a great honour for UAlg and for the members of the Organizing Committee to have this opportunity to put together this conference.

From the 32 received submissions, 30 papers were accepted. All submissions were double blind and were send to be reviewed by three members of the Technical Committee. All papers had at least one review feedback, most of them 2 or 3 reviews. The conference closing session will include the Best Paper Award and also the ceremony of the APRP Master Thesis Award.

An invited lecture by Prof. Norbert Krüger, Maersk Mc-Kinney Moller Institute for Production Technology, University of Southern Denmark, will present a talk on Deep Hierarchies in Human and Computer Vision.

We are very happy to have the support of the following sponsors: Eva Hotel which helped us in the hotel and dinner conference logistics, and SPIC – Creative Solution for all the layouts and graphics.

On behalf of the organising committee, thank you to all the people involved to this event, namely, the members of the Technical Committee, the Portuguese Association for Pattern Recognition, APRP, specially its president, Prof. Jaime S. Cardoso and to the University of the Algarve – Instituto Superior de Engenharia, with a special thanks to Prof. Ilídio Mestre, director of the institute which will held the conference. Finally, we would like to thanks the CINTAL – *Centro de Investigação Tecnológica do Algarve*, and the precious help from Dr^{a}. Gisela Oliveira, with all the work related to the registrations and invoices.

We hope you enjoy this year's edition of RECPAD.

Interactive/automated method to count bacterial colonies

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Abstract

The number of colonies in a culture is counted to calculate the concentration of bacteria in the original broth; however, manual counting can be tedious, time-consuming and imprecise. Automation of colony counting has been of increasing interest for many decades, and these methods have been shown to be more consistent than manual counting. Significant limitations of many algorithms used in automated systems are their inability to recognize overlapping colonies as distinct and to count colonies on the plate boundary. This study proposes an interactive semi-automated counting system and a fully automated counting system using image processing methods which overcomes these problems. The proposed systems 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. The results obtained are compared with other methods found in the literature.

1 Introduction

Bacterial culturing on solid agar plates (Petri dishes) is a fundamental process in microbiology, which is widespread for clinical laboratory exams, environmental control, food and beverage safety assessment.

The number of colonies in a culture is usually counted manually to calculate the concentration of bacteria based on the assumption that each colony has raised from one single bacterium (colony forming unit, CFU). However, this process is time-consuming (sometimes, the human who counts the colonies need to realize the procedure during many hours or even days), tedious (it is a monotonous procedure) and error prone (with the fatigue, the human being has more tendency to not do the right evaluation). The counting results obtained depend on the human conducting the count. This variability is one of the sources of error in the colony counting process that, along with methodological differences between different laboratories or even within a laboratory, can result in considerable fluctuations in results [1]. Due to this, for cultures with high density of colonies, manual counting mostly uses estimation methods, making an extrapolation from a small section of the Petri dish. Automating the detection, counting and analysis of CFU offers significant benefits to eliminate the risk of subjectivity, bias and human error, increasing speed and accuracy, and delivering unprecedented data archiving and retrieval capabilities.

Most automated counting systems perform adequately when the colonies are well spaced, large, circular in shape and with good contrast from the background. When these assumptions are violated, most automated colony analysis systems can rapidly lose reliability, accuracy and utility. These obstacles include the need to handle confluent growth or growth of colonies that touch or overlap other colonies; the identification of each colony as a unit in spite of differing shapes, sizes, textures, colours, light intensities; the exclusion of colonies around periphery of the plate reducing statistical accuracy.

To address the above problems, the goal of this study is to design and implement a cost-effective, software-centred system that accepts general digital camera images as its input, for detecting as well as enumerating bacterial colonies in a fully automatic manner. An interactive semi-automatic system is also proposed to overcome any error from fully automatic system. The proposed systems are capable to reduce the manpower and time required for counting colonies while producing correct colony counting.

2 Methods

In this study, we use a database of images available from [2] that contains 21 different images of bacterial cultures.

First, the original image is pre-processed in order to remove noise artefacts and to identify the dish area. The RGB image (colour images), shown in Figure 1, was converted into greyscale images. To remove the background, a mask was created from the original image and multiplied by the greyscale image.

Figure 1: Original image, retrieved from Chiang's database [2].

The second step of the method was to separate the central area of the Petri dish from the rim area. The greyscale image, in Figure 2(a), shows that the background of the central area is lighter than the surrounding of the rim area. Therefore, the central area, Figure 2(b), and the rim area, Figure 2(c), can be separated using thresholding processes.

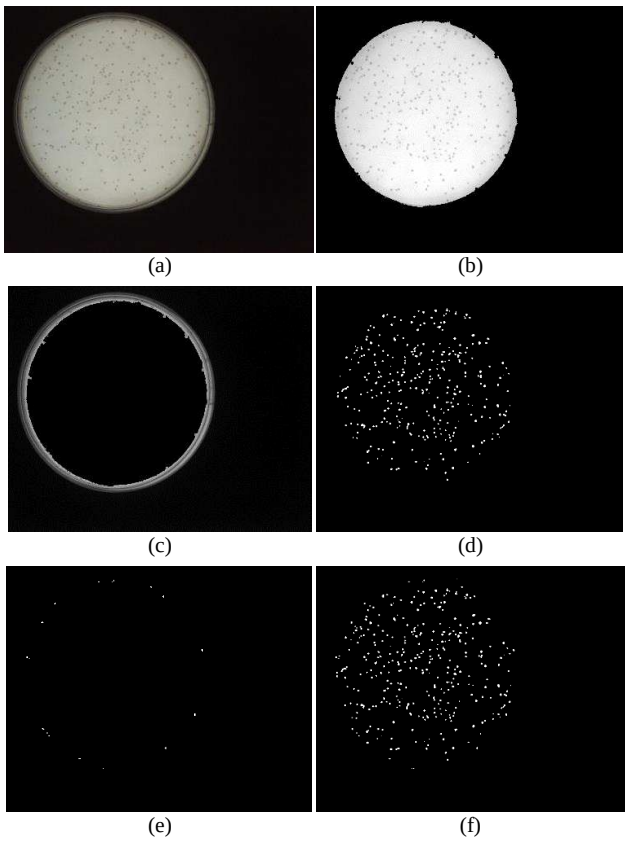


Figure 2: a) Original image; b) Central area; c) Image of the rim area; d) Colonies detected in central area; e) Colonies detected in the rim area; f) Sum of all detected colonies.

To obtain the Figure 2(d), a morphological close operation was applied, using a radius capable of cleaning the colonies from the central area. This image represents only the background from the Petri dish and

was subtracted by Figure 2(a). The final result in Figure 2(d) is an image where, supposedly, appears only the colonies.

To extract the colonies from the rim area was applied the bottom-hat transform. Then, knowing that the rim is longer and narrower than the colonies, some characteristics of the objects were calculated to obtain only colonies in the image, Figure 2(e). The Figure 2(f) represents the sum of the colonies of the central area and the colonies of the rim area.

In the next step, we obtained the area and eccentricity of each colony of the segmented image (Figure 2(f)). If an object has an area equal or smaller than the mean area of all the objects and an eccentricity lower 0.5 it is considered as an isolated colony, if not, it is considered as an overlapped colony.

The two methods (automatic and semi-automatic) use the same code to count the single colonies. It was applied the same method (to count colonies) used by Brugger [3], where a Bayes classifier is applied to count the final number of bacterial colonies. A Bayes classifier is a simple probabilistic classifier based on applying Bayes theorem. Geometric properties, such us ratio between major and minor axis length of the group are used to verify the number of colonies contained on the image.

2.1 Automatic Method

To separate the clustered colonies it was used the watershed operation that computes a label matrix identifying the watershed regions of the input matrix, which can have any dimension. The elements obtained are integer values greater than or equal to 0. The elements labelled 0 do not belong to a unique watershed region. These are called watershed pixels. The elements labelled 1 belong to the first watershed region, the elements labelled 2 belong to the second watershed region, and so on.

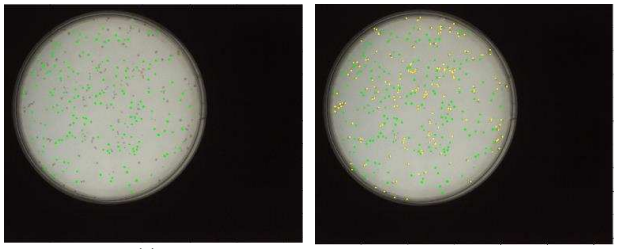
Although the watershed operation worked well to objects with few colonies, Figure 3(a), when the cluster has an aggregation of colonies, the watershed do not divide it uniformly, Figure 3(b). To overcome this issue, the mean area of all the unit colonies on Figure 2(f) was calculated. Thus, the larger areas, such as shown in Figure 3(b), were divided by the mean area obtained previously. These results were added to the count of watershed operation, and then added to the count of colonies.

(a) (b)

Figure 3: Results of the watershed transformation.

2.2 Semi-automatic Method

In the semi-automatic method, the algorithm counts all the separated colonies identifying them with the green colour, as shown in Figure 4 (a). The user marks each overlapped colony with a yellow point by clicking the mouse over the colony, or in the cluster of colonies, and decides how many colonies are in the clustering, as shown in Figure 4 (b). This number is then added to the number of isolated colonies, yielding the total number of colonies in the Petri dish.



(a) (b)

Figure 4: (a) Beginning of semi-automatic method, where the colonies on green are counted; (b) Result of semi-automatic counting, where the yellow points show the user's mouse clicks;

3 Discussion and Conclusion

To evaluate the performance of the two proposed methods, 21 images of colonies in Petri dishes were used. These images were counted automatically by the proposed systems and also manually by Biomedical Engineering students. The results obtained were compared with others 3 automatically counting systems (NICE [4], Clono Counter [5] and Chiang et al. method [2]).

The statistical results of the proposed systems were calculated after the automatic and semi-automatic counting. The statistical results of the systems NICE, Clono Counter and Chiang were obtained from [2]. The statistical results of precision, recall, F-measure and absolute percentage error (APE) comparing the methods are presented in Table 1.

Analysing the results of the automatic method, the value of precision is greater when comparing to the other three methods but the value of recall is the worst. The value of F-measure is greater than the value of Clono counter and worse than the other methods. Relatively to the APE, the automatic proposed system is 16,414% bigger than Chiang (3,37%) and NICE (7,2%) and smaller than Clono Counter (24,94%).

Table 1: Comparative results of precision, recall, F-measure and APE.

Method	Precision	Recall	F-measure	APE (%)
Automatic	0.99	0.85	0.91	16.41
Semi-automatic	0.99	0.99	0.99	0.79
Chiang	0.96	0,96	0,96	3.37
NICE	0.96	0,91	0,93	7.2
Clono Counter	0.79	0,95	0,85	24.94

Regarding the semi-automatic proposed system the results are better than all the existent methods. The statistical results obtained in this method (precision, recall, F-measure and APE) are close to perfection. It happens because even if the first phase of the semi-automatic method misses one colony, the interactive phase compensates this gap.

Comparing the aforementioned methods, the semi-automatic proposed method presents the best results.

References

[1] J. M. Bewes and N. S. McKenzie, "Automated cell colony counting and analysis using the circular Hough image transform algorithm (CHiTA)," *Physics in Medicine and Biology*, vol. 53, pp. 5991-6008, 2008.

[2] P. J. Chiang, Z. S. H. M. J. Tseng and C. H. Li, "Automated counting of bacterial colonies by image analysis," *Journal of Microbiological Methods*, vol. 108, pp. 74-82, 2015.

[3] S. D. Brugger, C. Baumberger, M. Jost, W. Jenni and U. Brugger, Automated counting of bacterial colony forming units on agar plates, *Plos one*, vol. 7, 2012.

[4] M. L. Clarke, R. L. Burton,A. N. Hill, M. Litorja, M. H. Nahm and J. Hwang, Low-cost, high-throughput, automated counting of bacterial colonies, *Cytometry A*, 77(8):790-797, 2010.

[5] M. Niyazi, I. Niyazi, and C. Belka, Counting colonies of clonogenic assays by using densitometric software. *Radiation Oncology*, 2(1), 1-3, 2007