ระบบตรวจสอบการก่อเกิดไบโอฟิล์มของ *Listeria monocytogenes* บนสายพานลำเลียง

นายกิตติชัย ปัญจวัฒน์

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต
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คณะวิทยาศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย
ปีการศึกษา 2555

INSPECTION SYSTEM OF LISTERIA MONOCYTOGENES BIOFILM FORMATION ON CONVEYER BELT

Mr.Kitthichai Pahnchawatt

A Thesis Submitted in Partial Fulfillment of the Requirements
for the Degree of Master of Science Program in Computer Science and Information Technology
Department of Mathematics and Computer Science
Faculty of Science, Chulalongkorn University
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By	Mr. Kitthichai Pahnchawatt
Field of Study	Computer Science and Information Technology
Thesis Advisor	Assistant Professor Rajalida Lipikorn, Ph.D.

Accepted by the Faculty of Science, Chulalongkorn University in Partial Fulfillment of the Requirements for the Master's Degree.

…………..……………………………………Dean of the Faculty of Science

(Professor Supot Hannongbua, Dr.rer.nat.)

THESIS COMMITTEE

…………..…………………………………………………….. Chairman

(Assistant Professor Nagul Cooharojananone, Ph.D.)

…………..…………………………………………………….. Thesis Advisor

(Assistant Professor Rajalida Lipikorn, Ph.D.)

………………………………………….…………………… External Examiner

(Suriya Natsupakpong, Ph.D.)

กิตติชัย ปัญจวัฒน์ : ระบบตรวจสอบการก่อเกิดไบโอฟิล์มของ *Listeria monocytogenes* บนสายพานลำเลียง. (INSPECTION SYSTEM OF LISTERIA MONOCYTOGENES BIOFILM FORMATION ON CONVEYER BELT) อ. ที่ปรึกษาวิทยานิพนธ์หลัก : ผศ.ดร. รัชลิดา ลิปิกรณ์,68 หน้า

งานวิจัยนี้นำเสนอวิธีการใหม่เพื่อทำให้บริเวณของส่วนที่เบลอในภาพชัดขึ้น ให้เหมาะสำหรับการหาและนับจำนวนของอาณานิคมไบโอฟิล์มของ *Listeria monocytogenes* เนื่องจากกล้องจุลทรรศน์มีข้อจำกัดในการโฟกัสได้แค่จุดเดียวในหนึ่งภาพตัวอย่าง ดังนั้นจึงเกิดส่วนที่เบลอนอกจุดโฟกัสในแต่ละภาพและมีขนาดความเบลอที่แตกต่างกันไป งานวิจัยนี้นำเสนอเทคนิค การกรองด้วยการปรับเส้นโค้งโดยใช้ลีสต์สแควร์แบบเป็นช่วงๆ และ เกรเดียนต์เพื่อทำให้ส่วนที่เบลอชัดขึ้น ขั้นตอนสุดท้าย เราจะใช้เทคนิด วิธีการวอเตอร์เชร์ด และ การดำเนินการเชิงสัณฐานวิทยาในการแยกอาณานิคมของแบคทีเรียที่ซ้อนกันอยู่ออกจากกัน ในการทดลองครั้งนี้เราทดลองกับภาพ 30 ภาพของ *Listeria monocytogenes* ที่ได้จากสายพานลำเลียง และ 90 ภาพของ *Listeria monocytogenes* ที่เราทำขึ้นในเคสพิเศษต่างๆ จากผลการทดลองที่ปรากฏ ได้ว่างานวิจัยฉบับนี้สามารถทำให้ภาพในส่วนที่เบลอชัดขึ้นซึ่งนำไปสู่ผลการนับจำนวนแบคทีเรีย ที่แม่นยำมากยิ่งขึ้นในกรณีภาพเบลอ

ภาควิชา คณิตศาสตร์และวิทยาการคอมพิวเตอร์ ลายมือชื่อนิสิต

สาขาวิชา วิทยาการคอมพิวเตอร์และเทคโนโลยีสารสนเทศ ลายมือชื่อ อ.ที่ปรึกษาวิทยานิพนธ์หลัก

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##5373602523 : MAJOR COMPUTER SCIENCE AND INFORMATION TECHNOLOGY
KEYWORDS : LISTERIA MONOCYTOGENES COLONY COUNTING/NON-LINEAR PIECEWISE LEAST-SQUARE CURVE FITTING FILTER/WATERSHED ALGORITHM/OUT OF FOCUS BLUR IMAGE

KITTHICHAI PAHNCHAWATT : INSPECTION SYSTEM OF LISTERIA MONOCYTOGENES BIOFILM FORMATION ON CONVEYER BELT. ADVISOR :ASST.PROF. RAJALIDA LIPIKORN,Ph.D,68 pp.

This thesis proposes a novel method to enhance out-of-focus regions in microscopic images and detect Listeria Monocytogenes(L. monocytogenes) in biofilm on a conveyor belt from an image with the aim to count the colonies of L. monocytogenes. Consider that a microscope can focus on a single point in the sample, this limitation makes each image contain both in-focus and out-of-focus regions. These out-of-focus regions are blurred with different blurring scale, thus the proposed thesis technique uses nonlinear piecewise least-square curve-fitting filter and the gradient to enhance the out-of-focus regions. Finally, watershed algorithm and morphological operations are used to segment and count the colonies. The proposed thesis was evaluated on a set of 30 L. monocytogenes microscopic images and a set of 90 synthetic test images. The counting results reveal that our proposed method can improve the quality of an image by enhancing the out-of-focus regions while preserving other regions which leads to more accurate counting results.

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Department : Mathematics and Computer Science Student's Signature

Field of Study : Computer Science and Information Technology Advisor's Signature

Academic Year : 2012

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CONTENTS

Page

ABSTRACT IN THAI iv

ABSTRACT IN ENGLISH v

ACKNOWLEDGEMENTS vi

CONTENTS vii

LIST OF TABLES v

LIST OF FIGURES vi

CHAPTER I INTRODUCTION 1

1.1 Background and Rationale 1

1.2 Objectives 3

1.3 Scope of Works 3

1.4 Problem Formulation 3

1.5 Expected Results 5

CHAPTER II EXISTING APPROACHES AND THEORETICAL BACKGROUND 6

2.1 Automatic Bacteria Colony Counting 6

2.2 Thresholding and Segmentation 7

CHAPTER III METHODOLOGY 10

3.1 Thresholding Using the Proposed Piecewise Least-Square Curve-fitting filter 13

3.2 Enhancing Using the proposed Relative Least-Square Curve-fitting Value 15

3.3 Segmentation and Counting 16

CHAPTER IV EXPERIMENTAL RESULTS AND DISCUSSION 19

4.1 The Sample of Experimental Results 20

4.2 The Counting Comparison between the Hand Counting Results and the Proposed Method 30

CHAPTER V CONCLUSION 33

REFERENCES 34

APPENDICES 36

Appendix A 37

BIOGRAPHY 68

LIST OF TABLES

TABLE Page

1 Kurtosis and skewness results 20
2 The counting results of 30 image data 28
3 The comparison results 32

LIST OF FIGURES

Figure		Page
1	Stages in the intracellular life-cycle of L. monocytogenes	1
2	Scanning electron micrograph of L. monocytogenes	2
3	Colonies of typical L. monocytogenes as they appear when grown on *Listeria*-Selective agar	2
4	A sample L. monocytogenes image from conveyer belt of chicken factory	4
5	The result of using Otsu's method with Figure 4	4
6	Vertical offset	7
7	Linear least square curve fitting	8
8	Non-linear least square curve fitting	8
9	The result of watershed segmentation algorithm	9
10	The original image before applying watershed segmentation algorithm	10
11	The result of distance transform	10
12	The result of marker extraction	11
13	The result of flooding process	11
14	The original image with out-of-focus regions, (b) the result of using Otsu's thresholding, and (c) the result of using the proposed nonlinear piecewise least-square curve- fitting filter	12
15	Workflow of the proposed method	13
16	(A) Sample intensities of row 900 with Otsu's thresholding,(b) intensities of row 900 with cubic least-square curve-fitting, (c) intensities of row 900 with the proposed non-linear piecewise least-square curve fitting, (d) $LC_r(i,j)$, (e) $LC_c(i,j)$, and (f) $LL(i,j)$ Respectively	17
17	(A) the histogram of $LL(i,j)$, (b) $M(i,j)$, (c) $N(i,j)$, (d) $MN(i,j)$, (e) the histogram of $MN(i,j)$, and (f) the binary image of (d)	18
18	The result of watershed algorithm	19
19	The original sharp image	21
20	The result from using the proposed method with Figure 19	21
21	The result from using non-piecewise least square curve fitting with Figure 19	21
22	The result from using Otsu's method with Figure 19.	21
23	The original sharp image blurred by Gaussian-PSF with standard deviation 5	22
24	The result from using the proposed method with Figure 23	22

Figure Page

25 The result from using non-piecewise least square curve fitting with Figure 23 22
26 The result from using Otsu's method with Figure 23 22
27 The original sharp image blurred by Gaussian-PSF with standard deviation 10 23
28 The result from using the proposed method with Figure 27 23
29 The result from using non-piecewise least square curve fitting with Figure 27 23
30 The result from using Otsu's method with Figure 27 23
31 The original sharp image blurred by Gaussian-PSF with standard deviation 15 24
32 The result from using the proposed method with Figure 31 24
33 The result from using non-piecewise least square curve fitting with Figure 31 24
34 The result from using Otsu's method with Figure 31 24
35 The original sharp image partially blurred by Gaussian-PSF with standard deviation 5 25
36 The result from using the proposed method with Figure 35 25
37 The result from using non-piecewise least square curve fitting with Figure 35 25
38 The result from using Otsu's method with Figure 35 25
39 The original sharp image partially blurred by Gaussian-PSF with standard deviation 10 26
40 The result from using the proposed method with Figure 39 26
41 The result from using non-piecewise least square curve fitting with Figure 39 26
42 The result from using Otsu's method with Figure 39 26
43 The original sharp image partially blurred by Gaussian-PSF with standard deviation 15 27
44 The result from using the proposed method with Figure 43 27
45 The result from using non-piecewise least square curve fitting with Figure 43 27
46 The result from using Otsu's method with Figure 43 27
47 The original image result used in counting comparison 30
48 The original image that used in hand counting 30
49 The result of the proposed method for Figure 48(counting result 234) 30
50 The result of Otsu's method for Figure 48(counting result 176) 30
51 The original image with subtle blur level 31
52 The result of the proposed method for Figure 51(counting result 228) 31
53 The result of Otsu's method for Figure 51(counting result 167) 31
54 The original image with moderate blur level 31
55 The result of the proposed method for Figure 55(counting result 189) 31
56 The result of Otsu's method for Figure 55(counting result 129) 31
57 The original image with massive blur level 31

Figure		Page
58	The result of the proposed method for Figure 57(counting result 166)	31
59	The result of Otsu's method for Figure 57(counting result 103)	31
60	The original image with very massive blur level	31
61	The result of the proposed method for Figure 60(counting result 146)	31
62	The result of Otsu's method for Figure 60(counting result 88)	31
63	The results of testing data name 1-1	38
64	The results of testing data name 1-2	39
65	The results of testing data name 1-3	40
66	The results of testing data name 2-1	41
67	The results of testing data name 2-2	42
68	The results of testing data name 2-3	43
69	The results of testing data name 3-1	44
70	The results of testing data name 3-1	45
71	The results of testing data name 4-1	46
72	The results of testing data name 4-2	47
73	The results of testing data name 4-3	48
74	The results of testing data name 4-4	49
75	The results of testing data name 4-5	50
76	The results of testing data name 5-1	51
77	The results of testing data name 5-2	52
78	The results of testing data name 6-1	53
79	The results of testing data name 6-2	54
80	The results of testing data name 6-3	55
81	The results of testing data name 6-4	56
82	The results of testing data name 6-5	57
83	The results of testing data name 7-1	58
84	The results of testing data name 8-1	59
85	The results of testing data name 8-2	60
86	The results of testing data name 8-3	61
87	The results of testing data name 8-4	62
88	The results of testing data name 9-1	63
89	The results of testing data name 9-2	64
90	The results of testing data name 9-3	65

Figure		Page
91	The results of testing data name 9-4	66
92	The results of testing data name 10-1	67

CHAPTER I

INTRODUCTION

1.1 Background and Rationale

L. monocytogenes is a bacterial pathogen that causes serious illness in humans. The concentration of L. monocytogenes is a major concern for the food industry because they can grow on food-processing equipment. L. monocytogenes can create biofilm on stainless steel surfaces that protect them against treatments and can be isolated from surfaces after cleaning and disinfection. Several predictive modelling programs have been proposed to estimate the growth of L. monocytogenes, and one of the key controlling factors to determine the exposure to this organism is the initial numbers of bacteria. The plate count method is the standard method used in microbiology to estimate cell numbers which results in colony-forming units per gram which may undercount the number of living cells presented in a sample[1][2][3]. Other methods are the conventional direct viable count (c-DVC) and the in situ conventional direct viable count (c-DVC)[4], however, none of these methods count bacteria cells from an image taken by a digital camera through a microscope that contains out-of-focus regions.

1.2 Objectives

We aim to enhance and filter blur regions in an image in order to increase accuracy on counting biofilm bacteria colonies in out-of-focus images, we use the characteristic of L. monocytogenes and L. monocytogenes colonies, as shown in Figures 1-3, to detect and segment L. monocytogenes colonies.

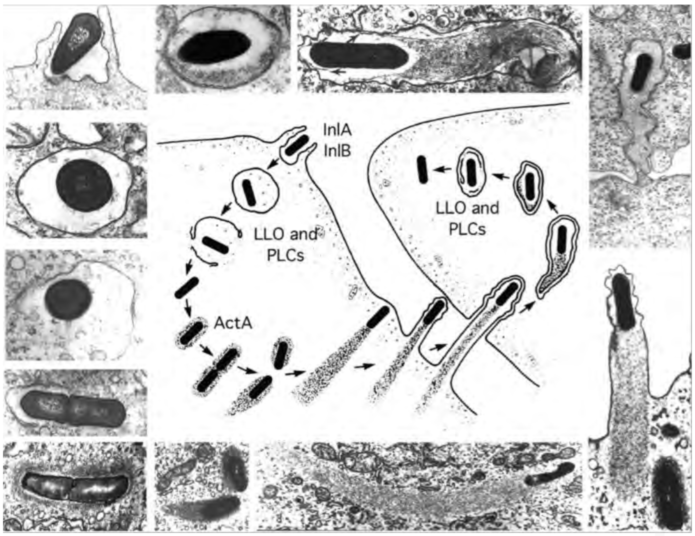

Figure 1 Stages in the intracellular life-cycle of L. monocytogenes [1]

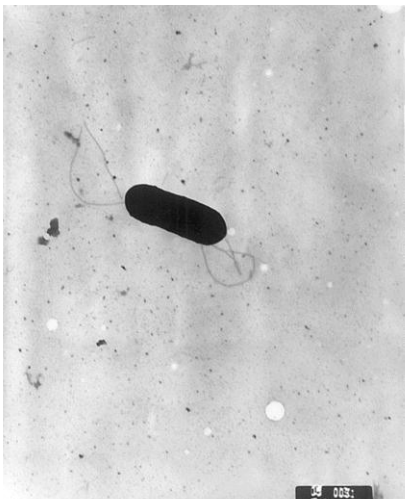

Figure 2 Scanning electron micrograph of L. monocytogenes[2]

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1 (Center) Cartoon depicting entry, escape from a vacuole, actin nucleation, actin-based motility, and cell-to-cell spread. (Outside) Representative electron micrographs from which the cartoon was derived. LLO, PLCs, and ActA are all described in the text. The cartoon and micrographs were adapted from Tilney and Portnoy (1989).

2 L. monocytogenes cells have diameter 0.5 micrometer and length 0.5-2 micrometer, L. monocytogenes also due to food poisoning, meningitis, septicemia and abortion in the human.

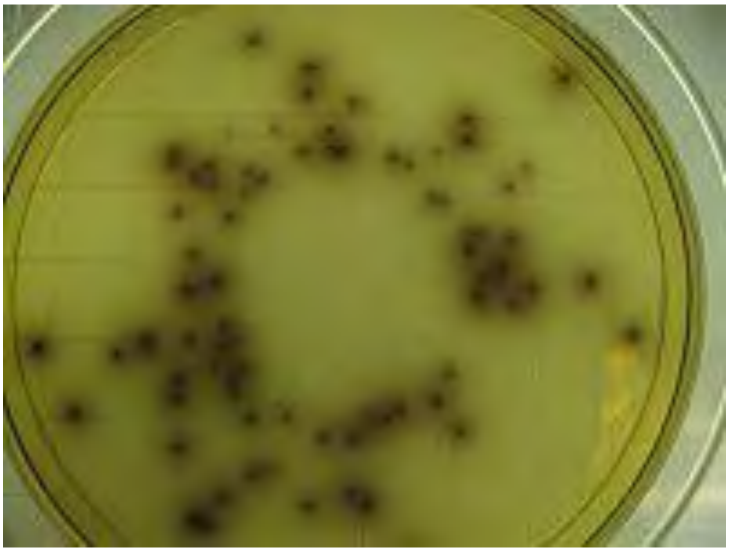


Figure 3 colonies of typical L. monocytogenes as they appear when grown on *Listeria*-selective agar

1.3 Scope of the Works

- Out-of-focus must not cover the whole area.
- Not too massive blur, colonies must be able to be counted by human eyes
- For developed out of focus blur images only
- For develop microscopic images only

1.4 Problem Formulation

As introduction above, many researches on topic of counting bacteria colony avoid blur images on scope of work because linear thresholding cannot work for blur images [5][6][7][8][9]. Berbedo used morphological operations to solve this problem but it still has the problem on overlapping colonies thus it still does not work on massive blur images because they contain overlapping colonies [10].

Although, we take a photo from microscope carefully, we cannot avoid the out-of- focus problem because the device has only single focus; therefore, I would like to develop a method to deal with this problem.

Figure 4 is an example of an image with out-of-focus regions which is used to test the proposed method. The result of Otsu's method which is linear thresholding cannot work on this case as shown in Figure 5.

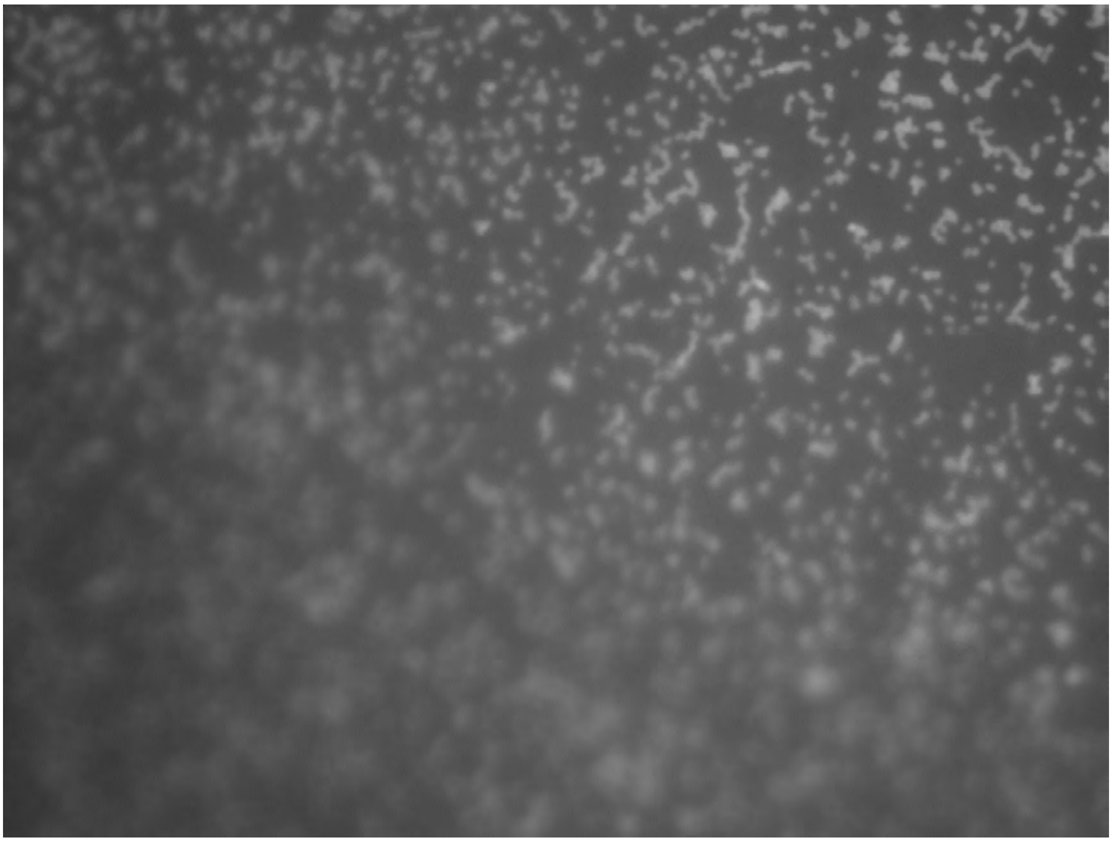


Figure 4 a sample L. monocytogenes image from conveyer belt of chicken factory

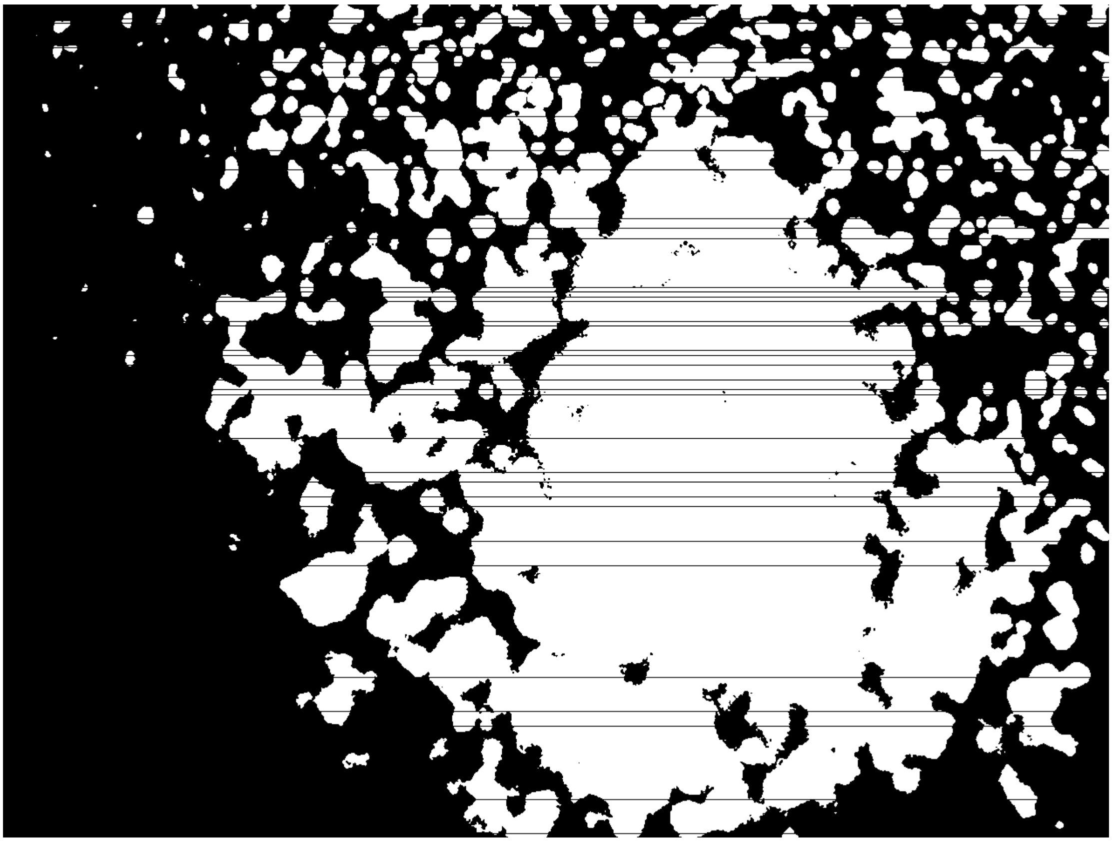


Figure 5 the result of using Otsu's method with Figure 4

1.5 Expected Outcomes

- This proposed method can automatically count bacteria colony correctly.
- This proposed method can deal with any blur position, area and large scale of blurring.

CHAPTER II

EXISTING APPROACHES AND THEORETICAL BACKGROUND

2.1 Automatic Bacteria Colony Counting

Men, et al. proposed the counting method of heterotrophic bacteria that used iterative grayscale-weighted thresholding to find the threshold value and used connected region labeling to count the colonies, however, this method cannot deal with blurred images[5]. Zhang, et al. proposed an automated bacteria colony counting system using Otsu's thresholding, watershed algorithm and enumeration technique on detection, segmentation and counting process, respectively[6]. The images were divided into two groups: achromatic and chromatic images. For chromatic images, color similarity in HSV color space was used to detect the boundary of a colony, while frequency distribution of colony size was used to detect the boundary for achromatic images. The drawback of this method is the use of linear thresholding which does not work on images with out-of-focus regions. Later, Ates, et al. proposed a new automatic bacteria colony counting system[7] which combined Otsu's thresholding, compactness ratio, and watershed segmentation algorithm together, however, the problem still arises on images with in-focus and out-of-focus regions due to linear thresholding method[8]. Shen, et al. presented an automatic colony counting system that used their proposed optimal thresholding and mark counting methods to reduce time complexity to O(n)[9]. However, this method cannot handle images with blurred regions. Barbedo, et al. proposed to use top-hat thresholding with morphological operations to segment bacteria and count colonies but it has problem with overlapped objects[10].

2.2 Thresholding and Segmentation

This chapter presents some technical details that are necessary to understand later parts of this thesis. The main techniques of this thesis are the least square curve fitting and watershed algorithm, the first technique is used to filter bacteria colony area, especially out-of-focus area and the second technique is used to segment all cluster colonies as we show on the following.

a. Least Square Curve Fitting

Least square curve fitting is the simplest mathematical procedure for finding the best curve of a given data set, Produced by sum square $R^2 \equiv \sum_{i=1}^{m}\left[L_r(i,j) - \left(c_n x_j^n + c_{n-1}x_j^{n-1} + \cdots + c_1 x_j + c_0\right)\right]^2$ with the condition that $\frac{\partial R^2}{\partial c_k} = 0\,, for\ k = 0, \ldots \ldots, n$, where R^2 is the least square error, c_k is the coefficient of polynomial, and n is the degree of polynomial.

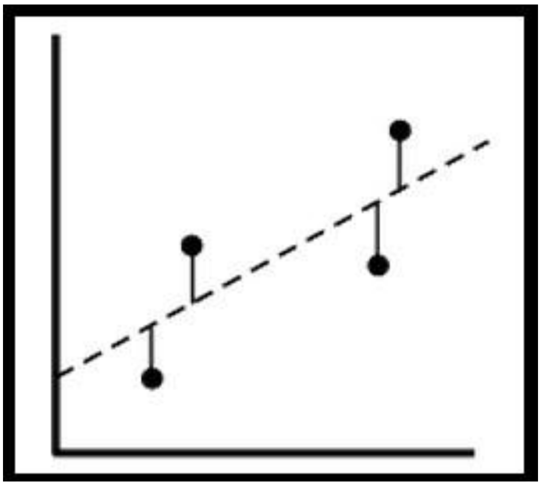


Figure 6 vertical offset

This proposed method represents non-linear least square curve fitting (n > 2) to filter bacteria colony, To create this curve fitting, we select vertical offset style as shown in Figure 6 to find the best curve. Figures 7-8 below show the different between linear and non-linear least square curve fitting.

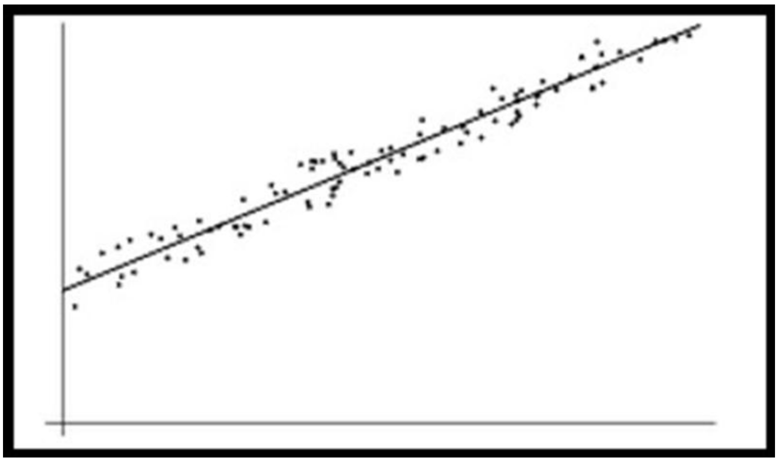


Figure 7 linear least square curve fitting

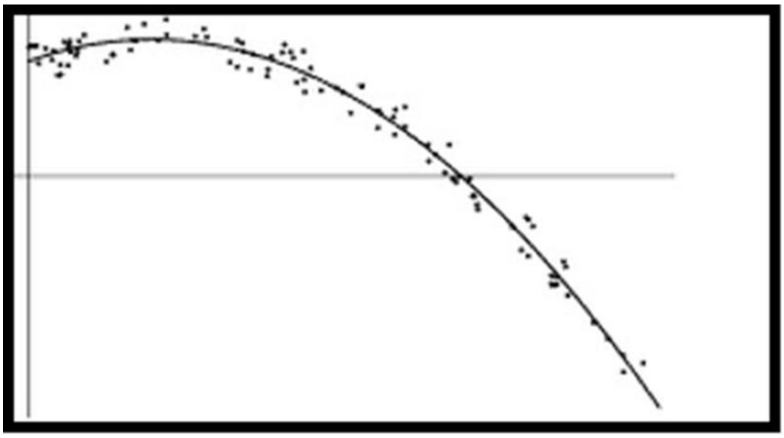


Figure 8 non-linear least square curve fitting

b. Watershed Definition and Algorithm

Definition 2.1 (Watershed transform)

$Let\ f\ \in C(D) have\ minima\ \{m_k\}_{k\in I}, for\ some\ index\ set\ I.\ \ The\ catchment\ basin$ $CB(m_i)\ of\ a\ minimum\ m_i\ is\ defined\ as\ the\ set\ \ of\ \ point\ x\ \in D\ which\ are\ closer\ to$ $m_i\ than\ any\ other\ regional\ minimum\ m_j$:

$$CB(m_i) = \{x \in D\ |\forall j\ \in I\{i\}: f(m_i) + T_f(x, m_i) < f(m_i) + T_f(x, m_j)\}$$

$The\ watershed\ of\ f\ is\ the\ set\ of\ points\ which\ do\ not\ belong\ to\ any\ catchment$ $basin$:

$$Wshed(f) = D\ \cap \left(\cup\, CB(m_i)\right)^c$$

As shown on Figure 9 the black areas are $CB(m_i)$ and the white lines are $Wshed(f)$.

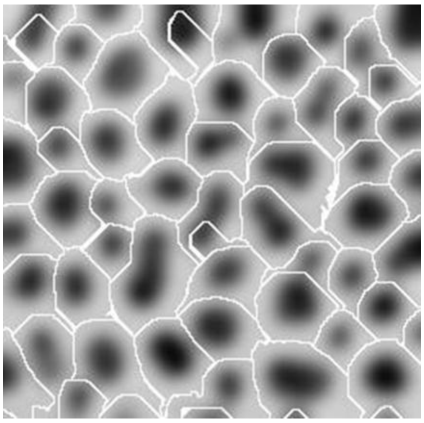


Figure 9 the result of watershed segmentation algorithm

The watershed algorithm consist of four step on the followings

Figure 10 the original image before applying watershed segmentation algorithm

1. Perform the distance transform on an image to find the center point of each artifact for the next step. Figure 11 shown the result when uses distance transform to Figure 10.

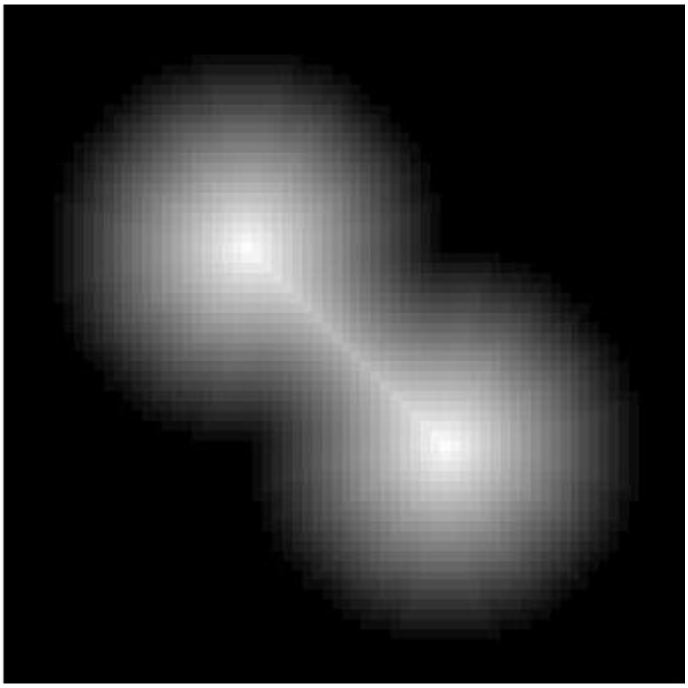


Figure 11 the result of distance transform

2. Do marker extraction, this step will mark center point of each step which obtained from step 1 as shown in Figure 12.

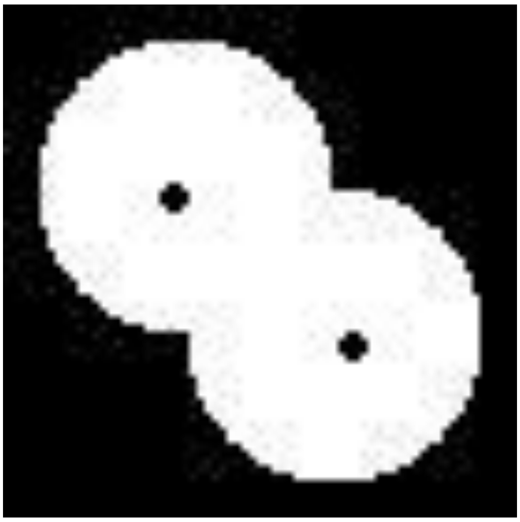


Figure 12 the result of marker extraction

3. Do background marking, this step marks one point of background, mostly peoples choose to mark the background near the corner points.
4. Do flooding process, this step applies watershed transform to find the segment line as define in the definition 2.1. The result of this step is as shown in Figure 13.

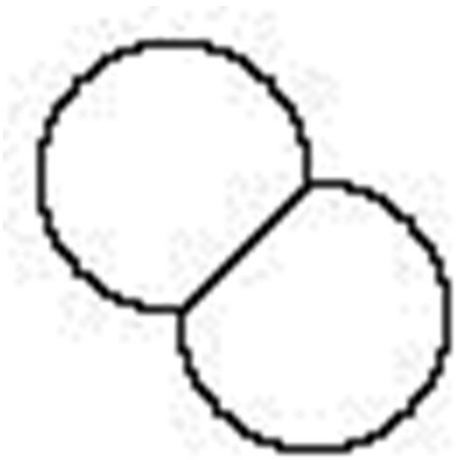
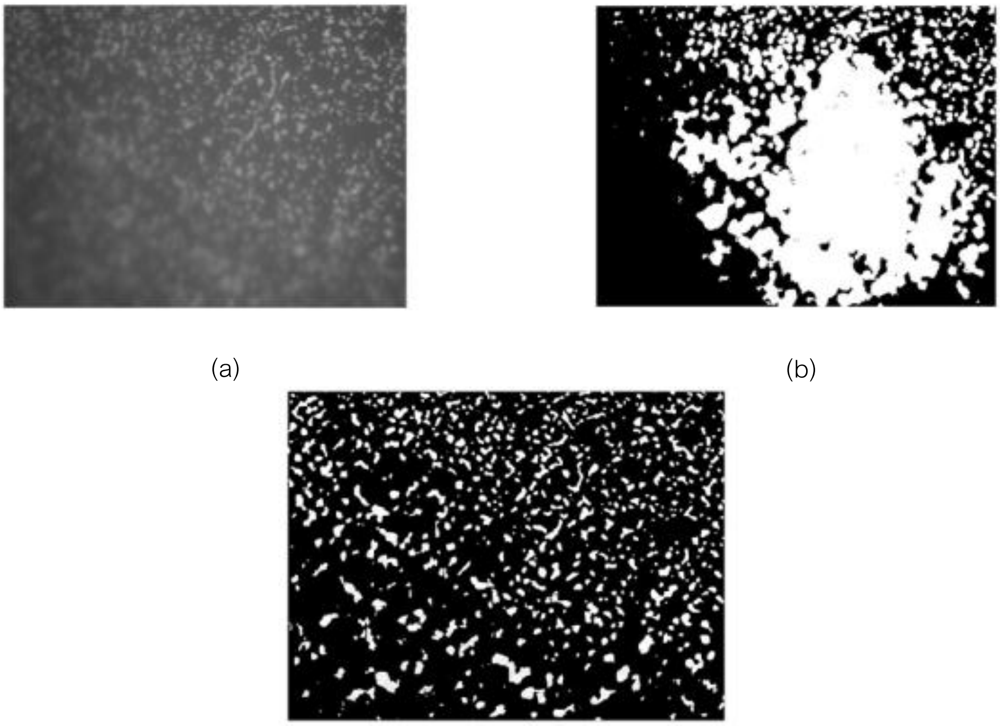


Figure 13 the result of flooding process

CHAPTER III

METHODOLOGY



(a) (b) (c)

Figure 14 (a) the original image with out-of-focus regions, (b) the result of using Otsu's thresholding, and (c) the result of using the proposed nonlinear piecewise least-square curve- fitting filter

The main objective of this thesis is to propose a new filter to enhance and sharpen an image especially in the out-of-focus regions in order to improve the performance and the efficiency of the counting system. As we can see from Figure 14(a) that the original microscopic image contains out-of-focus regions and if we use linear thresholding method such as Otsu's thresholding to enhance an image, the result image as shown in Figure 14(b) does not work. This thesis thus proposes a new method that can enhance out-of-focus

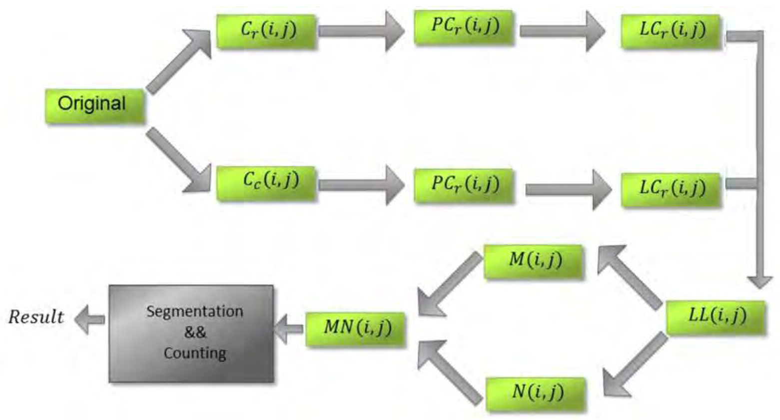


Figure 15 Workflow of the proposed method

regions and gives a better result as shown in Figure 14(c). The proposed L. monocytogenes colony counting system consists of the following steps: (1) thresholding using the proposed piecewise least-square curve-fitting filter, (2) enhancing using the proposed relative least-square curve-fitting values, and (3) segmenting and counting L. monocytogenes colonies using watershed algorithm and morphological operations.

Before counting L. Monocytogenes colonies, all the images, which were taken through a microscope, must be converted to gray scale images and the mean filter is applied to remove noise or artifact from the images.

3.1 Thresholding Using the Proposed Piecewise Least-square Curve-fitting Filter

Since each image must be taken through a microscope which can focus on a single point in the sample, this limitation makes each image contain both in-focus and out-of-focus regions. It also makes intensities of an image to vary in a wide range and it can be noticed that the variation is not linear. In order to enhance and sharpen an image especially those out-of-focus blurred regions, the nonlinear piecewise least-square curve-fitting filter is applied to an image horizontally and vertically. Let $Im(i,j)$ represents an image, the proposed filtering method can be performed as follows:

1. Let $L_r(i,j)$ represents each row of an image as shown in Figure 16(a), we plot the intensities from each row of an image and use the nonlinear least-square curve fitting to find the best curve that fits the intensities from

$$L_r(i,j) \approx c_n x_j^n + c_{n-1} x_j^{n-1} + \cdots + c_1 x_j + c_0$$

$$R^2 \equiv \sum_{i=1}^{m} \left[L_r(i,j) - \left(c_n x_j^n + c_{n-1} x_j^{n-1} + \cdots + c_1 x_j + c_0\right)\right]^2 \quad (1)$$

with the condition that $\frac{\partial R^2}{\partial c_k} = 0\, , for\ k = 0, \dots \dots, n$, where R^2 is the least square error, c_k is the coefficient of polynomial, and n is the degree of polynomial.

2. From Figure 16(b), let $C_r(i,j)$ represents the value of the least-square curve at pixel (i,j), and L_r represents the intensity curve. L_r, is then divided into several segments at the zero crossing points where the gradient becomes positive and the actual intensity, $L_r(i,j)$, is equal to the value of the least-square curve, $C_r(i,j)$. We then use the nonlinear least-square curve fitting to find the best curve for each segment as shown in Figure 16(c). The nonlinear piecewise least-square curving fitting filter can be calculated from Eq.(1). Any pixel whose intensity is below the curve is set to background.
3. Step 1 and 2 are repeated for each row of an image and these new intensities are combined to obtain a new image, $LC_r(i,j)$ as shown in Figure 16(d).
4. Let $L_c(i,j)$ represents each column of an image, we plot the intensities from each column of an image and use the nonlinear least-square curve fitting to find the best curve that fits the intensities which can be calculated using similar equation as Eq.(1)
5. Since the variation of intensities is not linear, the intensity curve is divided into several segments. Let $C_c(i,j)$ represents the value of the least-square curve at pixel (i,j). The points where we divide the intensity curve can be calculated by

considering zero crossing where the gradient becomes positive and the actual intensity is equal to the value of the least-square curve, $C_c(i,j)$. We then use the nonlinear least-square curve fitting to find the best curve for each segment. Any pixel whose intensity is below the curve is set to background.

6. Step 4 and 5 are repeated for each column of an image and these new intensities are combined to obtain a new image, $LC_c(i,j)$ as shown in Figure 16(e).
7. We define a new operation, ⋒ to merge $LC_r(i,j)$ and $LC_c(i,j)$ to obtain a new image, $LL(i,j)$ as shown in Figure 16(f) such that

$$LL(i,j) = LC_r(i,j) \Cap LC_c(i,j) \; = \begin{cases} \max\big(lc_r(i,j), lc_c(i,j)\big) \\ , lc_r(i,j) and\ lc_c(i,j) \neq 0 \\ 0,\ otherwise \end{cases} \tag{2}$$

3.2 Enhancing Using the Proposed Relative Least-square Curve-fitting Values

After a new image, $LL(i,j)$, is obtained, the histogram of $LL(i,j)$ as shown in Figure 17(a) indicates that the contrast of an image needs to be adjusted by applying the following steps:

1. Let $LL_r(i,j)$ represents each row of an image, $LL(i,j)$. Any pixel whose intensity is not equal to 0(background) is enhanced to be equal to

$$M_r(i,j) = \begin{cases} \frac{LL_r(i,j)}{C_r(i,j)}, & LL_r(i,j) \neq 0 \\ 0, & otherwise \end{cases} \tag{3}$$

2. Repeat step 1 to enhance the intensities of pixels in every row. After all rows are enhanced, they are integrated to form a new image, $M(i,j)$, and the intensities of the new image are normalized to be in the range [0, 1] as shown in Figure 17(b).

3. Let $LL_c(i,j)$ represents each column of an image, $LL(i,j)$. Any pixel whose intensity is not equal to 0(background) is enhanced to be equal to

$$N_c(i,j) = \begin{cases} \frac{LL_c(i,j)}{C_c(i,j)}, & LL_c(i,j) \neq 0 \\ 0, & otherwise \end{cases} \tag{4}$$

4. Repeat step 3 to enhance the intensities of pixels in every column. After all columns are enhanced, they are integrated to form a new image, $N(i,j)$, and the intensities of the new image are normalized to be in the range [0, 1] as shown in Figure 17(c).
5. Use the operation, $\Cap$, defined in the previous step to merge $M(i,j)$ and $N(i,j)$ to obtain a new image, $MN(i,j)$ and its histogram as shown in Figure 17(d)-(e).

3.3 Segmentation and Counting

The last step of our proposed method is to convert an image, $MN(i,j)$, to a binary image as shown in Figure 17(f) and use watershed algorithm together with morphological operations to segment L. Monocytogenes.

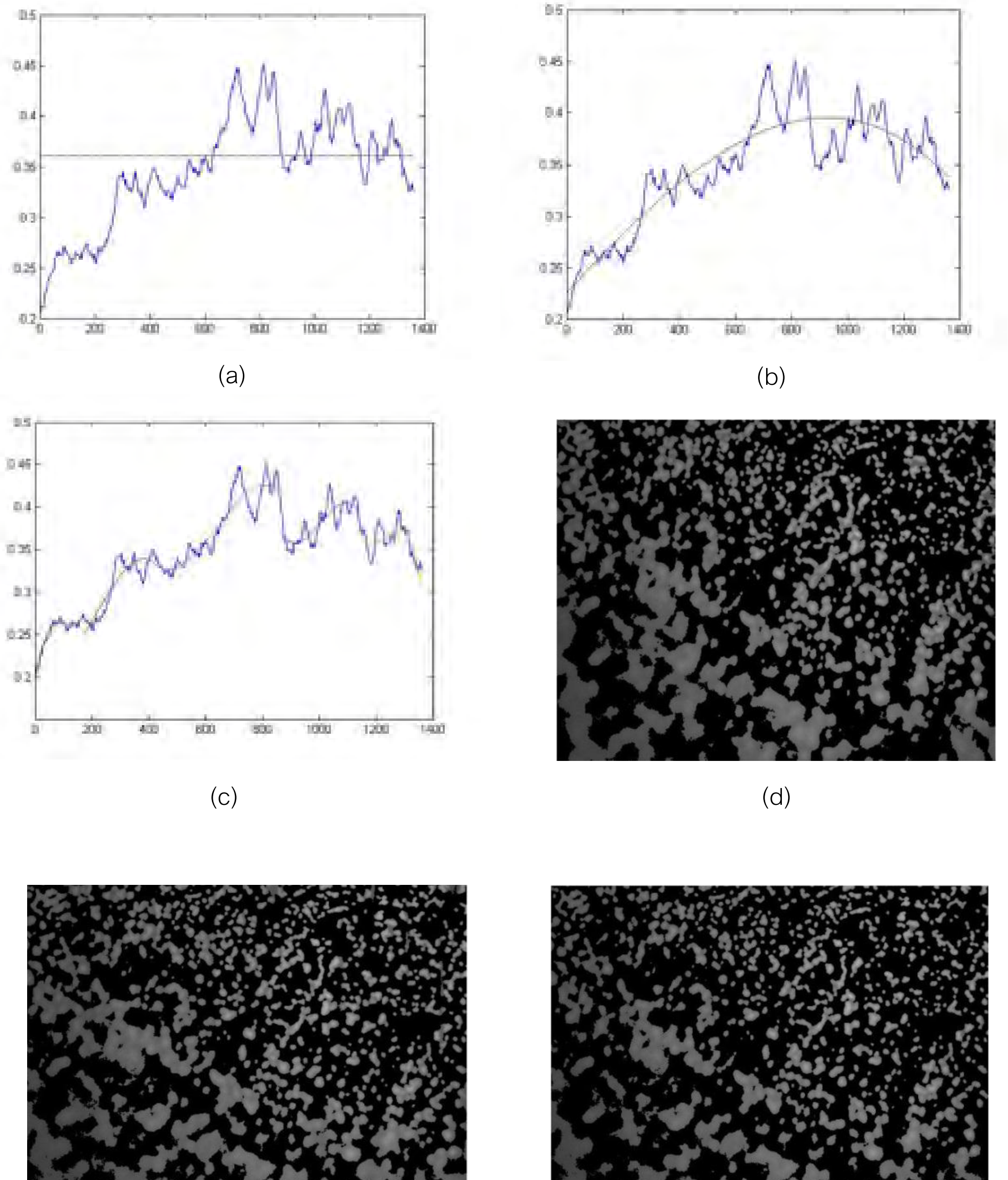


Figure 16 (a) Sample intensities of row 900 with Otsu's thresholding,(b) intensities of row 900 with cubic least-square curve-fitting, (c) intensities of row 900 with the proposed nonlinear piecewise least-square curve fitting, (d) $LC_r(i,j)$, (e) $LC_c(i,j)$, and (f) $LL(i,j)$. respectively

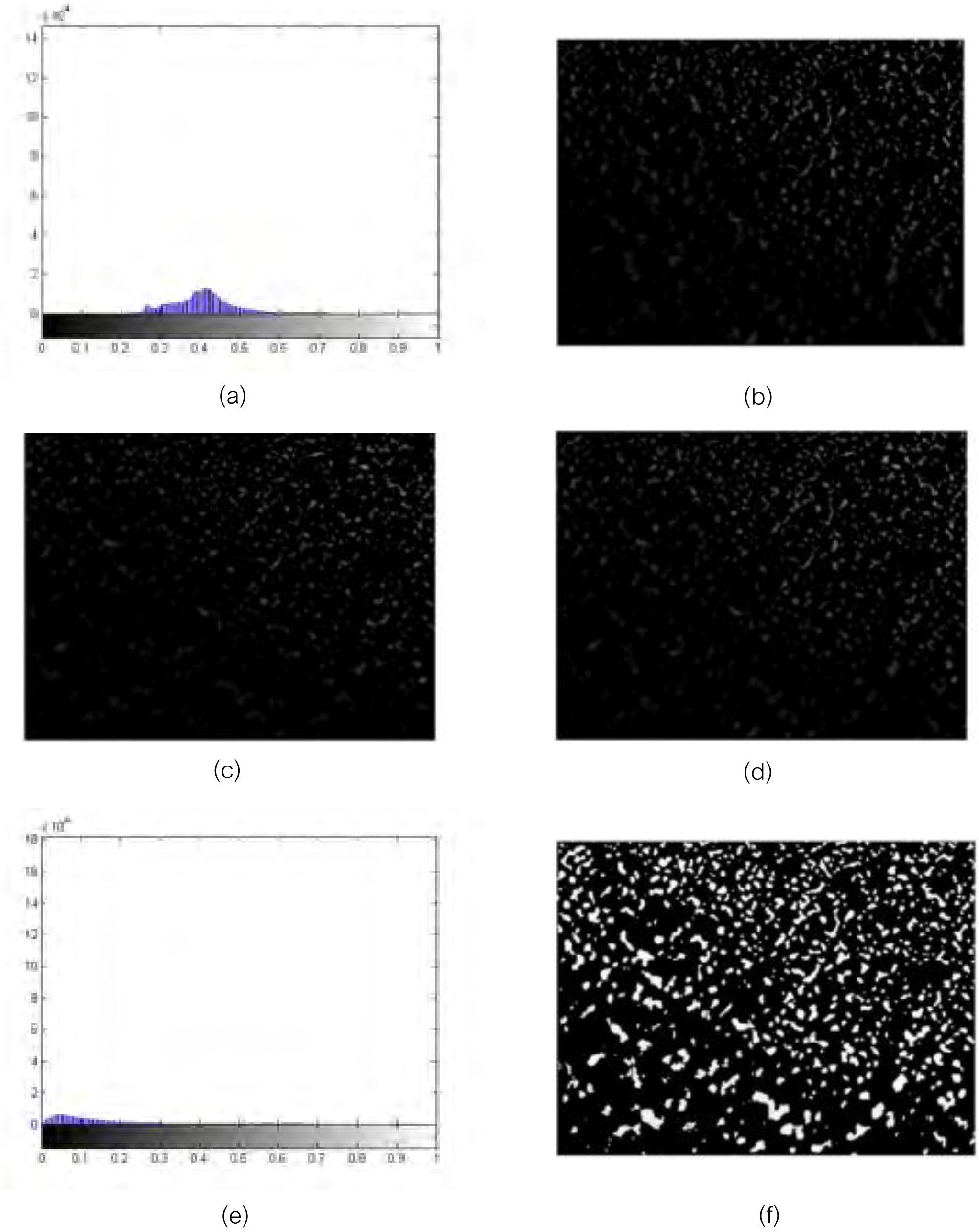


Figure 17 (a) the histogram of $LL(i,j)$, (b) $M(i,j)$, (c) $N(i,j)$, (d) $MN(i,j)$, (e) the histrogram of $MN(i,j)$, and (f) the binary image of (d)

CHAPTER IV

EXPERIMENTAL RESULTS AND DISCUSSION

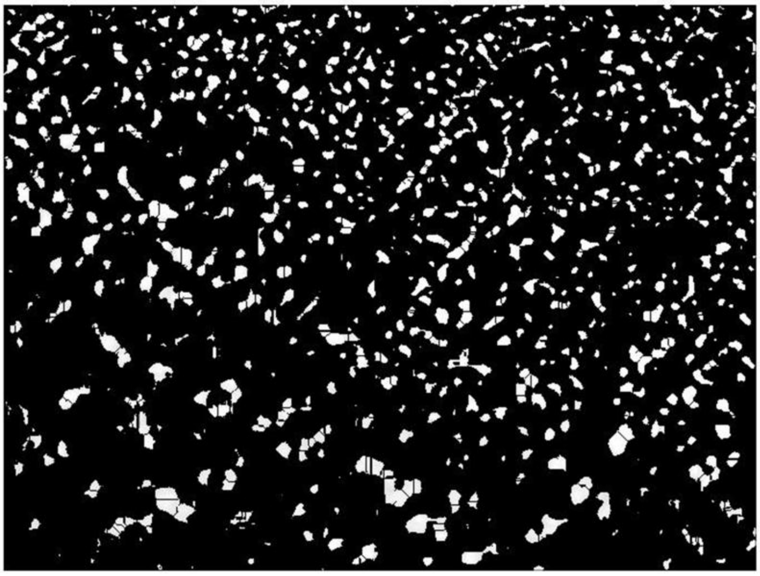


Figure 18 the result of watershed segmentation

The proposed method was tested on a data set of 30 L. monocytogenes microscopic images with out-of-focus regions taken from a conveyer belt and a set of 90 synthetic test images. The synthetic test images were generated by randomly convolving one quarter of the original sharp image with Gaussian Point Spread Function with standard deviation or sigma equal to 5, 10, and 15, respectively. The synthetic test images were used to measure the accuracy of the counting system after enhancing the images by our proposed method.

From Figure 18, it can be noticed that the nonlinear piecewise least-square curve fitting filter can enhance the out-of-focus regions while preserving the in-focus regions. After enhancing the images, we then compared the counting results between the original image and the enhanced ones. The results are shown in Table1 where the second column(Mean) represents the average counting result from 90 test images with respect to sigma and the third column(S.D.) represents standard deviation of the counting results for each sigma when comparing with 1320 cell count from the original image. We also evaluated our proposed method based on kurtosis and skewness and the results reveal that our proposed method can enhance any levels of out-of-focus blurred regions and give similar counting results.

TABLE I. Kurtosis and skewness results[1]

Sigma	Mean	S.D.	Kurtosis	Skewness
5	1264.3	7.08	2.694	0.3080
10	1198.1	10.18	3.1058	0.5609
15	1168.3	16.2	2.6403	0.4375

4.1 The Sample of experimental results

This section shows the thresholding results of the proposed method with normal least square curve fitting thresholding and Otsu's method thresholding. Figure 19 shows the original sharp image, and Figures 20-22 show the results of thresholding Figure 19 by using the proposed method ,non-piecewise least square curve fitting thersholding, and Otsu' thresholding method, respectively.

[1] Kurtosis and skewness of the counting results are calculate from each result in case of applying Gaussian-PSF with sigma 5,10 and 15 images in each case. the original image that used in synthetic test is shown In Figure 19

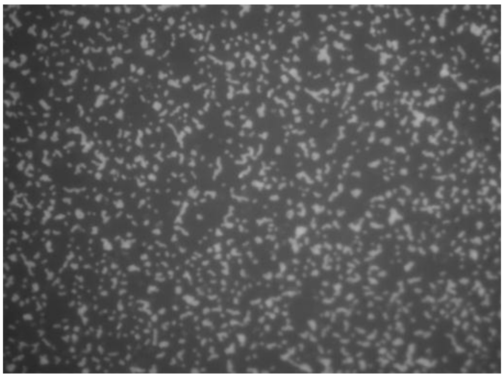


Figure 19 the original sharp image

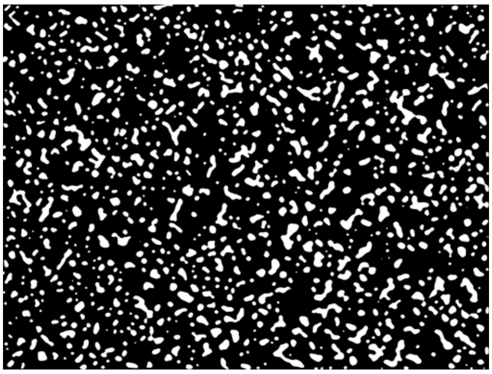


Figure 20 the result from using the proposed method with Figure 19

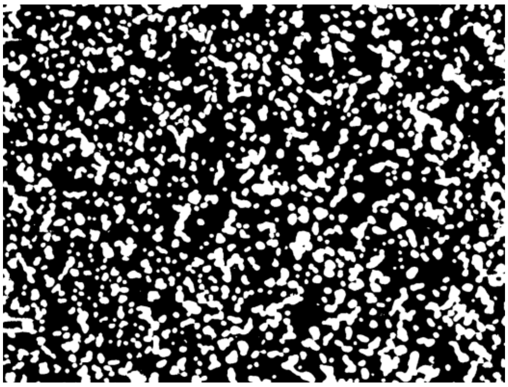


Figure 21 the result from using non-piecewise least square curve fitting with Figure 19

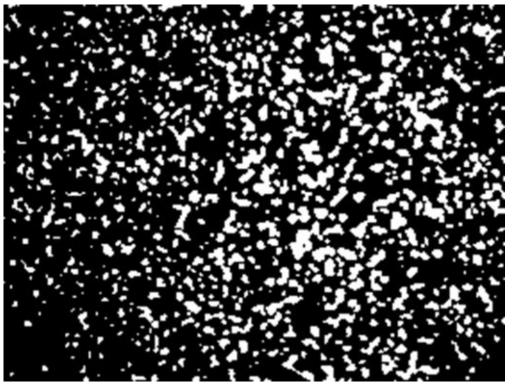


Figure 22 the result from using Otsu's method with Figure 19

Similar sample results are shown in Figures 23-26,27-30,31-34,35-38,39-42 and 43-46. Each group of figures used the same original image but we generated blurred images to data with Gaussian–Point spread function (PSF) with standard deviation(STD) 5,10 and 15 respectively as shown in Figures 23-34 and generated partial blurred images with Gaussian–PSF with standard deviation 5,10 and 15 respectively as shown in Figures 35-46.

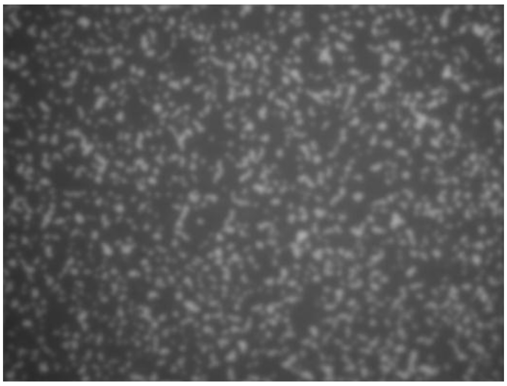


Figure 23 the original sharp image blurred by Gaussian-PSF with standard deviation 5

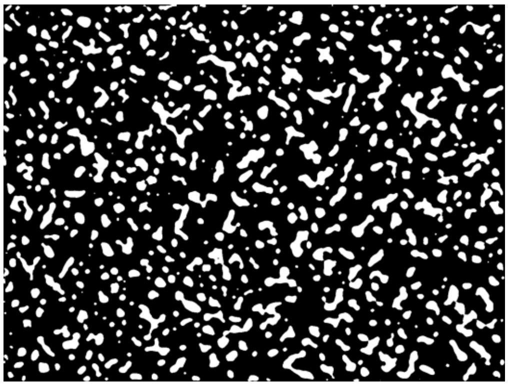


Figure 24 the result from using the proposed method with Figure 23

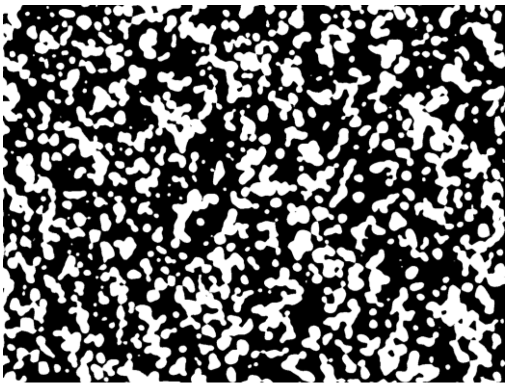


Figure 25 the result from using non-piecewise least square curve fitting with Figure 23

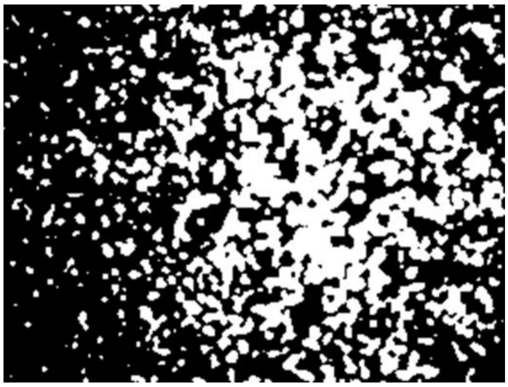


Figure 26 the result from using Otsu’s method with Figure 23

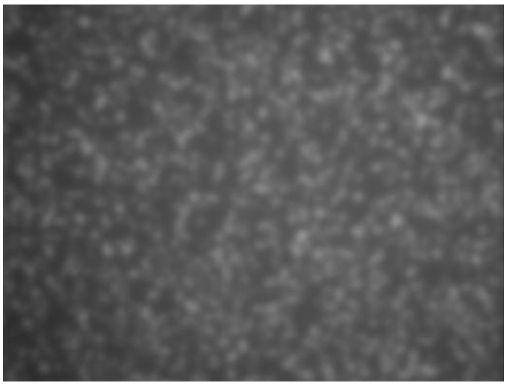


Figure 27 the original sharp image blurred by Gaussian-PSF with standard deviation 10

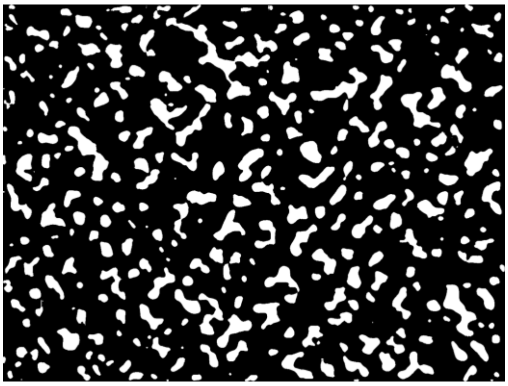


Figure 28 the result from using the proposed method with Figure 27

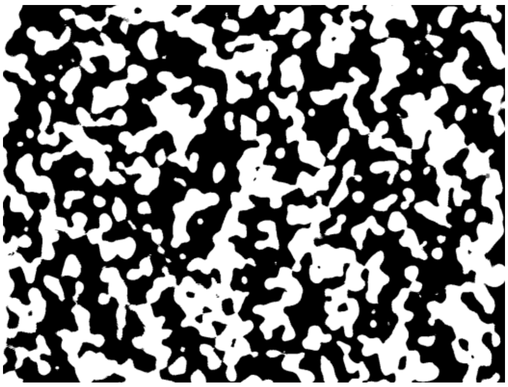


Figure 29 the result from using non-piecewise least square curve fitting with Figure 27

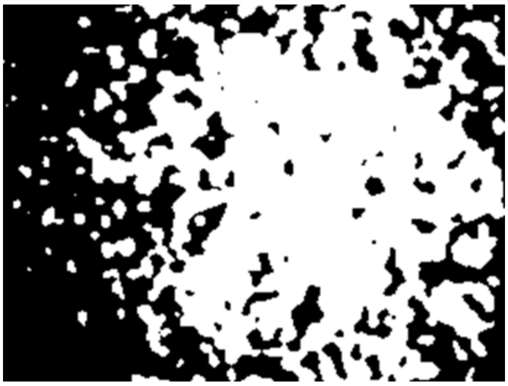


Figure 30 the result from using Otsu's method with Figure 27

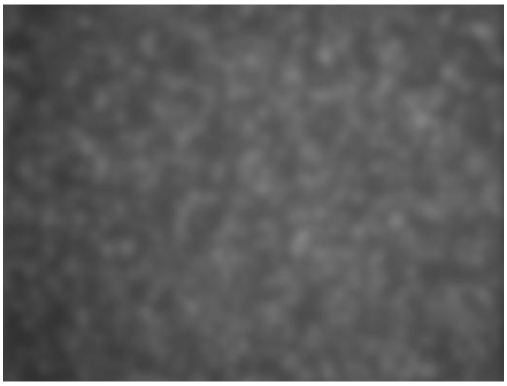


Figure 31 the original sharp image blurred by Gaussian-PSF with standard deviation 15

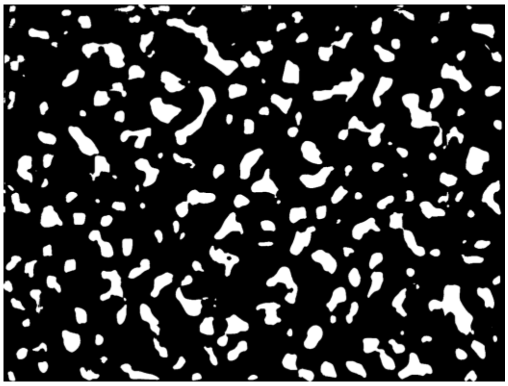


Figure 32 the result from using the proposed method with Figure 31

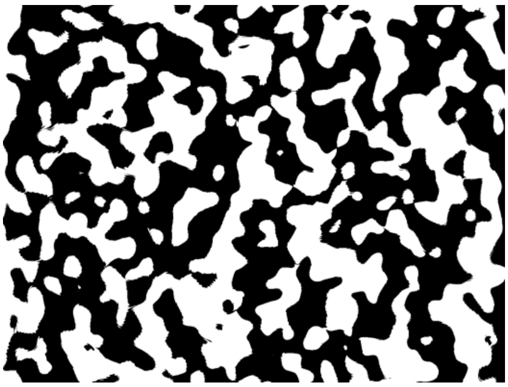


Figure 33 the result from using non-piecewise least square curve fitting with Figure 31

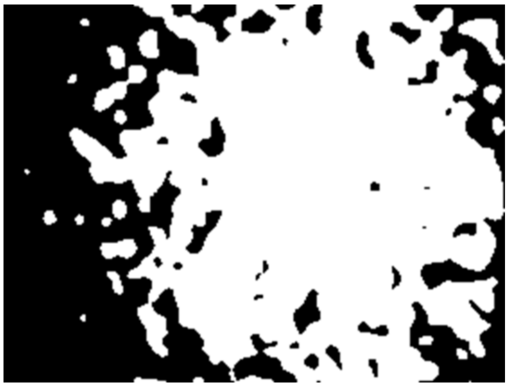


Figure 34 the result from using Otsu's method with Figure 31

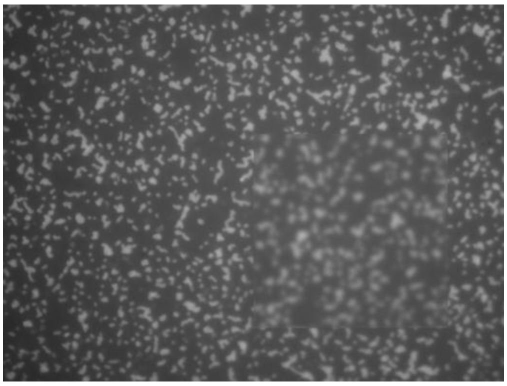


Figure 35 the original sharp image partially blurred by Gaussian-PSF with standard deviation 5

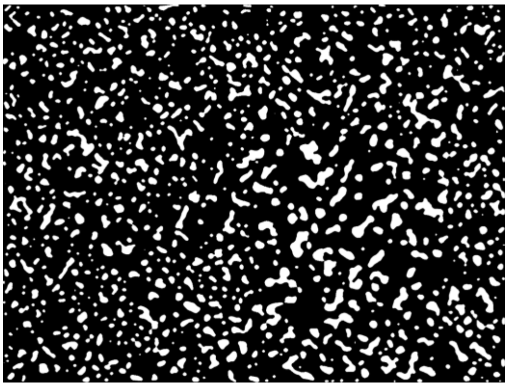


Figure 36 the result from using the proposed method with Figure 35

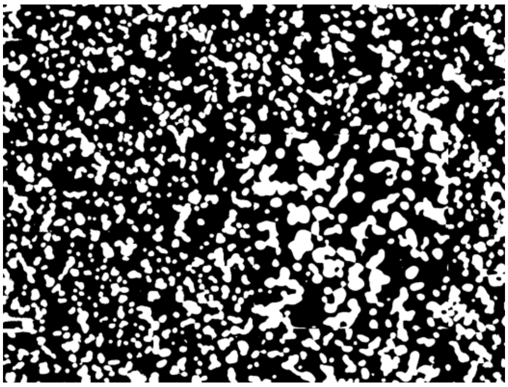


Figure 37 the result from using non-piecewise least square curve fitting with Figure 35

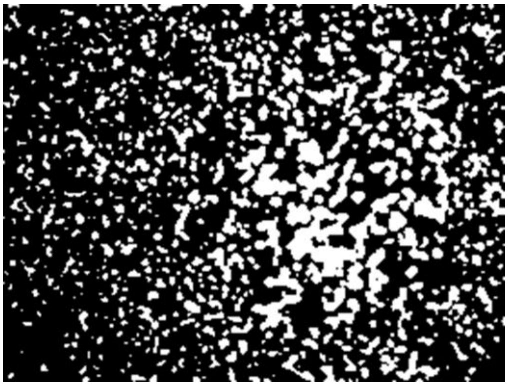


Figure 38 the result from using Otsu's method with Figure 35

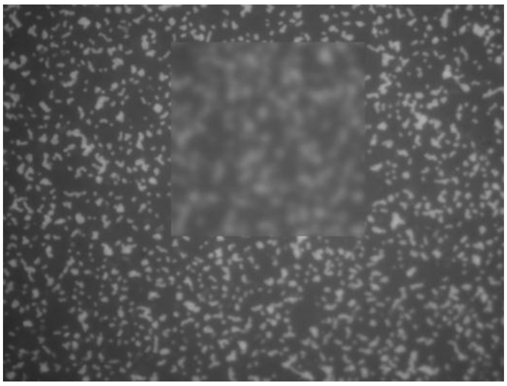


Figure 39 the original sharp image partially blurred by Gaussian-PSF with standard deviation 10

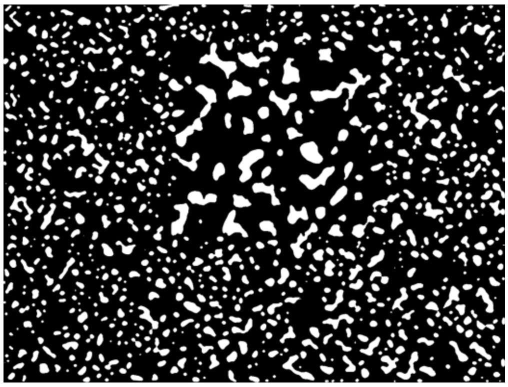


Figure 40 the result from using the proposed method with Figure 39

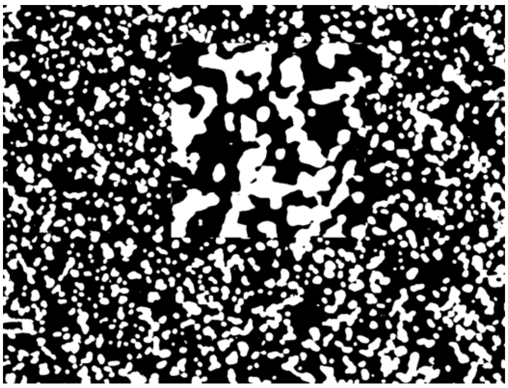


Figure 41 the result from using non-piecewise least square curve fitting with Figure 39

Figure 42 the result from using Otsu's method with Figure 39

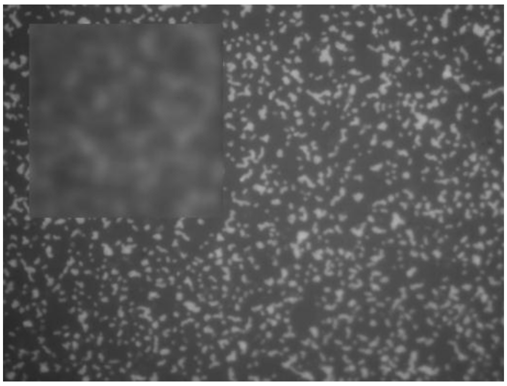


Figure 43 the original sharp image partially blurred by Gaussian-PSF with standard deviation 15

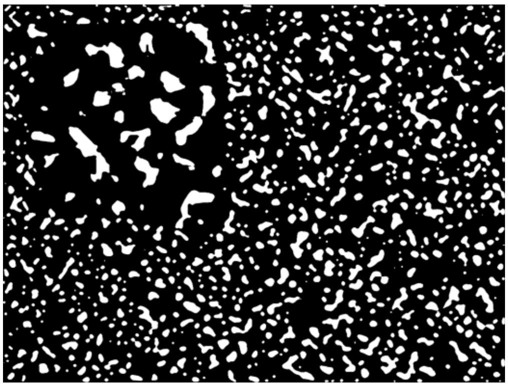


Figure 44 the result from using the proposed method with Figure 43

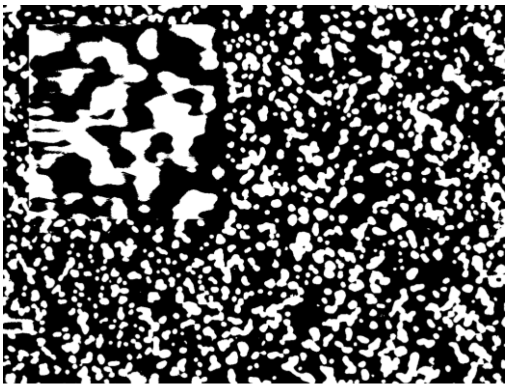


Figure 45 the result from using non-piecewise least square curve fitting with Figure 43

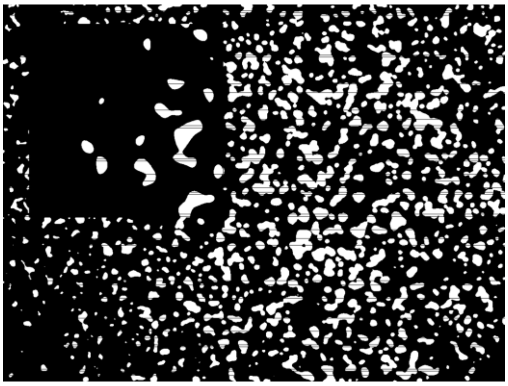


Figure 46 the result from using Otsu's method with Figure 43

According to the enhanced images as shown in Figures 63-92, Appendix A, the counting results of the 30 image data are shown in Table 2.

TABLE II. THE COUNTING RESULTS OF 30 IMAGE DATA.

Data name	Counting result	Data name	Counting result
1-1	1102	6-1	994
1-2	1233	6-2	1202
1-3	1010	6-3	1195
2-1	1257	6-4	1005
2-2	1382	6-5	1135
2-3	1241	7-1	1436
3-1	1536	8-1	976
3-2	1475	8-2	1032
4-1	757	8-3	1029
4-2	879	8-4	919
4-3	975	9-1	1021
4-4	911	9-2	1083
4-5	881	9-3	1136
5-1	1320	9-4	1020
5-2	1433	10-1	1357

From Table 2, the first digit of data name represents the name of an image and the second digit represents the out-of-focus position in the image data. Notice form Table 2 ,the counting results of the same images with different blurred regions are similar which can be concluded that the proposed method can handle blurred regions regardless of their position and degree of blurrness.

4.2 The Counting Comparison between the Hand Counting Results and the Proposed Method

In this section we evaluate the counting results by cropping the area of 200 x 300 pixels which is easier for hand counting. We then blur the image using Gaussian with four standard deviation: 2, 5, 7, and 10. On the left hand side of Figure 47 is the original image, the middle is the result of the proposed thresholding method, and the right hand side is the result of Otsu's thresholding method. In this comparison, the counting result from the proposed method is compared with the hand counting results and the counting results from the images enhanced by Otsu's method.

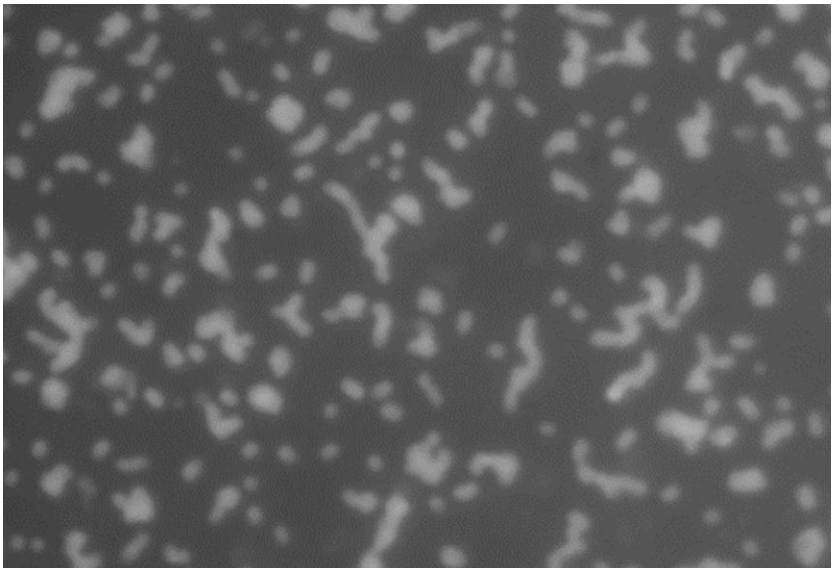


Figure 47 the original image used in counting comparison.

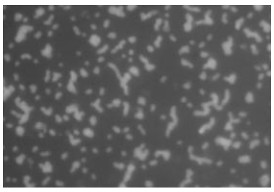


Figure 48 the original image that used in hand counting(counting result 210)

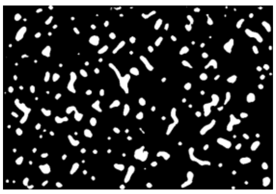


Figure 49 the result of the proposed method for Figure 48 (counting result 234)

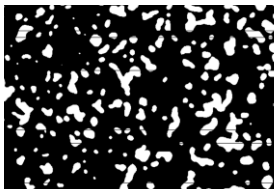


Figure 50 the result of Otsu's method for Figure 48 (counting result 176)

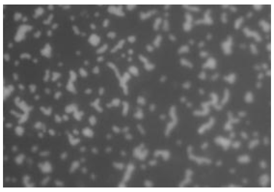


Figure 51 the original image with subtle blur level

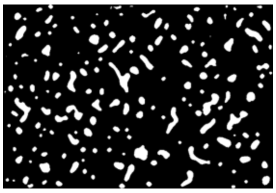


Figure 52 the result of the proposed method for Figure 51(counting result 228)

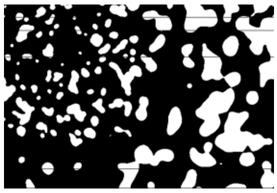


Figure 53 the result of Otsu's method for Figure 51(counting result 167)

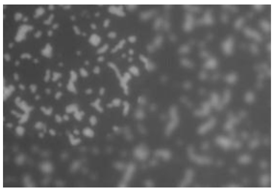


Figure 54 the original image with moderate blur level

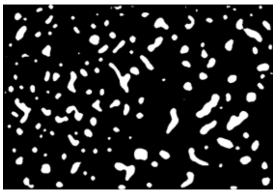


Figure 55 the result of the proposed method for Figure 54 (counting result 189)

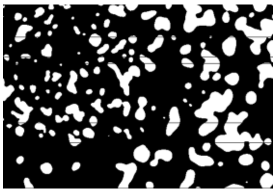


Figure 56 the result of Otsu's method for Figure 54 (counting result 129)

Figure 57 the original image with massive blur level

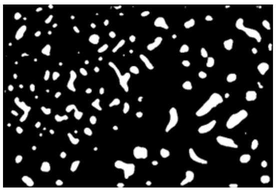


Figure 58 the result of the proposed method for Figure 57 (counting result 166)

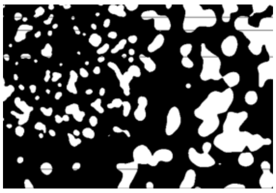


Figure 59 the result of Otsu's method for Figure 57 (counting result 103)

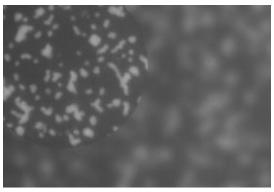


Figure 60 the original image with very massive blur level

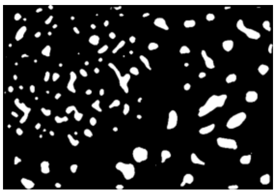


Figure 61 the result of the proposed method for Figure 60 (counting result 146)

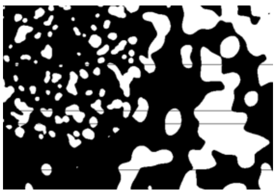


Figure 62 the result of Otsu's method for Figure 60 (counting result 88)

TABLE III. The comparing results

Original image data	Hand counting results	Proposed results	Otsu's results
NORMAL	210 COLONY	234	176
FEW BLUR		228	167
MODERATE BLUR		189	129
MASSIVE BLUR		166	103
VERY MASSIVE BLUR		146	88

According to the comparison results in Table 3 and Figures 47-62, we can conclude that the proposed method can enhance the out-of-focus regions in an image better than the Otsu's thresholding method and can reserve colony region better than linear thresholding.

CHAPTER V

CONCLUSION

This thesis proposes a novel method to enhance the out-of-focus regions of L. Monocytogenes images by using a nonlinear piecewise least-square curve-fitting filter. Figure 14 shows that the proposed method can enhance the out-of-focus blurred regions better than linear thresholding. Moreover, the kurtosis and skewness of the experimental results confirm that the proposed method makes the counting results to be quite accurate when comparing with the result from the original sharp image.

Table 2 shows that the proposed method can deal with various blurring scale and different position. The counting results of the same images with different blurring scale are not that much different.

Table 3 also confirms that the proposed method can filter bacteria colonies better than Otsu' method, however, this proposed method still has some problem that is the optimal threshold value is quite difficult to determine.

In future research, we will find the technique to determine the optimal threshold value to enhance the proposed method for more accuracy.

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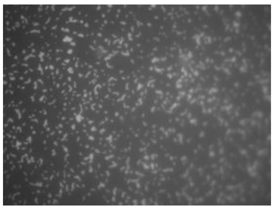
APPENDICES

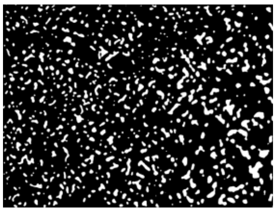
Appendix A

Results of 30 image data of L. monocytogenes and Histrograms

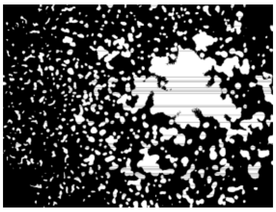
Data name 1-1.tif



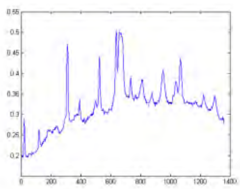
The original image 1-1.tif



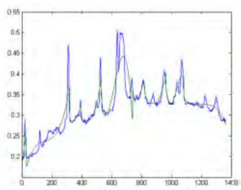
The result of the proposed method (counting result 1102)



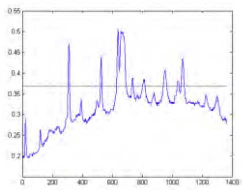
The result of Otsu's method



The plot of row 900 of original image



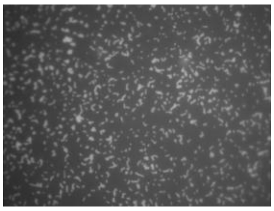
The plot of row 900 of original image with the proposed method



The plot of row 900 of original image with Otsu's method

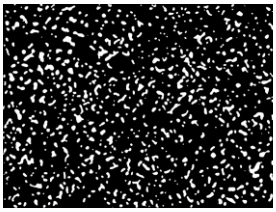
Figure 63 the results of test data name 1-1

Data name 1-2.tif

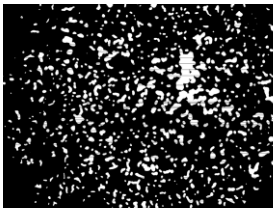


The original image

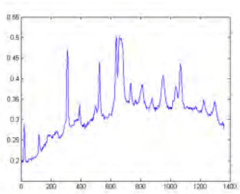
1-2.tif



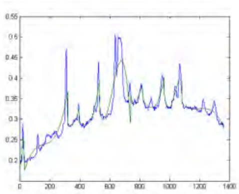
The result of the proposed method (counting result 1233)



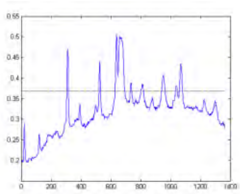
The result of Otsu's method



The plot of row 900 of original image



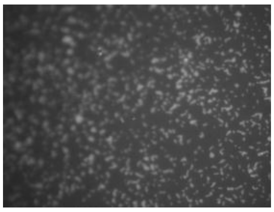
The plot of row 900 of original image with the proposed method



The plot of row 900 of original image with Otsu's method

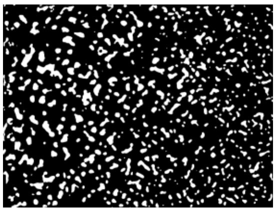
Figure 64 the results of testing data name 1-2

Data name 1-3.tif

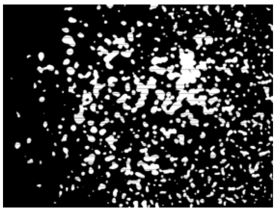


The original image

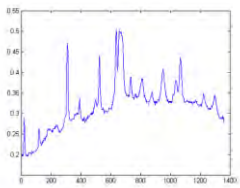
1-3.tif



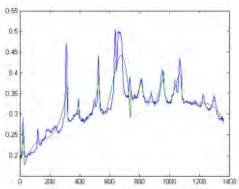
The result of the proposed method (counting result 1010)



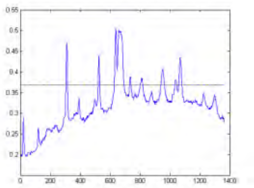
The result of Otsu's method



The plot of row 900 of original image



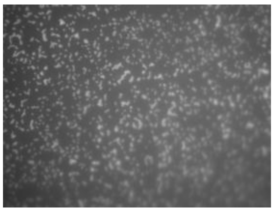
The plot of row 900 of original image with the proposed method



The plot of row 900 of original image with Otsu's method

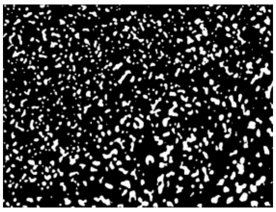
Figure 65 the results of test data name 1-3

Data name 2-1.tif

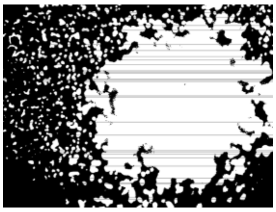


The original image

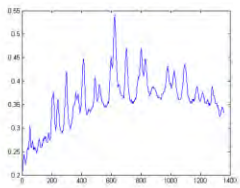
2-1.tif



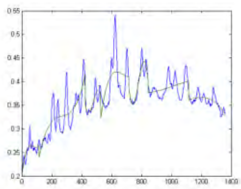
The result of the proposed method (counting result 1257)



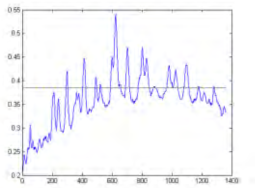
The 83 result of Otsu's method



The plot of row 900 of original image



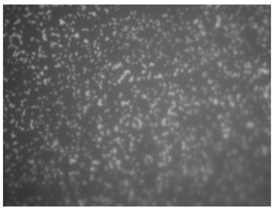
The plot of row 900 of original image with the proposed method



The plot of row 900 of original image with Otsu's method

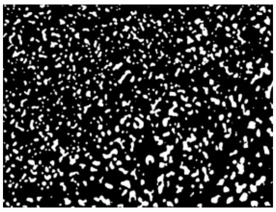
Figure 66 the results of test data name 2-1

Data name 2-2.tif

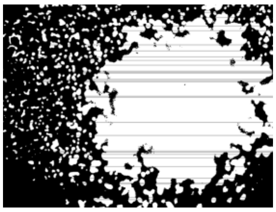


The original image

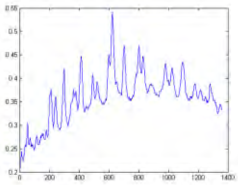
2-2.tif



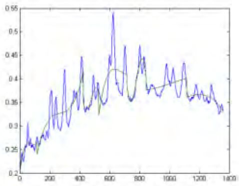
The result of the proposed method (counting result 1382)



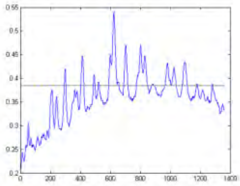
The result of Otsu's method



The plot of row 900 of original image



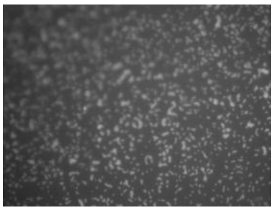
The plot of row 900 of original image with the proposed method



The plot of row 900 of original image with Otsu's method

Figure 67 the results of test data name 2-2

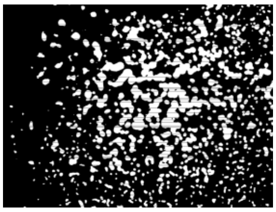
Data name 2-3.tif



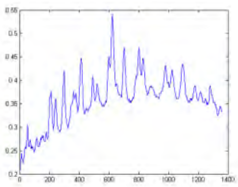
The original image

2-3.tif

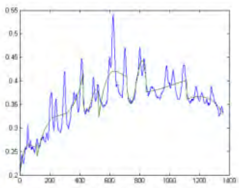
The result of the proposed method (counting result 1241)



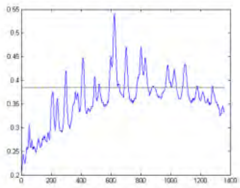
The result of Otsu's method



The plot of row 900 of original image



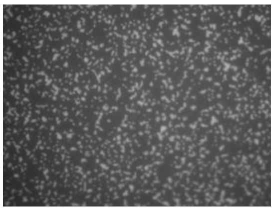
The plot of row 900 of original image with the proposed method



The plot of row 900 of original image with Otsu's method

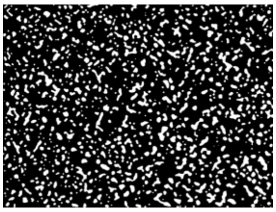
Figure 68 the results of test data name 2-3

Data name 3-1.tif

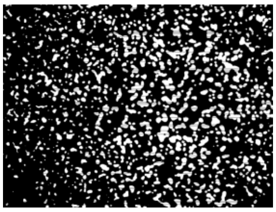


The original image

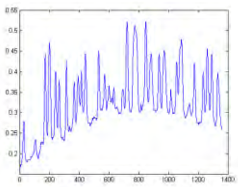
3-1.tif



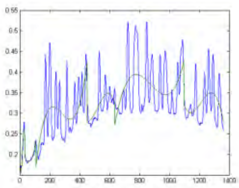
The result of the proposed method (counting result 1536)



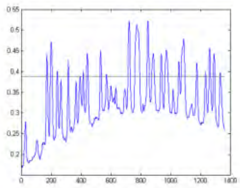
The result of Otsu's method



The plot of row 900 of original image



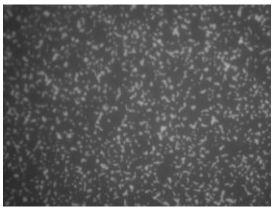
The plot of row 900 of original image with the proposed method



The plot of row 900 of original image with Otsu's method

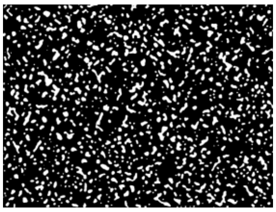
Figure 69 the results of test data name 3-1

Data name 3-2.tif

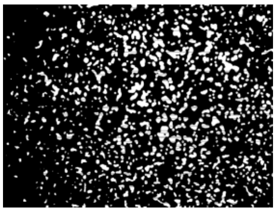


The original image

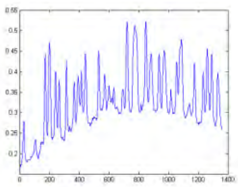
3-2.tif



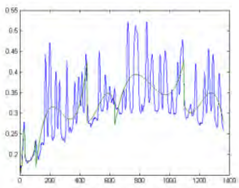
The result of the proposed method (counting result 1475)



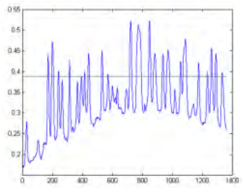
The result of Otsu's method



The plot of row 900 of original image



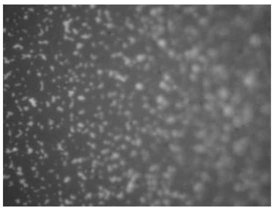
The plot of row 900 of original image with the proposed method



The plot of row 900 of original image with Otsu's method

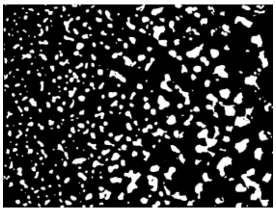
Figure 70 the results of test data name 3-2

Data name 4-1.tif

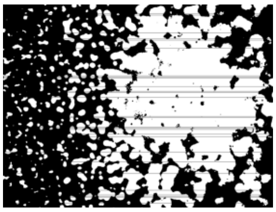


The original image

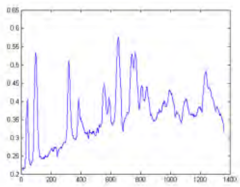
4-1.tif



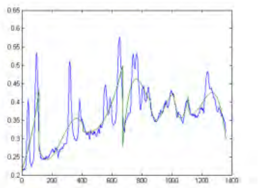
The result of the proposed method (counting result 757)



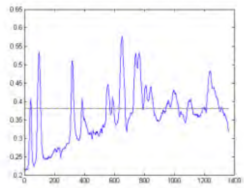
The result of Otsu's method



The plot of row 900 of original image



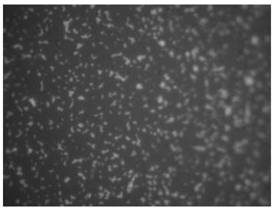
The plot of row 900 of original image with the proposed method



The plot of row 900 of original image with Otsu's method

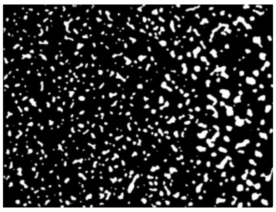
Figure 71 the results of test data name 4-1

Data name 4-2.tif

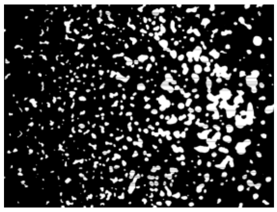


The original image

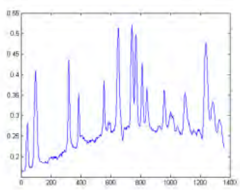
4-2.tif



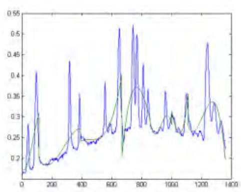
The result of the proposed method (counting result 879)



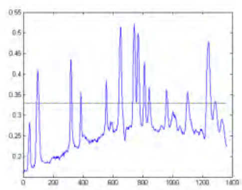
The result of Otsu's method



The plot of row 900 of original image



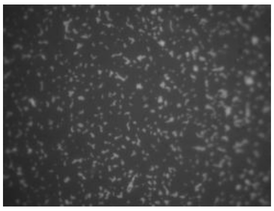
The plot of row 900 of original image with the proposed method



The plot of row 900 of original image with Otsu's method

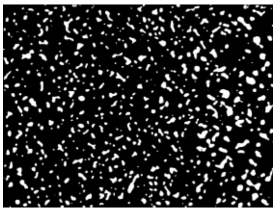
Figure 72 the results of test data name 4-2

Data name 4-3.tif



The original image

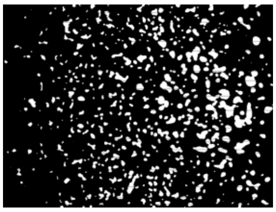
4-3.tif



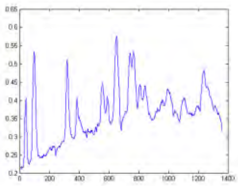
The result of the proposed

method (counting result

975)

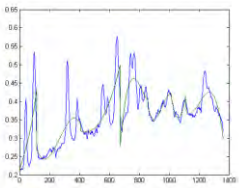


The result of Otsu's method



The plot of row 900 of

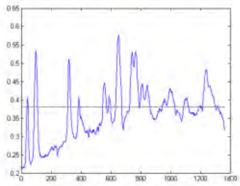
original image



The plot of row 900 of

original image with the

proposed method



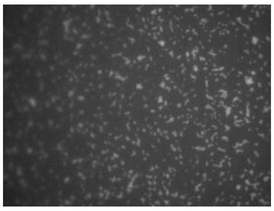
The plot of row 900 of

original image with Otsu's

method

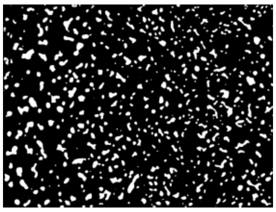
Figure 73 the results of test data name 4-3

Data name 4-4.tif

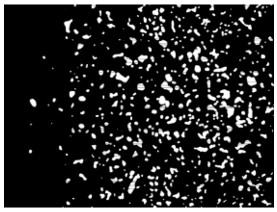


The original image

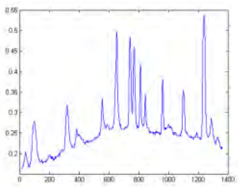
4-4.tif



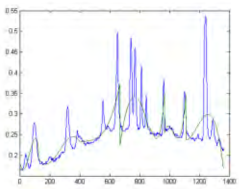
The result of the proposed method (counting result 911)



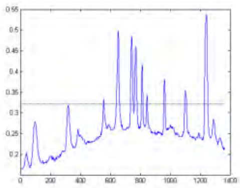
The result of Otsu's method



The plot of row 900 of original image



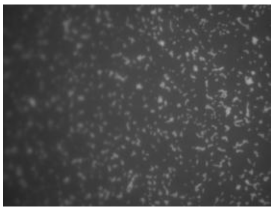
The plot of row 900 of original image with the proposed method



The plot of row 900 of original image with Otsu's method

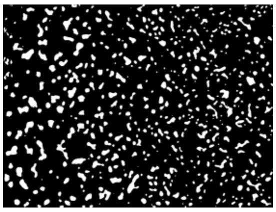
Figure 74 the results of test data name 4-4

Data name 4-5.tif

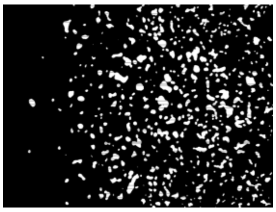


The original image

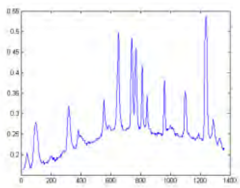
4-5.tif



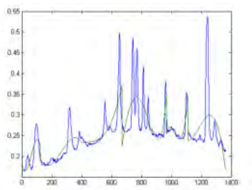
The result of the proposed method (counting result 881)



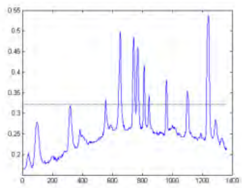
The result of Otsu's method



The plot of row 900 of original image



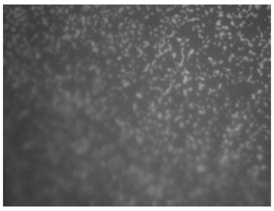
The plot of row 900 of original image with the proposed method



The plot of row 900 of original image with Otsu's method

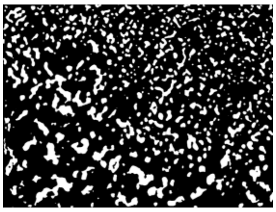
Figure 75 the results of test data name 4-5

Data name 5-1.tif

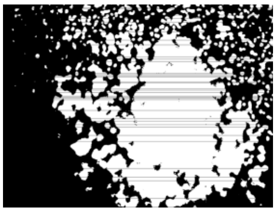


The original image

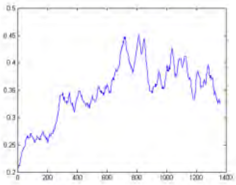
5-1.tif



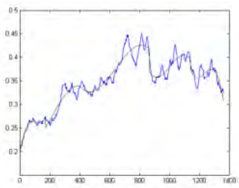
The result of the proposed method (counting result 1320)



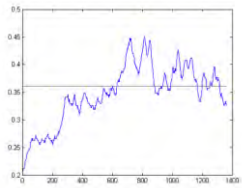
The result of Otsu's method



The plot of row 900 of original image



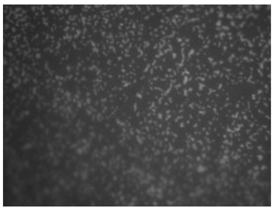
The plot of row 900 of original image with the proposed method



The plot of row 900 of original image with Otsu's method

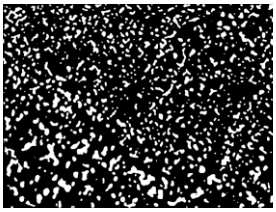
Figure 76 the results of test data name 5-1

Data name 5-2.tif



The original image

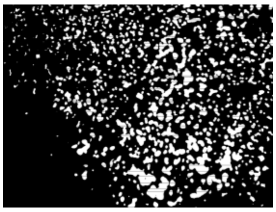
5-2.tif



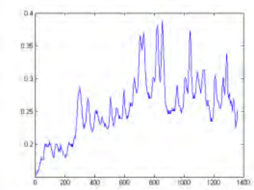
The result of the proposed

method (counting result

1433)

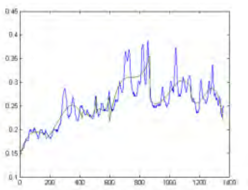


The result of Otsu's method



The plot of row 900 of

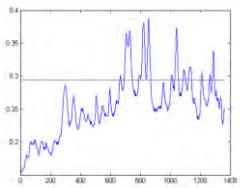
original image



The plot of row 900 of

original image with the

proposed method



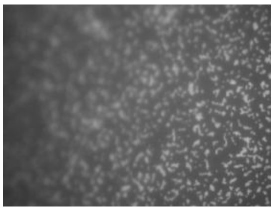
The plot of row 900 of

original image with Otsu's

method

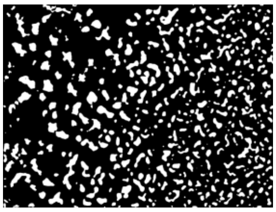
Figure 77 the results of test data name 5-2

Data name 6-1.tif

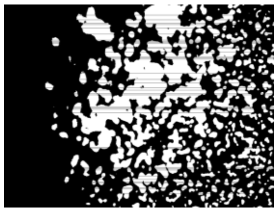


The original image

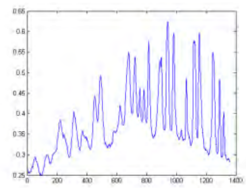
6-1.tif



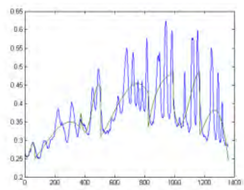
The result of the proposed method (counting result 994)



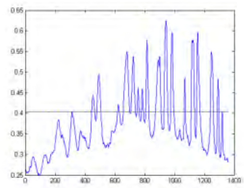
The result of Otsu's method



The plot of row 900 of original image



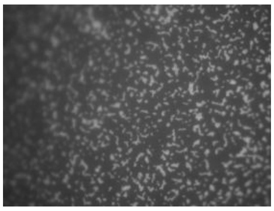
The plot of row 900 of original image with the proposed method



The plot of row 900 of original image with Otsu's method

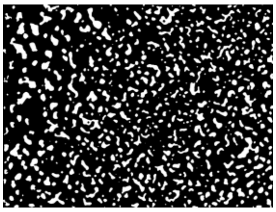
Figure 78 the results of test data name 6-1

Data name 6-2.tif

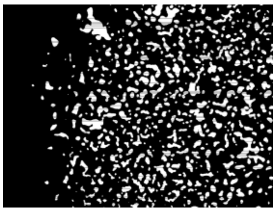


The original image

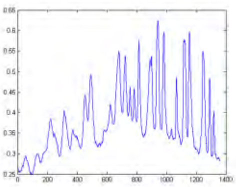
6-2.tif



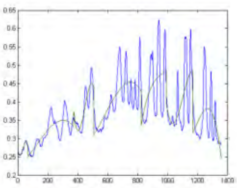
The result of the proposed method (counting result 1202)



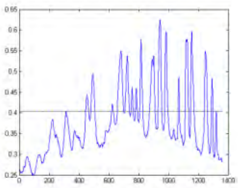
The result of Otsu's method



The plot of row 900 of original image



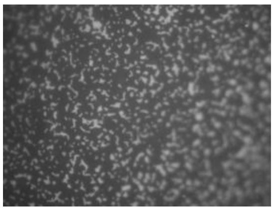
The plot of row 900 of original image with the proposed method



The plot of row 900 of original image with Otsu's method

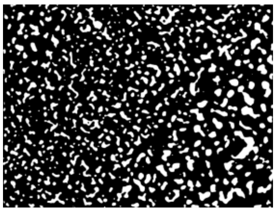
Figure 79 the results of test data name 6-2

Data name 6-3.tif



The original image

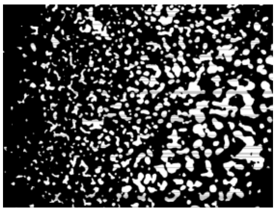
6-3.tif



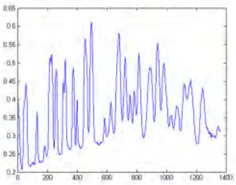
The result of the proposed

method (counting result

1195)

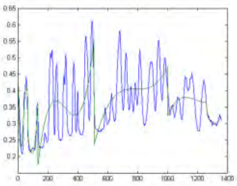


The result of Otsu's method



The plot of row 900 of

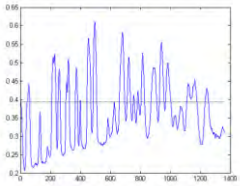
original image



The plot of row 900 of

original image with the

proposed method



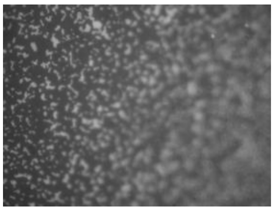
The plot of row 900 of

original image with Otsu's

method

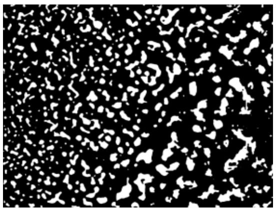
Figure 80 the results of test data name 6-3

Data name 6-4.tif

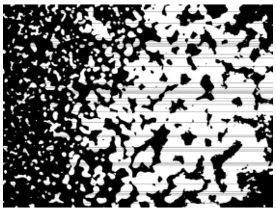


The original image

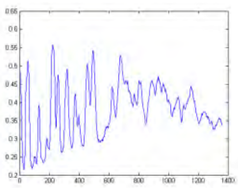
6-4.tif



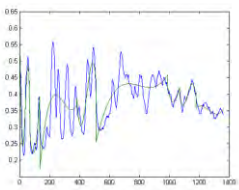
The result of the proposed method (counting result 1005)



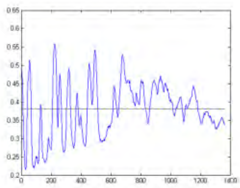
The result of Otsu's method



The plot of row 900 of original image



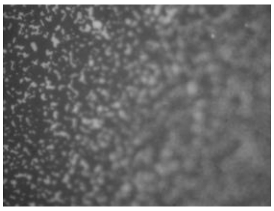
The plot of row 900 of original image with the proposed method



The plot of row 900 of original image with Otsu's method

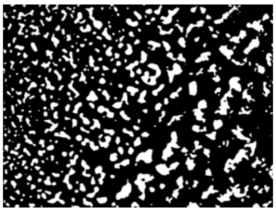
Figure 81 the results of test data name 6-4

Data name 6-5.tif

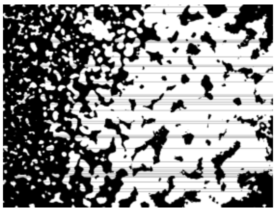


The original image

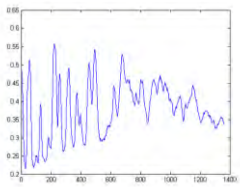
6-5.tif



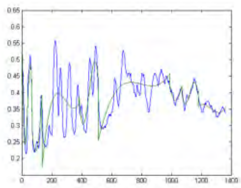
The result of the proposed method (counting result 935)



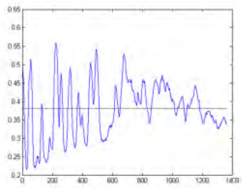
The result of Otsu's method



The plot of row 900 of original image



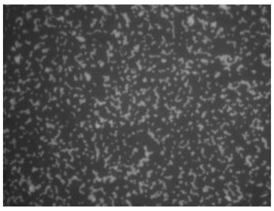
The plot of row 900 of original image with the proposed method



The plot of row 900 of original image with Otsu's method

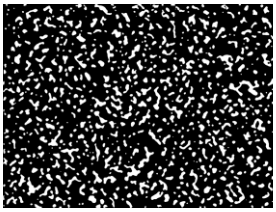
Figure 82 the results of test data name 6-5

Data name 7-1.tif

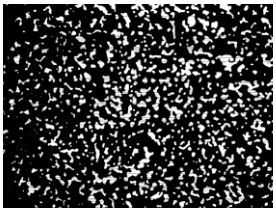


The original image

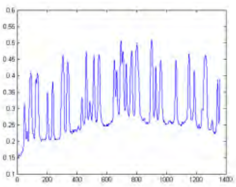
7-1.tif



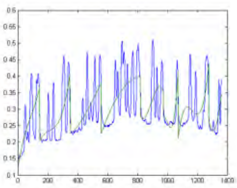
The result of the proposed method (counting result 1436)



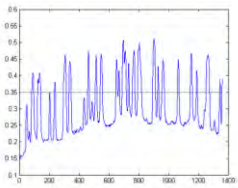
The result of Otsu's method



The plot of row 900 of original image



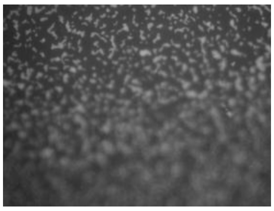
The plot of row 900 of original image with the proposed method



The plot of row 900 of original image with Otsu's method

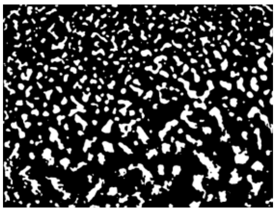
Figure 83 the results of test data name 7-1

Data name 8-1.tif

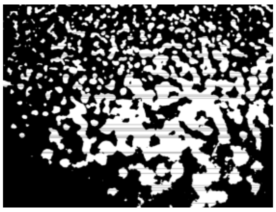


The original image

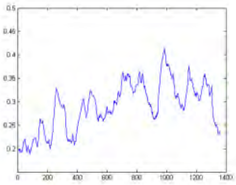
8-1.tif



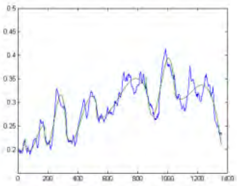
The result of the proposed method (counting result 967)



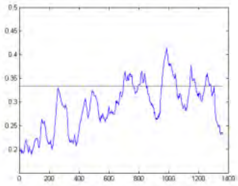
The result of Otsu's method



The plot of row 900 of original image



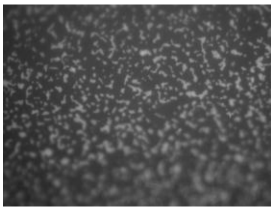
The plot of row 900 of original image with the proposed method



The plot of row 900 of original image with Otsu's method

Figure 84 the results of test data name 8-1

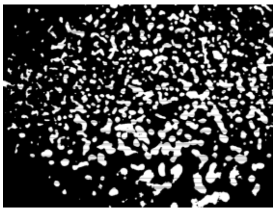
Data name 8-2.tif



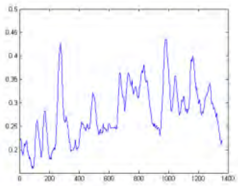
The original image

8-2.tif

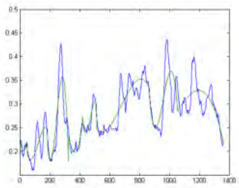
The result of the proposed method (counting result 1032)



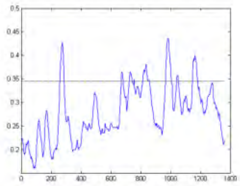
The result of Otsu's method



The plot of row 900 of original image



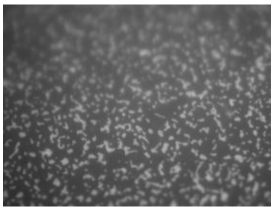
The plot of row 900 of original image with the proposed method



The plot of row 900 of original image with Otsu's method

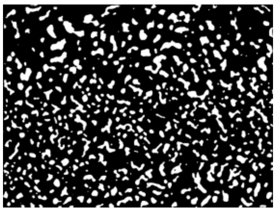
Figure 85 the results of test data name 8-2

Data name 8-3.tif

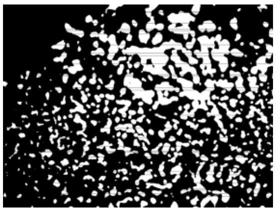


The original image

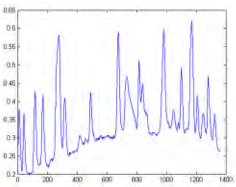
8-3.tif



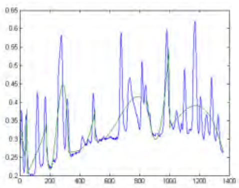
The result of the proposed method (counting result 1029)



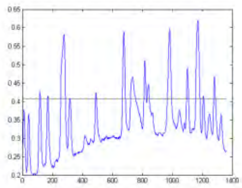
The result of Otsu's method



The plot of row 900 of original image



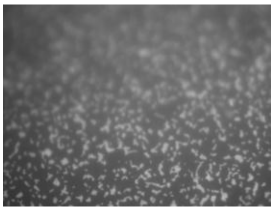
The plot of row 900 of original image with the proposed method



The plot of row 900 of original image with Otsu's method

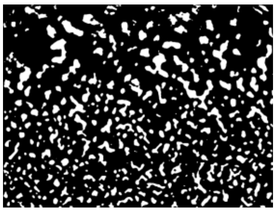
Figure 86 the results of test data name 8-3

Data name 8-4.tif

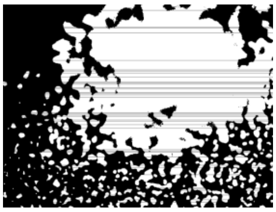


The original image

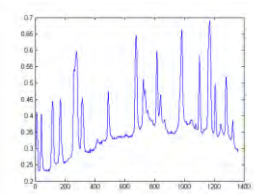
8-4.tif



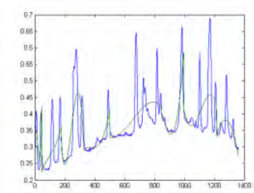
The result of the proposed method (counting result 919)



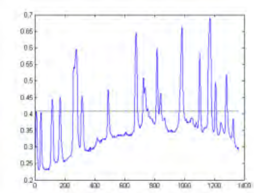
The result of Otsu's method



The plot of row 900 of original image



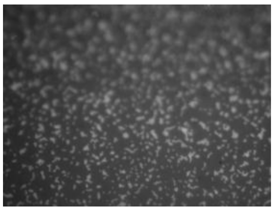
The plot of row 900 of original image with the proposed method



The plot of row 900 of original image with Otsu's method

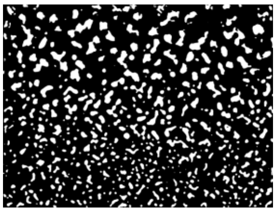
Figure 87 the results of test data name 8-4

Data name 9-1.tif

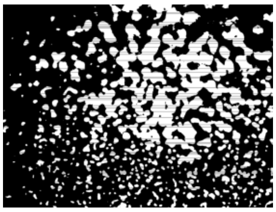


The original image

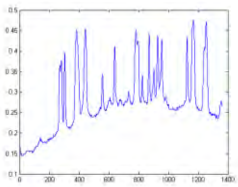
9-1.tif



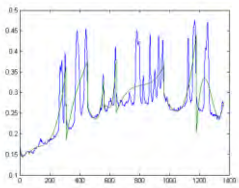
The result of the proposed method (counting result 1021)



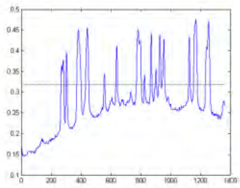
The result of Otsu's method



The plot of row 900 of original image



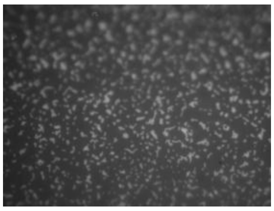
The plot of row 900 of original image with the proposed method



The plot of row 900 of original image with Otsu's method

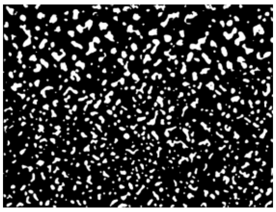
Figure 88 the results of test data name 9-1

Data name 9-2.tif

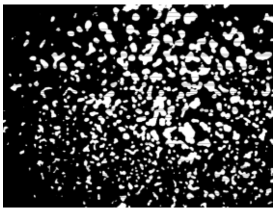


The original image

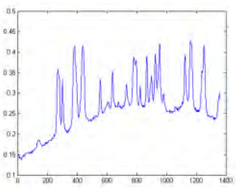
9-2.tif



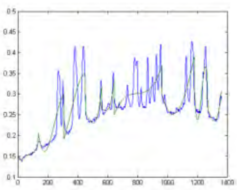
The result of the proposed method (counting result 1083)



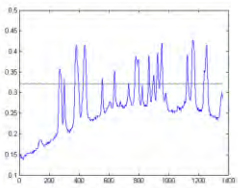
The result of Otsu's method



The plot of row 900 of original image



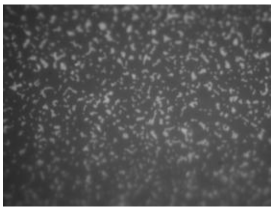
The plot of row 900 of original image with the proposed method



The plot of row 900 of original image with Otsu's method

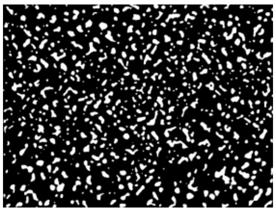
Figure 89 the results of test data name 9-2

Data name 9-3.tif

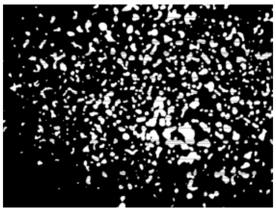


The original image

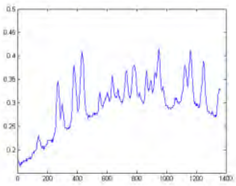
9-3.tif



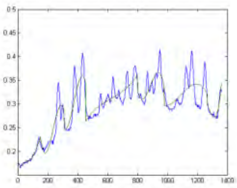
The result of the proposed method (counting result 1136)



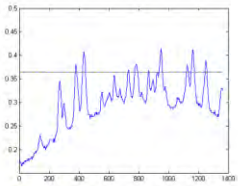
The result of Otsu's method



The plot of row 900 of original image



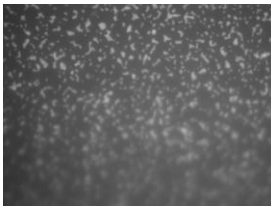
The plot of row 900 of original image with the proposed method



The plot of row 900 of original image with Otsu's method

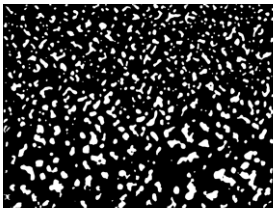
Figure 90 the results of test data name 9-3

Data name 9-4.tif

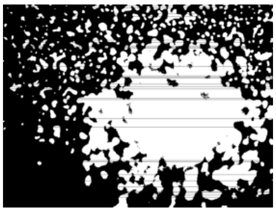


The original image

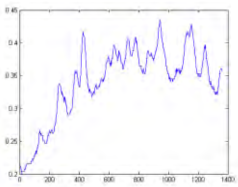
9-4.tif



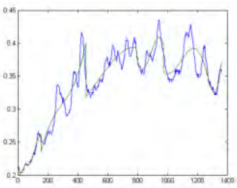
The result of the proposed method (counting result 1025)



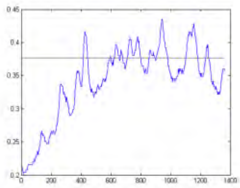
The result of Otsu's method



The plot of row 900 of original image



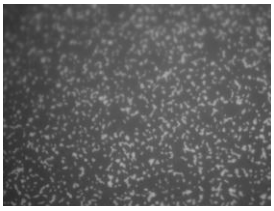
The plot of row 900 of original image with the proposed method



The plot of row 900 of original image with Otsu's method

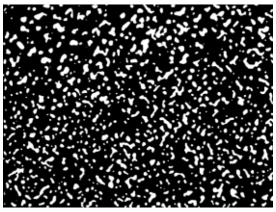
Figure 91 the results of test data name 9-4

Data name 10-1.tif

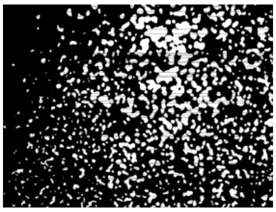


The original image

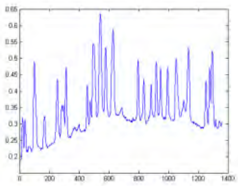
10-1.tif



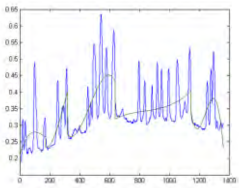
The result of the proposed method (counting result 1357)



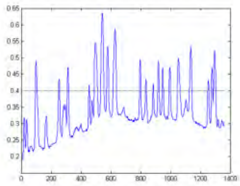
The result of Otsu's method



The plot of row 900 of original image



The plot of row 900 of original image with the proposed method



The plot of row 900 of original image with Otsu's method

Figure 92 the results of test data name 10-1

BIOGRAPY

Mr.Kitthichai Pahnchawatt was born on July 7th, 1987, in Bangkok, Thailand. He obtained his Bachelor's Degree in Mathematics from Department of Mathematics and Computer Science Faculty of Science , Chulalongkorn University in 2009.