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생물학적 영상 분석을 위한 자동 모바일 셀 계수 시스템☆

An Automatic Mobile Cell Counting System for the Analysis of Biological Image

서 재 준[1] 전 준 철[1*] 이 진 성[2]
Jaejoon Seo Junchul Chun Jin-Sung Lee

요 약

본 논문에서는 모바일 환경에서 미세세포 영상으로부터 셀을 자동 검출하고 계수하는 자동화 방법을 제시하였다. 셀 카운팅은 생물학 또는 병리학적 영상분석에 있어서 매우 중요한 과정이다. 과거에는 셀 카운팅은 수동적인 방법으로 진행되어 매우 지루하고 많은 시간을 필요로 하는 작업이었다. 이에 더하여 수동 계수 방법은 정확한 카운팅 결과를 도출하는데 어려움이 있었다. 따라서, 정확하고 일관된 셀 검출과 카운팅 결과를 생물학적인 영상으로부터 획득하기 위해서는 자동화방법이 필요하다. 제안된 다단계 셀 계수방법은 배양된 세포영상으로부터 셀을 자동으로 분할하고 분할된 셀의 위상학적 분석을 통하여 셀을 라벨링 한다. 셀 카운팅의 정확도를 높이기 위하여 워터쉐드 알고리듬에 의하여 서로 덩어리로 뭉쳐진 셀을 서로 분리하고 모폴로지 연산을 통하여 영상으로부터 획득한 개별 셀의 형태를 개선한다. 제안된 시스템은 모바일 환경에서 사용될 수 있도록 개발되었다. 따라서 셀 영상은 모바일 폰의 카메라로 획득하며 미세세포의 통계학적인 분석 데이터는 유비쿼터스 환경의 모바일 장치에 의해 전송 된다. 실험을 통하여 수동으로 계수한 셀의 숫자와 제안된 방법에 의해 자동 카운팅 된 셀의 수를 비교한 결과 제안된 방법이 매우 효과적이고 정확한 결과를 제시한다는 사실을 입증하였다.

☞ 주제어 : 자동 셀 계수, 셀 분할, 모폴로지 연산, 워터쉐드 알고리듬, HSV 컬러 모델

ABSTRACT

This paper presents an automatic method to detect and count the cells from microorganism images based on mobile environments. Cell counting is an important process in the field of biological and pathological image analysis. In the past, cell counting is done manually, which is known as tedious and time consuming process. Moreover, the manual cell counting can lead inconsistent and imprecise results. Therefore, it is necessary to make an automatic method to detect and count cells from biological images to obtain accurate and consistent results. The proposed multi-step cell counting method automatically segments the cells from the image of cultivated microorganism and labels the cells by utilizing topological analysis of the segmented cells. To improve the accuracy of the cell counting, we adopt watershed algorithm in separating agglomerated cells from each other and morphological operation in enhancing the individual cell object from the image. The system is developed by considering the availability in mobile environments. Therefore, the cell images can be obtained by a mobile phone and the processed statistical data of microorganism can be delivered by mobile devices in ubiquitous smart space. From the experiments, by comparing the results between manual and the proposed automatic cell counting we can prove the efficiency of the developed system.

☞ keyword : automatic cell counting, cell segmentation, morphological operation, watershed algorithm, HSV color model

1. Introduction

Automatic cell counting from biological or pathological imagery is a necessary step for analysing the images. Morphological cell analysis, which eventually provide the quantitative analysis of the cell, is a main issue for classifying the cells and identifying abnormality of those cell. Thus, recent researches in this area primarily focus on segmenting cell clumps from biological images, extracting morphological characteristic of the cells, identifying abnormal cells, and providing analyzing statistical analysis of the cells[1,2,3].

Nowdays the wide spread of ubiquitous smart space environments can lead the cell counting process applicable to

[1] Department of Computer Science, Kyonggi University, Gyeonggi-do, 443-760, Korea.
[2] Department of Life Science, Kyonggi University, Gyeonggi-do, 443-760, Korea.
[*] Corresponding author (jcchun@kyonggi.ac.kr)
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the mobile devices such as smart phones. The purpose of this research is to develop an efficient and reliable automated cell counting technique for the images of cell samples obtained by a camera attached on a smart phone. The statical analysis of the cells by the proposed method should support consistent and reliable results regardless of variation of surrounding environments. It is also provided through mobile device without any spatial or temporal constraints.

A numbers of automatic cell counting methods have been proposed. Those automatic methods we developed largely depend on the type of imagery available and the nature of the dataset. One of major difficulties in automatic counting cells is caused by the light variation when the images are captured. It definitely affects the bright variation of the cell and makes the conventional global threshold method not efficient [4]. It is known that morphology itself is not an efficient solution when the shape or size of the cell is inconstant from one class to another and also within a class [5]. A hybrid method which use local maxima detection and object recognition method was introduced for detecting cell from the image of yeast cells [6].

Meanwhile, a template matching method is adopted for detecting the locations of the cells by Young [7]. However, the constraints of the their method are computational burden of the template matching is expensive and needs prior knowledge of the size and shape of the cells. Moreover, their approach is working limited to the cells located non-overlapping fashion in the image.

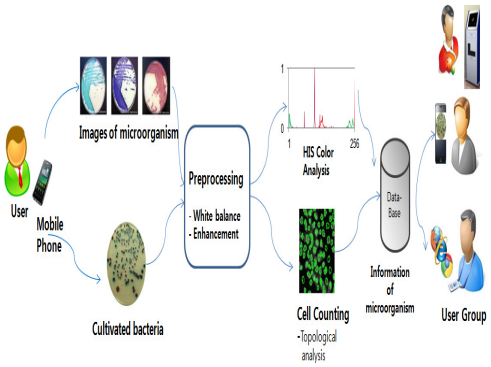
Other approaches used active contours [8] and level set method[9] for cell segmentation. Since the active contours method requires initialization of the boundary for each cell, it is difficult to make the process full automation. The other limitations of active contours and level set are not robust to the variation of background light, cell shape and clusters of cells.

The advantage of the proposed method is to produce accurate cell counting results based on a hybrid approach which is robust to light variation and cell overlapping involved cases. Moreover, it provides cell analysis on smart space environments. Thus, the availability of the system is excellent.

2. Proposed Approach

2.1 Overall Process

The proposed automatic cell counting technique consists of three major steps: HSV color-based object segmentation, morphological process for image enhancement and labeling for cell counting. In the first step, the images of microorganism such as glucuronidase or K. pneumoniae etc. on a culture medium are analyzed based on HSV color model to separate the cell organism from the background and to present as a binary image. In the second step, the converted binary cell image is enhanced by a morphological operator by removing noise and refining the boundary of the cell objects. The overlapped cells are separated by watershed algorithm. In the final step, the cell numbers are automatically counted by using a flood fill labeling. As illustrated in Figure 1. All of the phases for analyzing the images and providing the results are processed in ubiquitous environments.



(Figure 1) Schematic process for automatic cell counting and its service on smart mobile environments.

2.2 Automatic Cell Counting Method

In order to count the numbers of th cell from the biological images, the cell region is converted into binary object from the original image. In this work, we only use H(Hue) component of HSV color model. In order to minimize the affect caused by the light variation during capturing the original image, we adopt H color component

only from HSV model. The HSV color values can be easily derived from RGB color model as follows:

$$H = \cos^{-1}\left(\frac{1/2((R-G)+(R-B))}{\sqrt{(R-G)^2+(R-B)(G-B)}}\right) \quad (1)$$
$$S = 1 - \frac{3}{(R+G+B)}[\min[R,G,B]]$$
$$V = \frac{(R+G+B)}{3}$$

Once the binary cells are obtained by H value, then a morphological open operator is applied to the segmented cell region to enhance the quality of the image by removing noise between the cell regions while keep the original size of the cells. Using the processed binary cell images, we can obtain the contours of segmented cell regions for preparing the cell counting. To obtain the contour of the cell object, the center of each cell is first extracted by the distance transform[10] and the boundary of each cell is located based on the center. The center of the cell can be extracted from $I(p)$ the medial axis of the segmented cell region.

Once the center of the binary object is located by the distance transform, the contour of the single connected cell is acquired. The distance transform is the transformation that generates a map D whose value in each pixel p of the segmented region (O) is the smallest distance from this pixel to the background O^c. The image D, which is called a distance map, can be defined as follow:

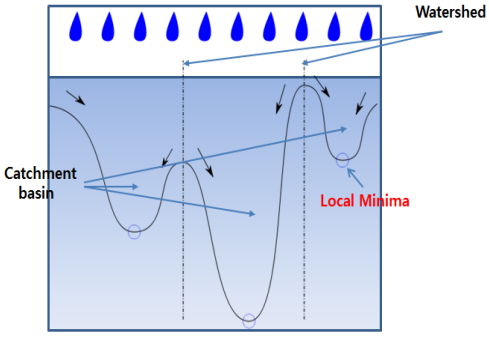
$$D(p) = \min\{d(p,q) | q \in O^c\} = \min\{d(p,q) | I(q) = 0\} \quad (2)$$

In general conventional cell segmentation method sometimes cannot separate cells completely because of the overlapped cells. This issue can be resolved by using watershed algorithm[11,12,13].

The watershed algorithm is a technique that makes a use of image gradient cues to segment images. It represents gray-scale image data as a measure of elevation and regards the image data as the DEM(Digital Elevation Model). If we use the gradient (relief) of the image to represent the topology, there is wall between objects of interest which is so called watershed. The regions separated by watershed are called catchment basins having lowest elevation where any drop of water that were to fall inside in the basin would eventually flow to.

A drop of water falling on a topographic gradient flows down to reach a local minima. Thus, the watershed of a gradient corresponds to the limits of the adjacent catchment basins of the drops of water as shown in Fig 2. This watershed algorithm can properly separate the overlapped segmented cells each other.

Even though overlapped cells are considered as a single region in the initial stage of segmentation, the watershed can properly disjoint the agglomerated cells into individual cell respectively.



(Figure 2) The general concept of watershed algorithm

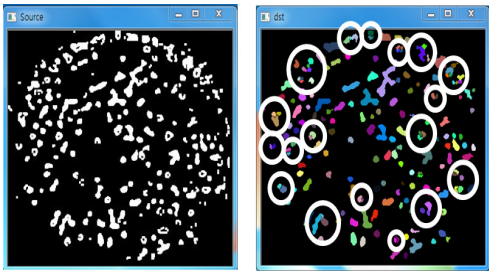
The basic definitions of watershed based on rainfall simulation which are used for cell segmentation are as follows. When a two-dimensional gray scale image f whose definition domain is denoted $D_f \in Z^2$ is given, f is supposed to take discrete values in a given range $[0, N]$, N being an arbitrary positive integer value[13].

Let G denote a square grid and p denotes a pixel in $G \in Z^2 \times Z^2$. Path of a water drop can be expressed as follows:

Definition 1: A path P of length L between two pixels α and β in image f is an (L + 1) ordered pair of pixels starting from α and ending in β.

$$P_{\alpha\beta} = \langle p_0, p_1, \cdots p_{L-1}, p_L \rangle | p_0 = \alpha, p_1 = \beta : \quad (3)$$
$$\forall p_i \in N_G(P_{i-1}), i = 1 \cdots L$$

Where, $N_G(p)$ is the set of the neighbouring pixels of pixel p , with respect to *G*. It is important to define the type of connectivity for the neighbourhood operation. Typically either 4 connectivity or 8 connectivity is used and we use 4 connectivity in this work. In terrain surface a water drop will flow along a steepest descent path until it reaches minimum. A digital image is analogous to a terrain surface in which, pixels with higher gray level make hills and pixels with lower gray level make lakes. Figure 3 shows a segmentation results with the watershed algorithm.



(Figure 3) Segmentation of the cell by the watershed algorithm.

In rainfall based watersheds every path starts from a pixel at higher altitude and end in a pixel at lowest altitude. A regional minimum is a pixel having gray level value smaller than any other pixels in the region. Thus, a steepest descending path $Dpath_{(p,m)}$ can be defined as follows:

Definition 2: Steepest descent path from a pixel '*p*' to minimum 'm' is a series of connected pixels originating from 'p'; such that every descendent pixel in $SDpath$ is smaller than its predecessor.

$$SDpath_{(p,m)} = P_{(p,m)} : p_i < p_{i-1} \quad \text{where } i = 0, 1 \cdots L \tag{4}$$

Suppose there are n regional minima $m_1, m_2 \cdots m_n$, the catchment basin can be defined by

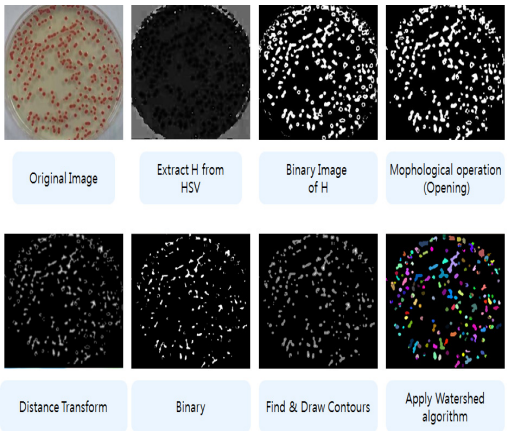
Definition 3: The catchment basin $CatchB_i$ associated with a regional minimum m_i is the set of pixels p of D_I such that a water drop falling at p flows down along the relief, following a certain descending path called the *downstream of* p, and eventually reaches M.

$$CathB_i = INF\{SDpath_{(p,m_1)}\} \tag{5}$$

Definition 4: The distance between two pixels p and q belonging to a certain domain $CatchB$ in an image is the minimum length of any path from p to q without leaving the domain $CatchB$.

$$Dist(p,q) = INF\{SDpath_{(p,q)} | p,q \in CatchB_1\} \tag{6}$$

Figure 4 illustrates the sample results from each step of image segmentation procedure using explained watershed algorithm.



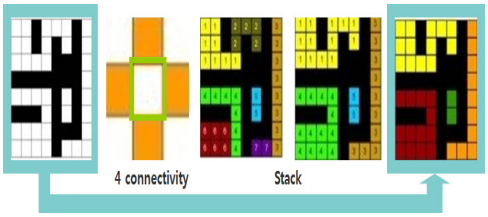
(Figure 4) Results in each stage of the proposed cell counting method

In general, the Flood Fill algorithm consists of three parameters such as a start node, a target color, and a replacement color. The algorithm looks for all nodes in the array which are connected to the start node by a path of the target color, and changes them to the replacement color. There are many ways in which the flood-fill algorithm can be structured, but they make use of a queue or stack data structure, explicitly or implicitly. In this paper, 4-way recursive Flood Fill algorithm is used for counting cells while filling out the detected cell regions a color. The Flood Fill algorithm is as follow.

/* **Algorithm of 4-Way Flood-Fill** */

```
void floodFill4 (int x, int y, int fill, int oldColor)
{
 if (getPixel(x,y) == oldColor) {
     setColor(fill);
     setPixel(x,y);
     floodFill4 (x-1, y, fill, oldColor);
     floodFill4(x, y+1, fill, oldColor);
     floodFill4(x, y-1, fill, oldColor);
 }
}
```

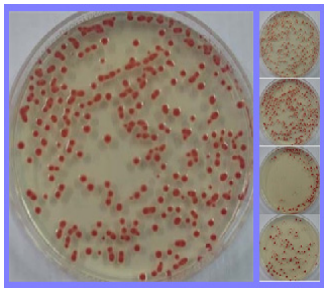
Figure 5 shows the way to fill the disconnected 4 different regions by different colors based on 4-way moves. When a closed cell area is fully filled with a certain color the cell counting number is increased by 1 automatically.



(Figure 5) 4-way Flood Fill algorithm to count cells

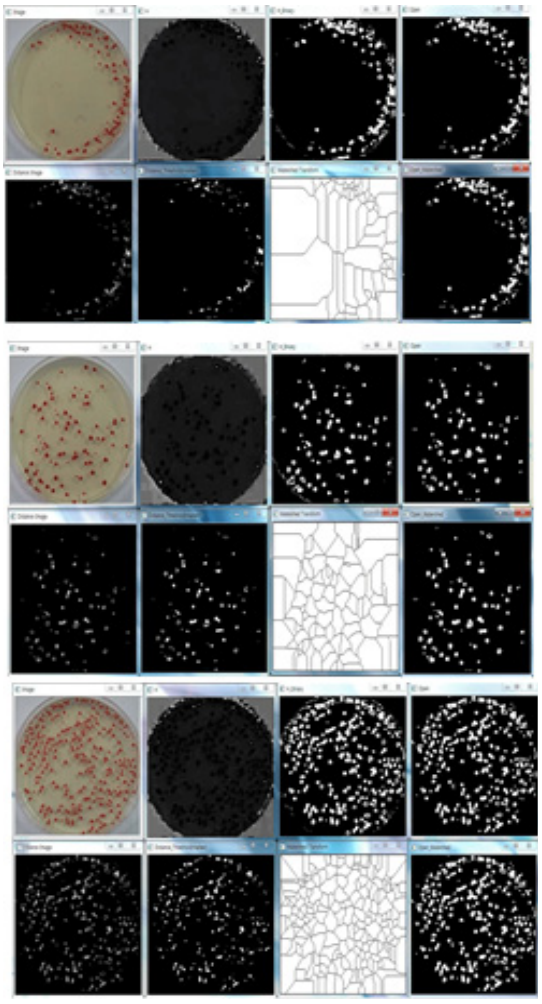
3. Experimental Results

In order to evaluate the proposed system, we have cultivated *K. pneumoniae* on the culture medium and obtained a sample image (360x300) by a camera attached smart phone. Figure 6 displays sample images and their labeling results. During the cultivation of the sample, the numbers of the *K. pneumoniae* on the culture medium have been increased.



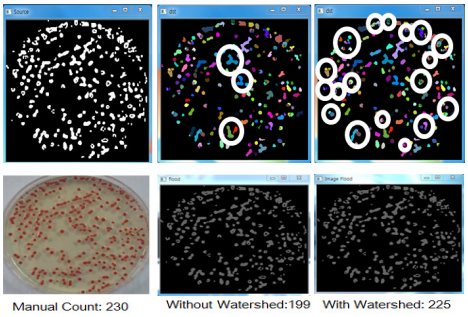
(Figure 6) Cultivated *K. pneumoniae*

Figure 7 shows the intermediate results of the proposed multistep process in automatic cell counting.



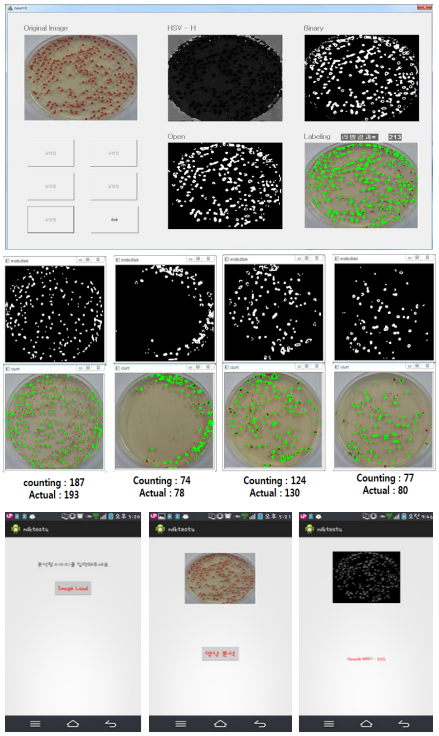
(Figure 7) Intermediate results of 3 different cultivated cells on the culture medium

From the morphologically processed binary image, watershed can disjoint most of overlapped cells into individual cell. Figure 8 shows the segmented binary image, unsuccessful instances of watershed, and successful instances of watershed marked by a circle from left to right. Comparative results by using manual cell counting and the proposed automatic cell counting are also displayed in Figure 8.



(Figure 8) Results of the segmented binary cell image, cells overlapped, and cells separated by watershed algorithm(top), Comparison between manual cell counting and automatic cell counting(bottom)

The automatic cell counting system is developed to be used in ubiquitous environments. When the sample cell images are captured by a smart phone, the cell counting can be processed both on server and the smart phone itself. The overall cell counting accuracy by the developing system is over 96% correct comparing with the ground truth data.



(Figure 9) The developed automatic mobile cell counting system

4. Conclusion

This paper introduces an automatic cell counting method from microorganism images on mobile environments. The proposed multi-step cell counting method automatically segments the cells from the image of cultivated microorganism and labels the cells by utilizing topological analysis of the segmented cells. In order to improve the accuracy of the cell counting, watershed algorithm is adopted for separating the overlapped cells. From the experiments, the proposed technique provides more accurate and consistent cell counting result comparing with previous cell counting approaches.

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◐ 저 자 소 개 ◑

서 재 준 (Jaejoon Seo)
2013년 경기대학교 컴퓨터과학과 졸업(학사)
2014년~현재 경기대학교 대학원 컴퓨터과학과 석사과정
관심분야 : 비실사적 렌더링, 의료영상처리, 컴퓨터그래픽스
E-mail : 1004aga@naver.com

전 준 철 (Junchul Chun)
1984년 중앙대학교 전자계산학과 (학사)
1986년 중앙대학교 대학원 전자계산학과 (석사)
1987년 2월~1988년 6월 삼성반도체 통신연구소 S/W 공학 연구실 연구원
1992년 The Univ. of Connecticut, 컴퓨터공학과 (석사)
1995년 The Univ. of Connecticut, 컴퓨터공학과 (박사)
1995년~현재 경기대학교 컴퓨터과학과 교수
2010년 7월~현재 경기대학교 CCSRC (콘텐츠융합 소프트웨어 연구센터) 센터장
2001년~2002년 2월 Michigan State Univ. 컴퓨터공학과 Pattern Recognition and Image Processing Lab. (PRIP) 방문 연구원
2009년~2010년 2월 Univ. of Colorado, Boulder, 컴퓨터과학과 Wellness Innovation and Interaction Lab. 방문 연구원
관심분야 : 비전기반 HCI, 컴퓨터 애니메이션, 증강현실, 의료영상처리 등
E-mail : jcchun@kgu.ac.kr

이 진 성 (Jin-Sung Lee)
1992년 경기대학교 생물학과 (학사)
1994년 성균관대학교 생물학과 (석사)
1999년 성균관대학교 생물학과 (박사)
2012년～현재 경기대학교 생명과학과 조교수
2001년~2007년 코아바이오시스템(주) 연구소장
2007년~2008년 (주)바이오닉스 상무이사
2008년~2012년 (주)나래바이오테크 기술개발총괄(CTO)
2007년~2008년 한국학술진흥재단 경영자문위원
2007년~2009년 농촌진흥청 전문위원
2012년~현재 (사)한국기술거래사회 부회장
관심분야 : 핵산기반 병원성 미생물 분자진단, 메타게노믹스활용 바이오에너지(에탄올)
E-mail : lejis@daum.net