

A region analysis approach for segmenting neural-cell images

Author

Yu, Donggang, Pham, Tuan D, Crane, Denis I

Published

2007

Conference Title

2007 ANNUAL INTERNATIONAL CONFERENCE OF THE IEEE ENGINEERING IN MEDICINE AND BIOLOGY SOCIETY, VOLS 1-16

DOI

[10.1109/IEMBS.2007.4353598](https://doi.org/10.1109/IEMBS.2007.4353598)

Downloaded from

<http://hdl.handle.net/10072/19120>

Link to published version

<http://www.ieee.org/portal/site>

Griffith Research Online

<https://research-repository.griffith.edu.au>

A Region Analysis Approach for Segmenting Neural-Cell Images

Donggang Yu, Tuan D. Pham, Denis I. Crane

Abstract—In this paper we present new algorithms based on region analysis of grey and distance differences of images that successfully circumvent these problems. Two key parameters of this analysis, window width and logical threshold, are automatically extracted for use in logical thresholding, and spurious regions are detected and removed through use of a hierarchical window filter. The efficacy of the developed algorithms is demonstrated here through an analysis of cultured brain neurons from newborn mice.

I. INTRODUCTION

A widely used approach is to challenge cultured neurons with relevant chemical agents and look for morphological changes that may indicate a process of neuronal cell death [1]. Image segmentation methods developed to date to analyze such changes have been limited by the low contrast of cells in unstained neuronal cultures and the unimodal histograms generated by these analyses.

A number of thresholding techniques have already been developed. These include global [2], [3], [4], [5] and local thresholding [6], [7] algorithms, multi thresholding methods [8], [9] and unimodal thresholding [10]. Despite these advances, it is still difficult to deal with images of very low quality, where major problems include variable background intensity due to nonuniform illumination, low local contrast due to smear or smudge, and shadows. These potential problems are apparent in a typical series of images shown in Fig. 1.

The most commonly used global thresholding method such as Otsu method, fuzzy c-mean clustering (FMC) and unimodal thresholding method do not work well for poor quality images because the spatial structure of the images is not taken into account.

In an effort to circumvent these highlighted problems raised above, we propose innovative segmentation algorithms of neuron cell images using a thresholding method based on logical level technique with difference analysis of both grey and distance in combination with a filtering window with constrained condition. The particular advantage of this approach is the capacity to binarize poor quality grayscale neuron cell images. The thresholding parameters of this method are automatically selected based on difference analysis of grey region.

D. Yu is with the Bioinformatics Applications Research Centre, James Cook University, Townsville, QLD 4811, Australia donggang.yu@jcu.edu.au

T.D. Pham is with the Bioinformatics Applications Research Centre, School of Maths, Physics, and Information Technology, James Cook University, Townsville, QLD 4811, Australia tuan.pham@jcu.edu.au

D.I. Crane is with School of Biomolecular and Physical Sciences, Griffith University, Nathan, Qld 4111, Australia d.crane@griffith.edu.au

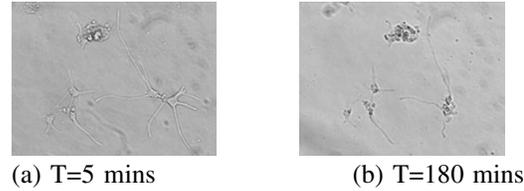


Fig. 1. Images obtained from monitoring a live, unstained, neuron in culture exposed to $350 \mu\text{M } \text{H}_2\text{O}_2$ over the times indicated.

II. CONSTRAINT-BASED LOGICAL LEVEL TECHNIQUE

Logical level techniques for segmenting document images have previously been developed [11], [12]. The principle of such techniques is compare the gray level of the processed pixel or its smoothed gray level with some local neighborhood averages. Suppose selected window width is represented by W . Let the start point of the image be upper-left and $f(i, j)$ be grey intensity of coordinates (i, j) , and it is eight neighboring.

Suppose each neighbor point (x, y) is the center of region $(2W + 1)^2$, then the average grey intensity $lp(k)$ of region $(2W + 1)^2$ is

$$lp(k) = \frac{\sum_{-W \leq m \leq W} \sum_{-W \leq n \leq W} f(x(k) + m, y(k) + n)}{(2W + 1)^2} \quad (1)$$

where if $k=0$, $x(0) = i$ and $y(0) = j + 1$; $k=1$, $x(1) = i - 1$ and $y(1) = j + 1$; $k=2$, $x(2) = i - 1$ and $y(2) = j$; $k=3$, $x(3) = i - 1$ and $y(3) = j - 1$; $k=4$, $x(4) = i$ and $y(4) = j - 1$; $k=5$, $x(5) = i + 1$ and $y(5) = j - 1$; $k=6$, $x(6) = i + 1$ and $y(6) = j$; $k=7$, $x(7) = i + 1$ and $y(7) = j + 1$.

Therefore grey region difference ($llp(k)$) between $lp(k)$ and $f(i, j)$ can be obtained by

$$llp(k) = lp(k) - f(i, j) \geq T, k = 0, \dots, 7 \quad (2)$$

where T is a predetermined parameter.

The logical level technique works as follows: Let $a = llp(0) \wedge llp(4)$, $b = llp(2) \wedge llp(6)$, $c = llp(1) \wedge llp(5)$ and $d = llp(3) \wedge llp(7)$, then

$$b(i, j) = \begin{cases} 1 & \text{if } (a \vee b \vee c \vee d) \text{ is true} \\ 0 & \text{otherwise} \end{cases} \quad (3)$$

where 1 and 0 represent object and 0 background in the resulting binary image respectively.

The logical level technique requires the calculation of two key parameters, window width W and region grey difference threshold T . However the predetermination of these parameters is difficult using existing logical level technique. As the essence of image segmentation is the determination of

grey regions, the approach we propose is a new method to determine the parameters W and T based on the automatic analysis of grey and distance difference. The basic principle is selecting W and T to represent most of objects of the images. Grey region can be defined as the region between each pair of neighbor grey peak and valley points in both horizontal and vertical directions. The peak point set, P_h (in the horizontal direction) can be determined as follows:

$$P_h = \{f_p(i(m), j(m))\}, \quad m = 1, \dots, k \quad (4)$$

where $f_p(i(m), j(m))$ is the m th the local maximum, and there are the total k local maxima. Similarly, the valley point set (all local minima), V_h can be found. Also, the peak and valley point sets, P_v and V_v (in the vertical direction) can be found.

Grey regions can be calculated based on determined peak and valley point sets, P_h, V_h, P_v and V_v . For each grey region two parameters are calculated. One is the grey difference between each pair of neighbor peak and valley points, representing as $H_g(m), m = 1, 2, \dots, k$, where k is region number for all rows. Other parameter is the distance difference between each pair of peak and valley points, representing as $H_d(m), m = 1, 2, \dots, k$. Furthermore, one new data set of grey region in which the number of points that have same grey difference and distance difference is found based on $H_g(m)$ and $H_d(m)$. It can be represented by $H_{dg}(m), m = 1, 2, \dots, kn$ (kn being the number of groups of data). Therefore, there are three elements in each group of data, distance difference, grey difference and region number in the group of data. We can sort $H_{dg}(m), m = 1, 2, \dots, kn$ based on region number in the group of the data set $H_{dg}(m)$ to find a decreasing data set, $H_{dgd}(m), m = 1, 2, \dots, kn$ where the suffix dgd represents a decreasing data set. Therefore, $H_{dgd}(1)$ the largest number of regions with the same grey and distance difference is largest. Based on the above procedure, we can find these data sets of the images in Fig. 1. If the first tk groups are summed

$$S_{tk} = \sum_{m=1}^{tk} H_{dgd}(m), \quad (5)$$

where tk represents the number of the selected groups of data in data set $H_{dgd}(m), m = 1, \dots, kn$. Parameter tk is selected to meet $(S_{tk}/k) \geq 0.7$, where k is total region number for all rows of an image. Based on such determinations, both the window parameter W and threshold T can be determined as mean region distance and region grey difference of tk groups of region respectively. That is

$$W_h = \frac{\sum_{m=1}^{tk} H_{dd}(m)}{tk}. \quad (6)$$

$$T_h = \frac{\sum_{m=1}^{tk} H_{gd}(m)}{tk}. \quad (7)$$

where $H_{dd}(m)$ and $H_{gd}(m)$ are the data set of region distance and grey difference of tk groups in horizontal direction respectively (for the rows of the image).

Similarly, the peak and valley points, the related analysis parameters, W_v (window parameter) and T_v (thresholding

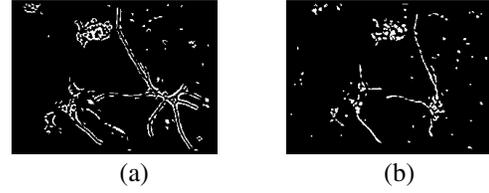


Fig. 2. Segmentation of cell images in Fig. 1 using innovative logical thresholding.

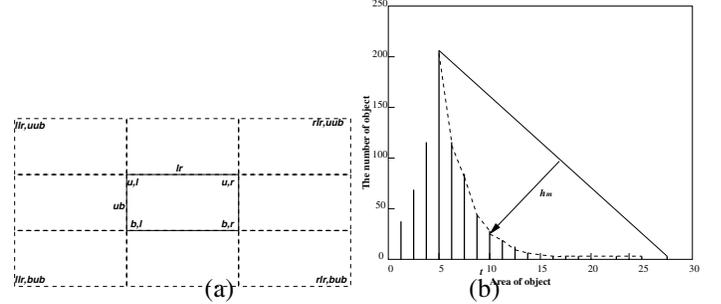


Fig. 3. Filtering window with one object region, and classification threshold based area histogram.

parameter) in the vertical direction of images can be found. The final window parameter is $W = (W_h + W_v)/2$, and the thresholding parameter is $T = (T_h + T_v)/2$. We can find all window parameters W and thresholds T for the images in Fig. 1 using the above algorithm. Based on the determined parameters W and T , and using logical thresholding algorithm, the images in Fig. 1 can be extracted. The result is shown in Fig. 2.

III. FILTERING WINDOW WITH CONSTRAINED CONDITION

Usually the valuable object image is large, with each part of the image close to its neighbor parts, whereas spurious regions are usually isolated, small regions. Therefore, we can detect and remove spurious region using a filtering window. The algorithm used for this purpose can be described as follows:

- (1) Find all regions of binarization image, which can be represented as $R(k), k = 1, \dots, rn$, where rn is the number of regions and $R(k)$ is k -th region's area size.
- (2) Sort $R(k), k = 1, \dots, rn$ based their area size in increasing order, and it is represented as $SR(k), k = 1, \dots, rn$.
- (3) From the first region:(3.1) find minimum rectangle which can cover the region, which is call as filtering window; (3.2) find a new region which consists of the region found in Step (3.1) and its eight neighbor regions; (3.3) detect whether there is the point of another object region in the new region, and then remove the processed region if not; (3.4) If there are points which are belong to other regions, detect whether the area of any one is larger than an area threshold which is represented with AT . If the detection result is yes, keep the processed region. Otherwise, remove the processed region and the points of other regions in the window.

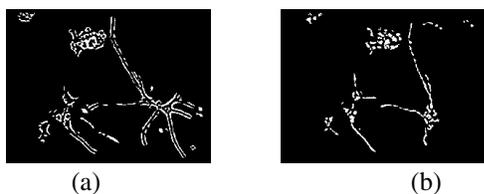


Fig. 4. The processed results of images from Fig. 1 using a filtering window with two object images.

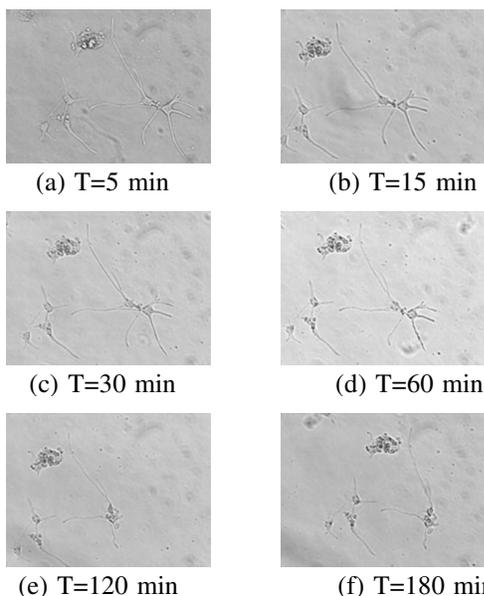


Fig. 5. Images obtained from monitoring a live, unstained, neuron in culture exposed to $350 \mu M H_2O_2$ over the times indicated.

Detection region is shown in Fig. 3(a), where $(l,u),(r,u),(l,b)$ and (r,b) are the coordinates of four corners of found minimum rectangle respectively, $lr = r - l$ and $ub = b - u$ are sizes of the rectangle respectively, and $llr = l - lr$, $rlr = r + lr$, $uub = u - ub$ and $bub = b + ub$.

Area threshold, AT , is determined as follows:

The idea is that the classification of object and back ground is based on the area size of regions. The classification threshold is finding maximum concave point of area histogram. It can be demonstrated in Fig. 3(b). For the example in Fig. 3(b), the maximum height is h_m , and corresponding threshold is $AT = t$.

Based on the above algorithm, the images in Fig. 2 can be processed to yield those shown in Fig. 4 where two filtering windows with one, and two neighbor object regions are used respectively.

IV. EXPERIMENTAL RESULTS

Dissociated cells were resuspended in Neurobasal-A medium (Gibco-Invitrogen) containing B27 supplement and 10 ng/mL nerve growth factor (Gibco-Invitrogen), and seeded onto coverslips coated with poly-L-lysine. Once neurons had attached, H_2O_2 ($350 \mu M$) was added and images captured at regular intervals over 3 hours. We have applied

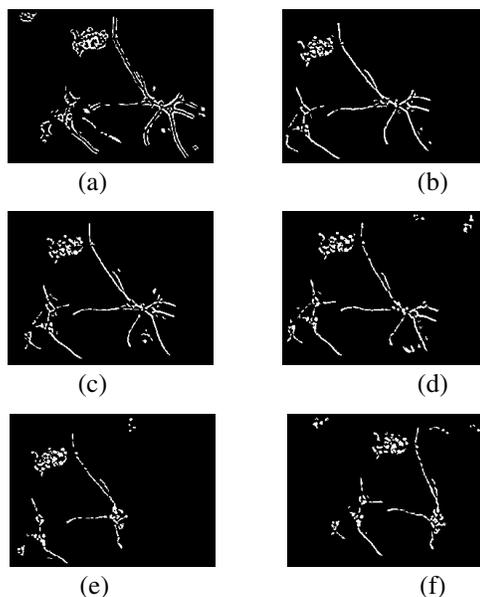


Fig. 6. Segmentation of images from Fig. 5 using proposed method.

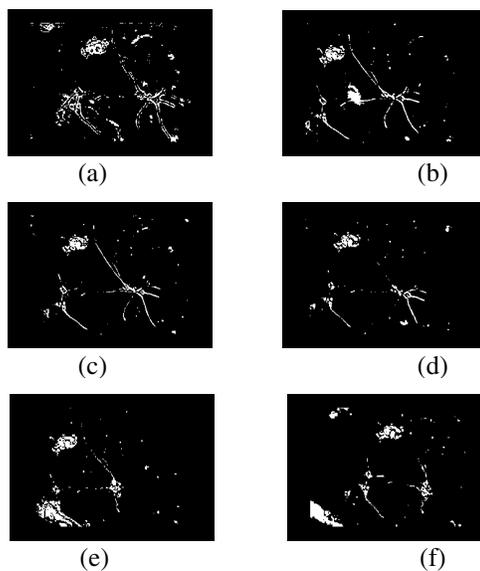


Fig. 7. Segmentation of images from Fig. 5 using Otsu's method.

the developed algorithm to a typical series of images shown in Fig. 5. The segmentation results are shown in Fig. 6. For example, when Otsu's method [2] is used, certain sections of cellular structure shown in Fig. 5 are lost that can be seen in Fig. 7, whereas some large non-cellular objects are extracted as shown in Figs. 7(b,e,f). Similar segmentation results are obtained when the FCM method [4] is applied to these images that are shown in Fig. 5: some sections of neuronal cell structure are lost (see Fig. 8), and many spurious large sized parts are extracted (see Figs. 8(a,b,e,f)). This is especially the case for Figs. 8(a,b), where it is difficult to find the sections of neuronal cell structure. When unimodel thresholding [10] is used on these same images, most of

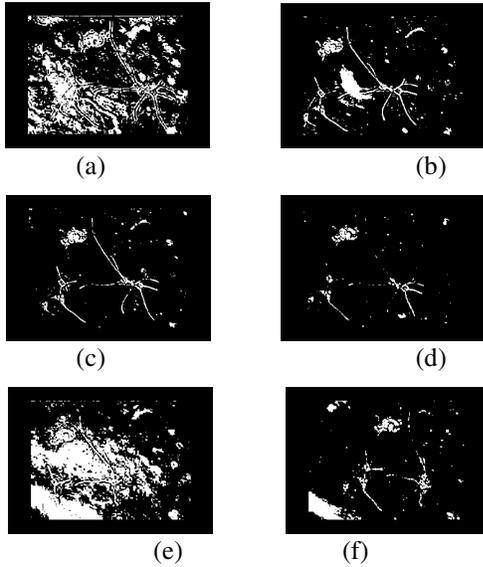


Fig. 8. Segmentation of images from Fig. 5 using the FCM method.

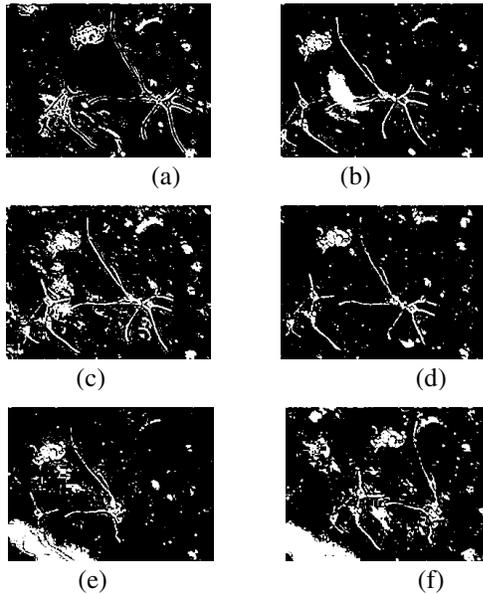


Fig. 9. Segmentation of images from Fig. 5 using unimodal thresholding.

cellular structure in Fig. 5 is segmented, but so are areas of non-cellular background which cannot be easily removed (see Fig. 9).

V. CONCLUSION

New algorithms based on region analysis have been developed for segmenting neural-cell images with very low quality. Two key parameters of this analysis, window width and logical threshold, are automatically extracted for use in logical thresholding. Spurious regions are detected and removed through use of a hierarchical window filter. Experimental results demonstrate that our algorithm is more efficient by comparing the result of proposed method (see Fig. 6) with those of other three methods (see Figs. (7-9)).

Acknowledgement- This work was supported by the Australia Research Council ARC-DP grant (DP0665598).

REFERENCES

- [1] P.K. Allani, T. Sum, SG. Bhansali, SK. Mukherjee, M. Sonee, A comparative study of the effect of oxidative stress on the cytoskeleton in human cortical neurons, *Toxicol. Appl. Pharmacol.*, **196**, (2004) 29–36.
- [2] N. A. Otsu, thresholding selection method from graylevel histogram, *IEEE Trans. Systems Man Cybernet*, **SMC8**, (1978) 62–66.
- [3] S.U. Lee, S.Y. Chung, R.H. Park, A comparative performance study of several global thresholding techniques for segmentation, *CVGIP*, **52**, (1990) 171–190.
- [4] Z. Chi, H. Yan and T. Pham, Fuzzy Algorithm: With Application to Image Processing and Pattern Recognition, *World Scientific Publishing Co., Singapore*, (1996).
- [5] T.D. Pham, D.I. Crane, Segmentation of neuronal-cell images from stained fields and monomodal histograms, *Proc. 27th Annual Int. Conf. IEEE Engineering in Medicine and Biology Society*, **3.5**, (2005) 7–13.
- [6] F. Deravi, S.K. Pal, Gray level thresholding using second order statistics, *Pattern Recognition Lett.*, **1**, (1983) 417–422.
- [7] Y. Nakagawa, A. Rosenfeld, Some experiments on variable thresholding, *Pattern Recognition*, **11**, (1979) 191–204.
- [8] S. Boukharouba, J.M. Rebordao, P.L. Wendel, An amplitude segmentation method based on the distribution function of an image, *Computer Vision Graphics Image Process*, **29**, (1985) 47–59.
- [9] N. Papamarkos, B. Gatos, A new approach for multilevel threshold selection, *CVGIP: Graphical Models Image Process*, **56**, (1994) 357–370.
- [10] P.L. Rosin, Unimodal thresholding, *Pattern Recognition*, **34**, (2001) 2083–2096.
- [11] M. Kamel, A. Zhao, Extraction of binary character/ graphics images from grayscale document images, *CVGIP: Graphical Models Image Process*, **55**, (1993) 203–217.
- [12] Y. Yang, H. Yan, An adaptive logical method for binarization of degraded document images. *Pattern Recognition* **33(5)**, (2000) 787–807.