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Reconstruction and Elimination of Optical Microscopic Background Using Surface Fitting Method

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One serious problem among the troubles to identify objects in an optical microscopic image is contour background due to non-uniform light source and various transparency of samples. To solve this problem, this paper proposed an elimination method of the contour background and compensation technique as follows. First, Otsu's optimal thresholding method extracts pixels representing background. Second, bilinear interpolation finds non-deterministic background pixels among the sampled pixels. Third, the 2D cubic fitting method composes surface function from pivoted background pixels. Fourth, reconstruction procedure makes a contour image from the surface function. Finally, elimination procedure subtracts the approximated background from the original image. To prove the effectiveness of the proposed algorithm, this algorithm is applied to the yeast *Zygosaccharomyces rouxii* and ammonia-oxidizing bacteria *Acinetobacter* sp. Labeling by this proposed method can remove some noise and is more exact than labeling by only Otsu's method. Futhermore, we show that it is more effective for the reduction of noise.

Key words: Otsu's method, Bilinear interpolation, *Acinetobacter* sp., *Zygosaccharomyces rouxii*

Introduction

The work observing objects through a microscope is not only tedious but also iterative. From 1960, these kinds of works are automated by using image processing technique and nowadays, the image analysis system is regarded as a necessary device for the optical microscopes (Castleman, 1996; Sonka et al., 1999). In cell engineering, digital image analysis is applied for several application fields. Pons et al. (1993) used a semi-auto image analysis system and analyzed morphological characteristics. Zalewski and Bildanalyse (1995) proposed an on-line monitoring system which monitors elements of circumstance in culturing yeast by using general optical microscope. Suhr and Wehnert (1995) developed in-situ microscope which can analyze cell growth on-line. Bloem et al. (1995) proposed a method for detecting position and counting the number of bacteria in solid foods automatically. The above stated studies need a procedure extracting characteristic information of each cell in order to estimate the growth state of cells. To extract the characteristic vectors of cells, separating work of the target object and its background in microscope image is very important. In the case of optical microscope, a background which is not distributed with constant gray-level causes variation of pure gray level for cells. The traditional methods to remove the background use a concept that after acquiring only the part of background in the captured image and storing it as a background image, they subtract the prestored background image from a obtained cell image (Pons et al., 1993). This concept is applicable

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only in the state of off line. In the case of on-line analysis system, in order to consider the parameter variations of image, a complicate analysis algorithm must be realized (Suhr and Wehnert, 1995; Bloem et al., 1995). But those kinds of methods cannot be realized easily in the fields of requiring a real time processing because of its long processing time.

In this paper, using high order surface fitting and interpolation, a background elimination method interpolating an image with unnecessary background element is proposed. The method obtains background data and reconstructs the background by calculating surface function minimizing least square error of the sampled data. It eliminates its background from the original image. Optimal thresholding elects pixels to be representative of the background and bilinear interpolation finds non-deterministic background pixels among the sampled pixels. Reconstruction procedure makes a contour image from surface function which was composed by the 2D cubic fitting method from pivoted background pixels. Elimination procedure subtracts the approximated background from the original image. When this algorithm is applied to yeast *Zygosaccharomyces rouxii* and ammonia-oxidizing bacteria *Acinetobacter* sp., the higher order surface fitting method can eliminate background suitably and estimate the object more precisely and obviously. Furthermore, we can see that it is more effective for the reduction of noise.

Reconstruction and elimination of background image measured by microscope

Fig. 1 shows original image of (a) *Zygosaccharomyces rouxii*, a kind of yeast and (b) *Acinetobacter* sp., a kind of ammonia-oxidizing bacteria.

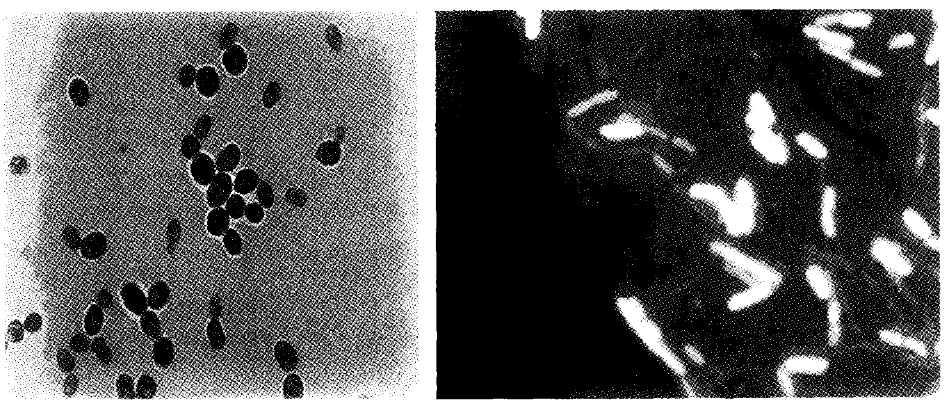
An image variation by this kind of non-uniformed background is easily found in microscope image because intensity for center of optical light is stronger than that of its outside. According to going from center to its outside, its intensity is slowly decreased. The non-uniformed property causes a distortion for internal gray level of object separated from background in image. In a distribution device using image processing or in application field identifying growth characteristics of microbial cells, since the internal gray level embeds the characteristics of an object such as density, color, and depth etc., the slope of background must be compensated before the parameters of the image is measured.

1. Two-dimensional 3rd order surface data fitting technique

Surface fitting is also used for noise removal when one of the noise components can fitted, computed and subtracted out. In this paper, for noise removal in background, it is assumed that the background of microscope image has non-uniformed property, and its surface slope can be expressed by 3rd order polynomial. Then, we partition the captured image in a constant interval, obtain the gray level representing its background, and decide an approximate function minimizing square error of these pixels. Also it is assumed that the approximated background function $f(x,y)$ is given by the following:

$$f(x,y) = c_0 + c_1x + c_2y + c_3xy + c_4x^2 + c_5y^2 + c_6x^2y + c_7xy^2 + c_8x^3 + c_9y^3 \quad (1)$$

where c_0, c_1, $\cdots$, c_9 are coefficients of polynomials



(a) *Zygosaccharomyces rouxii* (b) *Acinetobacter* sp.

Fig. 1 Original image

decided by least square method (Castleman, 1996).

Since 2nd order data fitting can be easily realized by matrix based calculation, using a matrix B expressing polynomial according to coordinate (x,y) of background pixels, column vector Y with gray level of background pixels and column vector C of coefficients decided by least square method, a column vector E meaning an error between practical background pixel value and its approximated value can be given by:

$$E=Y-BC \tag{2}$$

where

$$Y=\begin{bmatrix} g_{11} \\ g_{12} \\ \vdots \\ g_{1N} \\ g_{21} \\ \vdots \\ g_{MN} \end{bmatrix},\ C=\begin{bmatrix} c_0 \\ c_1 \\ \vdots \\ c_9 \end{bmatrix},\ B=\begin{bmatrix} 1 & x_1 & y_1 & x_1y_1 & \cdots & x_1y_1^2 & x_1^3 & y_1^3 \\ 1 & x_1 & y_2 & x_1y_2 & \cdots & x_1y_2^2 & x_1^3 & y_2^3 \\ \vdots & \vdots & \vdots & \vdots & \cdots & \vdots & \vdots & \vdots \\ 1 & x_M & y_1 & x_My_1 & \cdots & x_My_1^2 & x_M^3 & y_1^3 \\ \vdots & \vdots & \vdots & \vdots & \cdots & \vdots & \vdots & \vdots \\ 1 & x_M & y_N & x_My_N & \cdots & x_My_N^2 & x_M^3 & y_N^3 \end{bmatrix}$$

g_{ij} is a gray level of pixel (i,j) of the sampled background pixels with the size of $M \times N$. Matrix multiplication BC means background pixel value approximated by eq. (1) and subscripts M,N are sizes of data sampling for x and y axes respectively. The mean square error (MSE) of column vector E is given by (Castleman, 1996):

$$MSE=\frac{1}{MN}E^TE \tag{3}$$

Substituting eq. (2) into eq. (3), differentiating for C and setting the derivative to be zero, then the result is yielded as:

$$C=[B^TB]^{-1}B^TY \tag{4}$$

where the coefficient vector C minimizes the mean square error, and square matrix $[B^TB]^{-1}B^T$ is a pseudoinverse of B (Castleman, 1996).

2. Interpolation of blank region

The interpolation is the process of generating values for addresses that lies between two pixels. It means a process to extract background from original image after obtaining mask image. Then using the mask image, the part shaded by mask is regarded as a part of object and is excluded from the procedure detecting the background. When we extract the background value, since the input image is divided into 20×15 regions with 32×32 grids, the background value of each region is given as maximum gray level in its region. Because object size is larger than grid size, there are regions in which we cannot choose the background value. We grade the regions as blank regions and the representative value is estimated using interpolation method based on neighborhood background value. Then the matrices B and Y to be used for pseudoinverse calculation of eq. (4) have dimensions $R^{MN \times 1}$ and $R^{MN \times 10}$, respectively. In the case that we cannot obtain the background value because all the regions are shaded by object, we use the interpolation method usually. The region of O in Fig. 2 is one which we can decide a background value by mask of object. The value of this region can be decided by applying bilinear interpolation method (Castleman, 1996) based on background values of 1~8.

Fig. 3 shows a concept of bilinear interpolation method that newly produced pixel is a summation of values multiplying a weighting factor to 4 nearest pixels. Each weighting factor is proportional to distance between pixels.

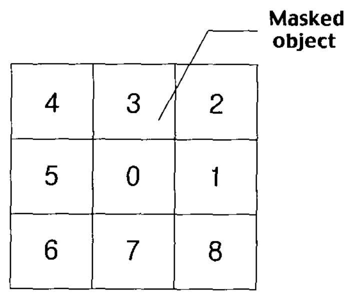


Fig. 2. A blank region problem of big masked object.

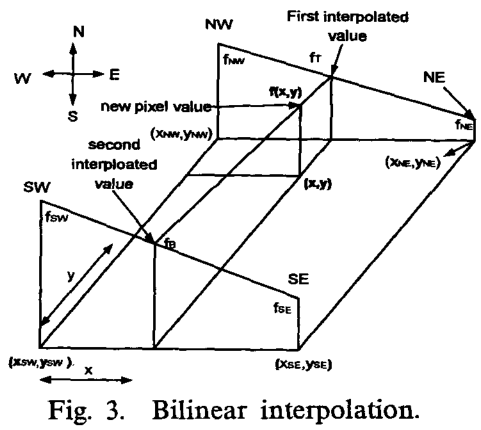


Fig. 3. Bilinear interpolation.

The bilinear interpolation is according to the following equations:

$$f(x,y) = f_T - \rho_y(f_B - f_T) \quad (5)$$

$$f_B = f_{SW} - \rho_x(f_{SE} - f_{SW}) \quad (6)$$

$$f_T = f_{NW} - \rho_x(f_{NE} - f_{NW}) \quad (7)$$

$$\rho_x = \frac{x - x_{SW}}{x_{SE} - x_{SE}} \quad (8)$$

$$\rho_y = \frac{y - y_{SW}}{y_{NW} - y_{SW}} \quad (9)$$

where $f(x,y)$ is approximate function of background and ρ_x, ρ_y are weighting factors for each directions, $f(x,y)$ is a pixel value obtained by inter-polation method, (x,y) coordinate of pixel, and *NW*, *NE*, *SW*, *SW* means directions of each (x,y).

3. Mask image and labeling

Generally, mask image can be obtained just by binarization procedure. The labeling is a basic procedure for image recognition. Since our work is a prestage for image analysis, we extract the mask image from original image. In the labeling procedure, an adaptive binarization procedure and a morphological filtering procedure are included. Then, isolated points and holes in object are eliminated. So, it is compatible for use of mask image (Sonka et al., 1999).

4. Compensation of image using background elimination

The frame image has size of 640×480 pixels and each pixel has 256 gray levels. In order to prove the effectiveness of the proposed background compensation, we used *Acinetobacter* sp. and *Zygosaccharomyces rouxii*. The practical experimental procedure is divided into 3 steps.

[Step 1] Input image from original image is produced. After applying noise reduction filter and distinguishing background and object, input and mask images are produced.

[Step 2] Obtain approximate function of background $f(x,y)$. Input image has 20×15 regions separated with 32×32 grids. The procedure of surface fitting is as follows; select one gray level representing the background in each region and get $f(x,y)$. Using the mask image, the region including only object is set by blank. Using the $f(x,y)$, produce approximate background image of 640×480 pixels, and the blank region is compensated by interpolation method.

[Step 3] Subtracting the approximate background image from original image, a compensated image is obtained. But since the compensated image has low gray level over all image, appropriate gray level compensation is realized.

Experimental Results and Discussion

1. Experimental devices

Fig. 4 and Table 1 show composition of experimental device and specification of image processing system. The object is set up on the guide plate of the microscope and it is captured by CCD camera attached at the microscope. The captured image is changed into digital image by frame grabber interface card and the image sequence is analyzed by the developed image processing program package.

2. Experimental results and discussion

Fig. 5~Fig. 14 show the experimental results applied to *Zygosaccharomyces rouxii* and *Acinetobacter* sp. using the proposed background interpola-

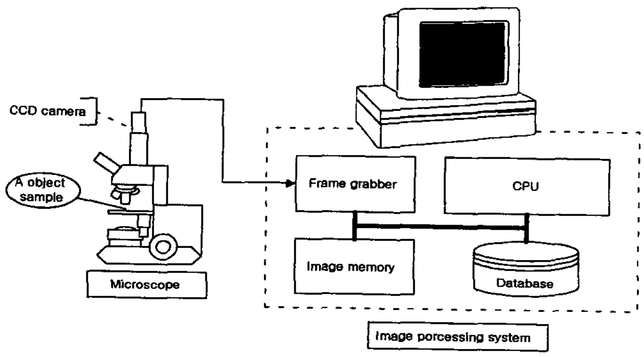
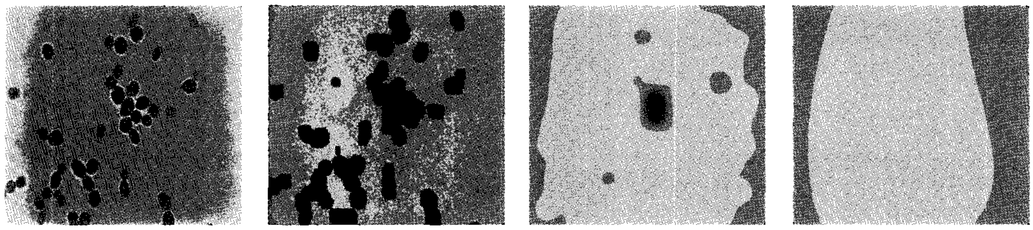


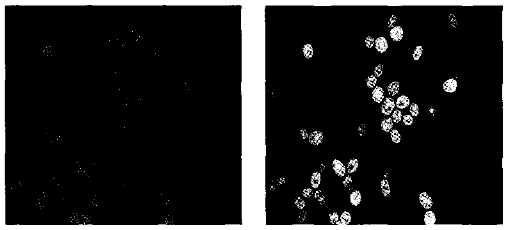
Fig. 4. Composition of Experimental Apparatus.

Table 1. Configuration of the image processing system

Items	Models	Spec.
Microscope	BX40, Olympus	Lens sets: ×1000
CCD Camera	SFA-410ED, Samsung	1/2 inch CCD cell 640×480 or more
Frame Grabber	MVB-03, Samsung	Mono, built-in DSP, VRAM, 4MB
Host Computer	GT53H, LG Electronics	Pentium Windows 95 64MB RAM

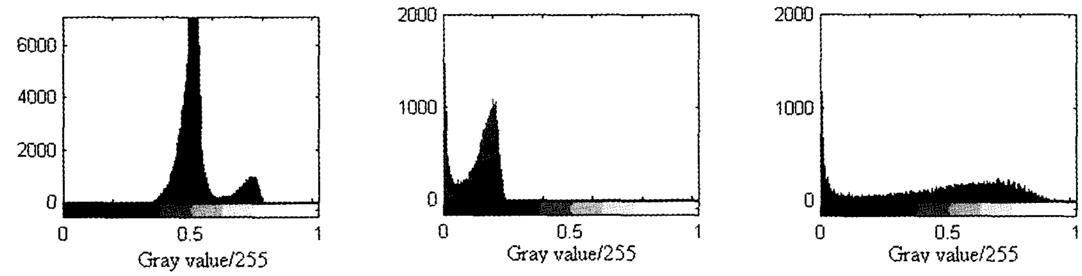


(a) Original image (b) Masked image (c) Sampled background image (d) Approximated background image



(e) Subtracted image (f) Adjusted image

Fig. 5. The resulted image for *Zygosaccharomyces rouxii*.



(a) Original image (b) Subtracted image (c) Adjusted image

Fig. 6. Histograms for *Zygosaccharomyces rouxii*.

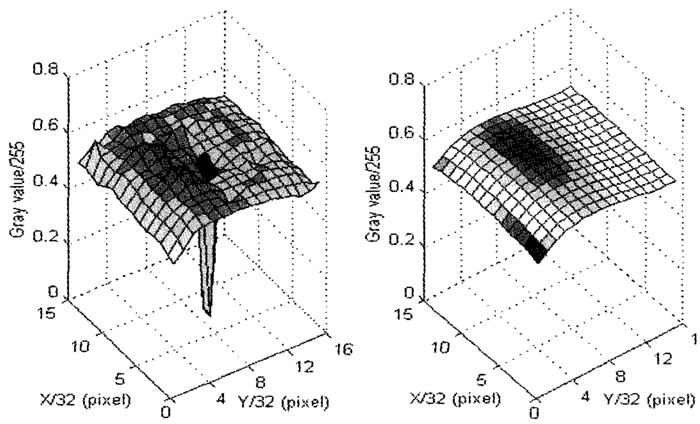
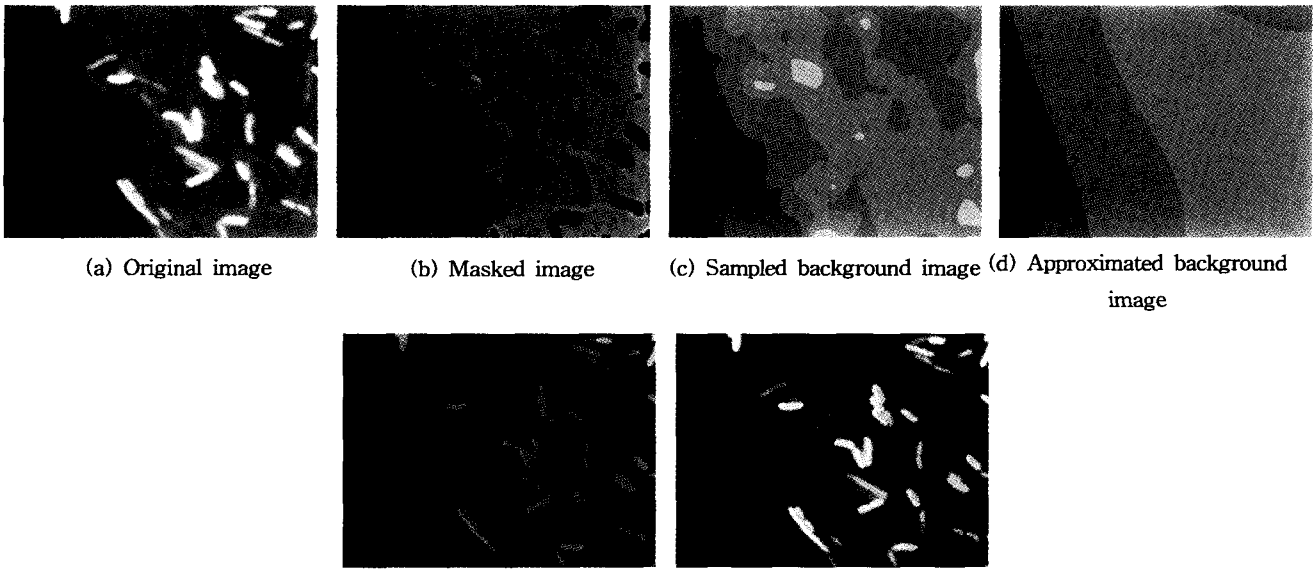


Fig. 7. Background's gray-level for *Zygosaccharomyces rouxii*.

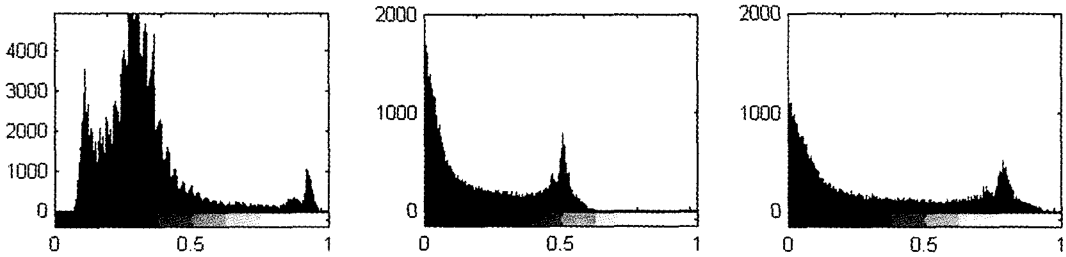
tion method. Fig. 5 and Fig. 8 show us a principle eliminating a background with slope in original images (Fig. 5(a) and Fig. 8(a)), respectively. Fig. 5(a) and Fig. 8(a) are input images with shaded-background of gentle slope for *Zygosaccharomyces rouxii* and *Acinetobacter* sp., respectively. Fig. 5(b) and Fig. 8(b) show masked images extracted from original images, respectively. Fig. 5(c) and Fig. 8(c) are sampled background images from masked images (Fig. 5(b) and Fig. 8(b)), respectively. Fig. 5(d) and Fig. 8(d) are approximated background images of Fig. 5(c) and Fig. 8(c), respectively. Fig. 5(e) and Fig. 8(e) are subtracted images that the approximated background images (Fig. 5(d) and Fig. 8(d)) are subtracted from the original images, respectively. Fig. 5(f) and Fig. 8(f) are adjusted images that highlight Fig. 5(e) and Fig. 8(e), respectively. Original images (Fig. 5(a) and Fig. 8(a)) are contaminated with a background images (Fig. 5(d) and Fig. 8(d)) with a slack slope, respectively. Fig. 6 shows histograms of Fig. 5(a), Fig. 5(e) and Fig. 5(f) for *Zygosaccharomyces rouxii*. Fig. 9 shows histograms of Fig. 8(a), Fig. 8(e) and Fig. 8(f) for *Acinetobacter* sp. Fig. 7 and Fig. 10 display sampled background's gray level (a) and approximate background's gray level (b) into three dimensional method that are taken with maximum gray value from Fig. 5(a) and Fig. 8(a), respectively. We can see that the backgrounds contain a lot of noise in Fig. 5(a) and Fig. 8(a), respectively. Fig. 11(a) and Fig. 12(a) show labeled images by only Otsu's method (Otsu, N. 1979) without applying the proposed method for



(a) Original image (b) Masked image (c) Sampled background image (d) Approximated background image

(e) Subtracted image (f) Adjusted image

Fig. 8. The resulted image for *Acinetobacter* sp.



(a) Original image (b) Subtracted image (c) Adjusted image

Fig. 9. Histograms for *Acinetobacter* sp.

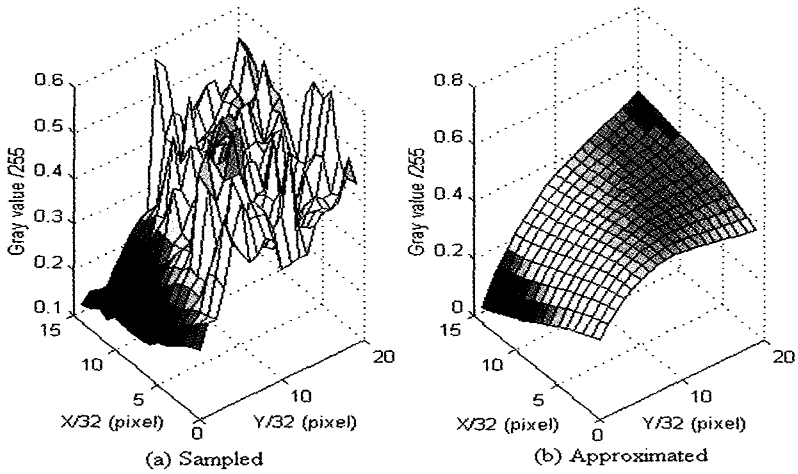
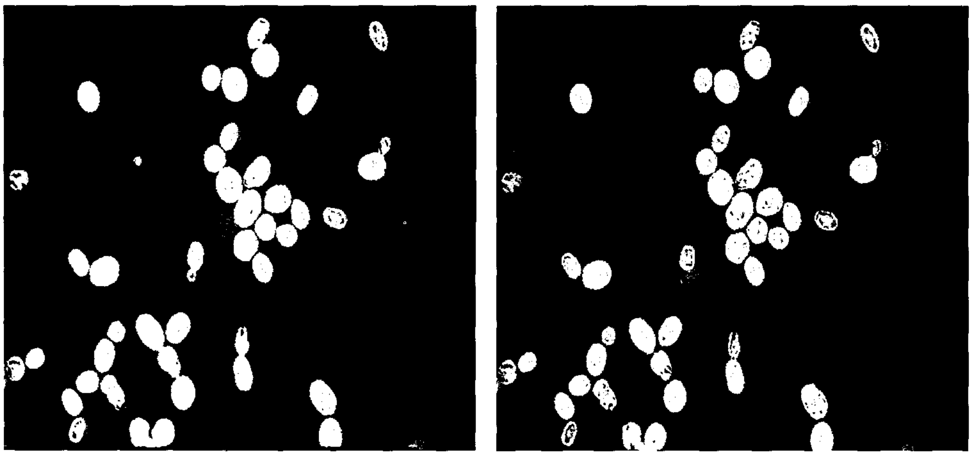
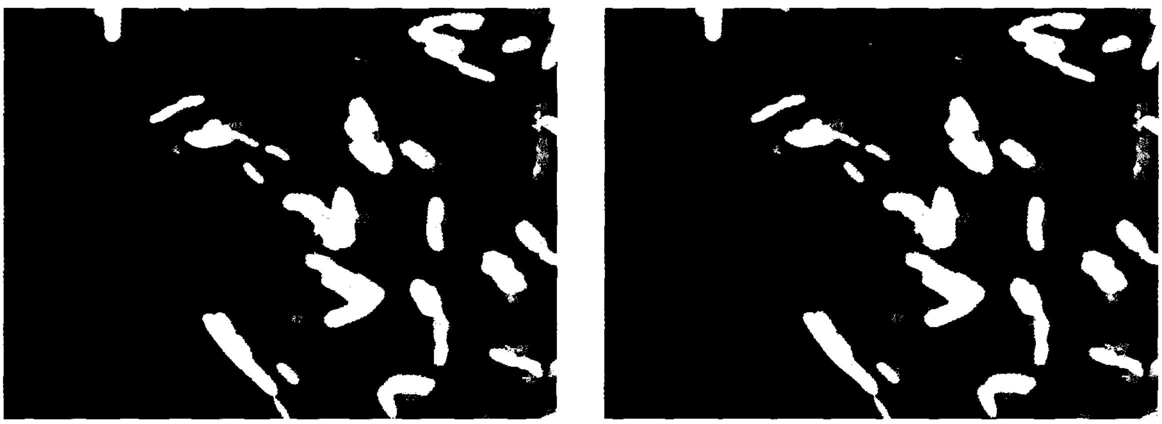


Fig. 10. Background's gray-level for *Acinetobacter* sp.



(a) Otsu's method (b) The proposed method

Fig. 11. Labeled images for *Zygosaccharomyces rouxii.*



(a) Otsu's method (b) The proposed method

Fig. 12. Labeled images for *Acinetobacter* sp.

Fig. 5(a) and Fig. 8(a), respectively. Fig. 11(b) and Fig. 12(b) are labeled images by the proposed method for Fig. 5(a) and Fig. 8(a), respectively. These results show that labeling by the proposed method is more exact for cell number counting than Otsu's method. Fig. 13 and Fig. 14 show the area distribution of the labeled images for Fig. 11 and Fig. 12, respectively. It is shown that labeled images by only Otsu's (1979) method contains a lot of noise, while labeled images by the proposed method remove some noise.

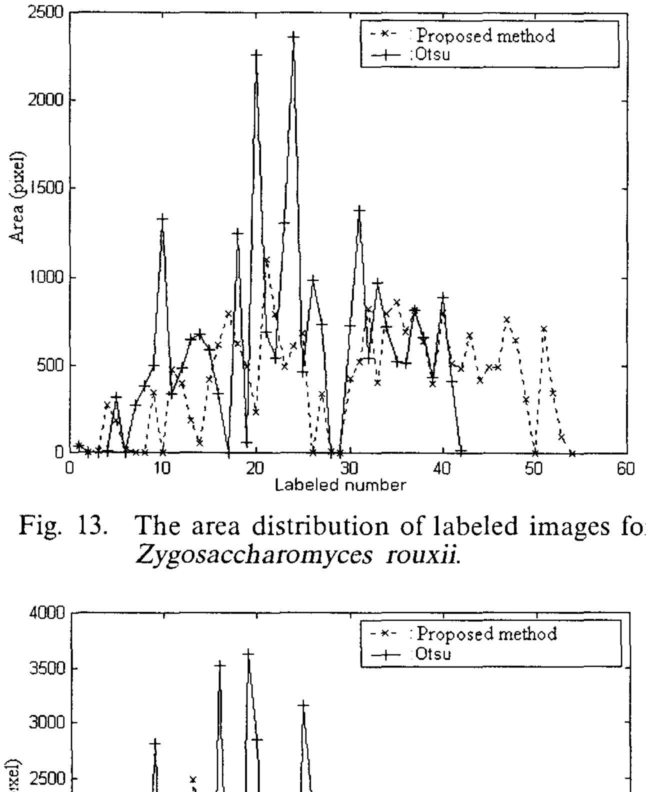


Fig. 13. The area distribution of labeled images for *Zygosaccharomyces rouxii*.

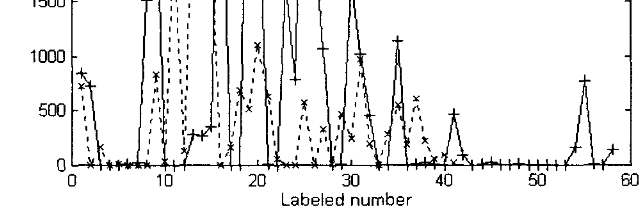


Fig. 14. The area distribution of labeled images for *Acinetobacter* sp.

Conclusion

This paper proposes the elimination method of contour background in order to get rid of the background from the input image based on a higher order surface fitting method. The elimination method used two dimension 3rd order surface data fitting technique and a bilinear interpolation method. The resultant compensated image is obtained through several procedures such as optimal thresholding, bilinear interpolation, reconstruction and elimination procedures. To prove the effectiveness of the proposed method, the proposed method is applied to *Acinetobacter* sp. and *Zygosaccharomyces rouxii*. Labeling by this proposed method can remove some noise and is more exact than labeling by only Otsu's method. But segmentation is not satisfactory. Futhermore, it is more effective for the reduction of noise.

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References

Bloem, J., M. Veninga and J. Shepher. 1995. Fully automatic determination of soil bacterium numbers, cell volumes, and frequencies of dividing cells by confocal laser scanning microscopy and image analysis. Appl. Environ. Microbiol., 61, 926~936.

Castleman, K.R. 1996. Digital image processing. Prentice Hall. Englewood Cliffs. New Jersey, pp. 123~125, 501~504 and 644~645.

Otsu, N. 1979. A threshold selection method from gray-level histograms. IEEE Trans. Syst. Man Cybern., 9, 62~66.

Pons, M.N., H. Vivier, J.F. Remy and J.A. Dodds. 1993. Morphological characterization of yeast by image analysis. Biotech. Bioeng., 42, 1352~1359.

Sonka, M., V. Hlavac and R. Boyle. 1999. Image, processing, analysis and machine vision, Chap. 5; Brooks/Cole Publishing Company. CA., 123~133.

Suhr, H. and G. Wehnert. 1995. In situ microscopy for on-line characterization of cell-populations in bioreactors, including cell-concentration measurements by depth from focus. Biotech. Bioeng., 47, 106~116.

Zalewski, K., E.E. Bildanalyse. 1995. System zur on-line charakterisierung von hefesuspensionen. Master of Diploma, TU-Berlin, VDI-Verlag GmbH.