Quantification of Neural Images using Grey Difference

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Abstract

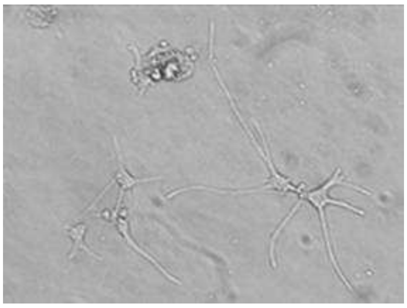
We present new algorithms for segmenting neuron images which are taken from cells being grown in culture with oxidative agents. Information from changing images can be used to compare changes in neurons from the Zellweger mice to those from normal mice. Image segmentation is the first and major step for the study of these different types of processes in neuron cells. It is difficult to do it as these neuron cell images from stained fields and unimodal histograms. In this paper we develop an innovative strategy for the segmentation of neuronal cell images which are subjected to stains and whose histograms are unimodal. The proposed method is based on logical analysis of grey difference. Two key parameters, window width and logical threshold, are automatically extracted to be used in logical thresholding method. Spurious regions are detected and removed by using hierarchical filtering window. Experiment and comparison results show the efficient of our algorithms.

Keywords: Neuron cell imaging, segmentation, grey difference, distance difference, filtering window.

1 Introduction

Information taken from images of neuron cells being grown in culture with oxidative agents allows life science researchers to compare changes in neurons from the Zellweger mice to those from normal mice. Neuron degeneration refers to the excessive damage or loss of neurons, or brain and spinal cord cells which perform different functions such as controlling movement, processing sensory information, and making decisions. Neuron degenerative diseases can cause devastating effects on an individual. It is clear that image analysis and recognition are useful tool to help our study of the neuron degeneration in a human disorder called Zellweger syndrome. In our study, the cells are from mice that are a model of the Zellweger syndrome, a severe neuron degenerative disorder characterised by death in the first 16 months after birth, severe dysmorphia, hypotonia, and other widespread tissue defects. This disorder arises because of defects in cellular organelles called peroxisomes, that are required for a number of essential cellular metabolic functions. We have hypothesised that the loss of peroxisomes in neurons results in these cells being susceptible to oxidative stress, because peroxisomes contain a number of important antioxidant enzymes, including catalase needed to break down hydrogen peroxide that is made in cells. In response to oxidative stress, we propose that these neurons will deteriorate. In morphological terms, we expect to see this initial deterioration as the contraction, and eventually loss, of processes of neurons grown in culture. Given the above motivation, image analysis by segmentation is an efficient way that allows us to measure the changes in cell process number and length from images taken of cells being challenged in culture with oxidative agents. The changes in neurons from the Zellweger mice can be compared to those from normal mice in a quantitative manner based on image analysis and recognition. Some neuron cell images subjected to 350 $\mu M H_2O_2$ with different time (t=5, 15, 30, 60, 120 and 180 mins) are shown in Fig. 1. So the first and important step is exact neuron images from original images which have stained fields and unimodal histograms. Many methods are investigated for image segmentation. Application of each approach can be useful for solving some particular problem. However, in general, segmentation of nontrival images is still one of the most difficult task in image processing.

In order to segment object images from poor quality images with shadows, nonuniform illumination, low contrast, large signal dependent noise, smear and smudge, it is essential to threshold the image reliably. Therefore, thresholding an intensity image into two levels is the first step and also a critical part in most image analysis systems as any error in this stage will propagate to all later processing, analysis, recognition etc. Although many thresholding techniques, such as global (Ostu 1978) (Lee, Chung & Park 1990) (Pham & Crane 2005) (Chi, Yan & Pham 1996) and local thresholding (Deravi & Pal 1983) (Nakagawa & Rosenfeld 1979) algorithms, multi thresholding methods (Papamarkos & Gatos 1994) and unimodal threshholding (Rosin 2001) have been developed in the past, it is still difficult to deal with images with very low quality. Major problems of segmenting poor quality images are variable background intensity due to nonuniform illumination, low local contrast due to smear or smudge and shadows. For example, one group of screening neuron cell images from stained fields and monomodal histograms are shown in Fig. 1. In this paper, we propose innovative segmentation algorithms of neuron cell images, which are a thresholding method based on logical level technique with difference analysis of the grey region

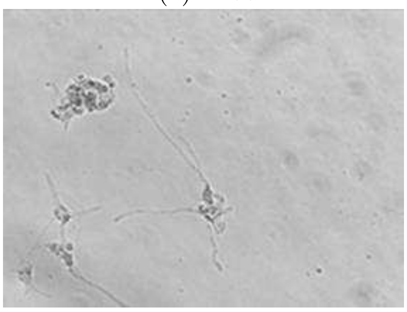


(1) t=5 mins

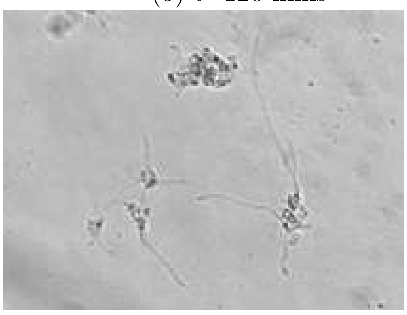
(2) t=15 mins

(3) t=30 mins

(4) t=60 mins



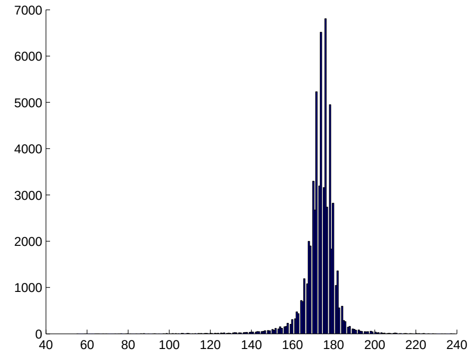
(5) t=120 mins



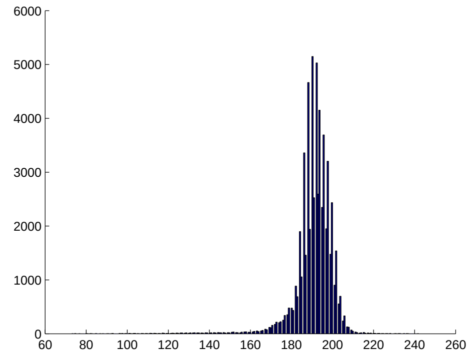
(6) t=180 mins

Figure 1: One example of neuron cell screening.

and filtering window with contained condition. This thresholding method is used to binarize poor quality grayscale neuron cell images. The thresholding parameters of this method are automatically selected based on difference analysis of grey region. In order to segment neuron cell images, binarized images are filtered by hierarchical filtering window with contained condition. Our method can deal with variable background intensity caused by nonuniform illumination, shadow, smear or smudge and low contrast without obvious loss of useful information. This paper is orgnized as follows. In Section 2, we briefly review related works on image thresholding techniques. In Section 3, segmentation algorithms of neuron cell images, logical level technique with difference analysis of grey region and filtering window with contained condition are described. In Section 4, illustrate the performance of the proposed methods with several experiments the experimental results of the proposed method by comparison with some related segmentation methods are given. We then conclude our analyzer in the final section.



(1)



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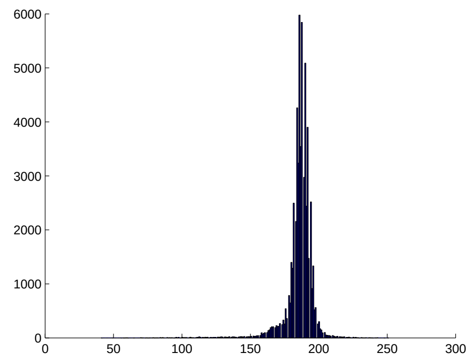


(3)

Figure 2: Unimodal histogram of images in Figs. 1(1-6).

2 Related work

The most commonly used global thresholding techniques are based on histogram analysis (Ostu 1978)(Lee et al. 1990). Threshold is determined from the measure that best separates the levels corresponding to the peaks of the histogram, each of which corresponds to image pixels of a different part like background or objects in the image. A threshold is an intensity value which is used as the boundary between two classes of a binary segmented image. These methods do not work well for the poor quality images with shadows, inhomogeneous backgrounds, complex background patterns which may have a histograms that contains a single peak. For example, the histograms of images in Figs. 1(1,5,6) are single peak (unimodal), which are shown in Fig. 2. In this case, a single threshold could not result in an accurate binary image. For example, if the neuron cell images in Figs. 1(1-6) are segmented by Otsu's method, then binarization results are shown in Fig. 3.

Distinct from thresholding method, k-Means clustering is involved to determine classes themselves, rather than a threshold value (Zhang 2000). Fuzzy c-Means Clustering is used to segment images, which is called as FCM (Chi et al. 1996). However these techniques use only intensity data of images to perform segmentations, and as the spatial structure of the images is not taken into account. For example, if the neuron cell images in Figs. 1(1-6) are processed by FCM thresholding, the binarization results are shown in Fig. 4.

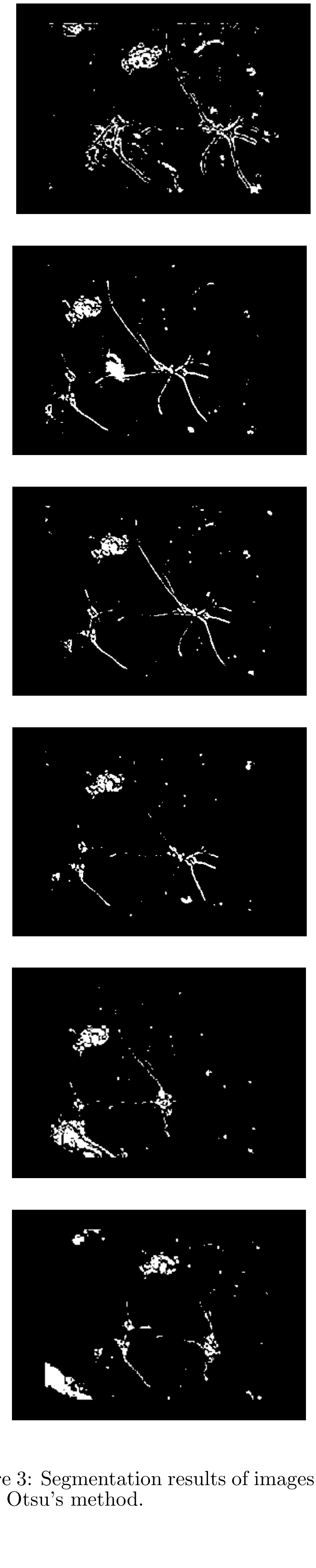
One unimodal thresholding is approached by (Rosin 2001). The threshold point is selected as the histogram index value that maximises the perpendicular distance between the straight line (drawing from the peak to the high end of the histogram) and histogram line. However, the method is available under several assumptions. Therefore, unimodal thresholding cannot segment the neuron cell images in Figs. 1(1-6), and their segmentation results are shown in Fig. 5.

3 Logical level technique with difference analysis of grey region and filtering window with contained condition

3.1 Logical level technique

Logical level technique are developed to be used to segment document images images by Kamel, Zhao (Kamel & Zhao 1993), Yang and Yan (Yang & Yan 2000). After analyzing integrated function algorithm (Trier & Taxt 1995), Kamel and Zhao proposed Logical level technique. The basis idea is comparing the gray level of the processed pixel or its smoothed gray level with some local averages in the neighborhoods, and the comparison results are regarded as derivatives. Therefore, pixel labeling, detection and extraction using the derivatives, the logical bound on the ordered sequences and the window width range can be adopted. This technique processes each pixel by simultaneously comparing its gray level or its smoothed gray level with four local averages in the selected window region. Suppose selected window is "W". The window region is $(2W+1)^2$. Let the start point of the image be upper-left and $f(i,j)$ be grey intensity of coordinates (x,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



(1) (2) (3) (4) (5) (6)

Figure 3: Segmentation results of images in Figs. 1(1-6) by Otsu's method.

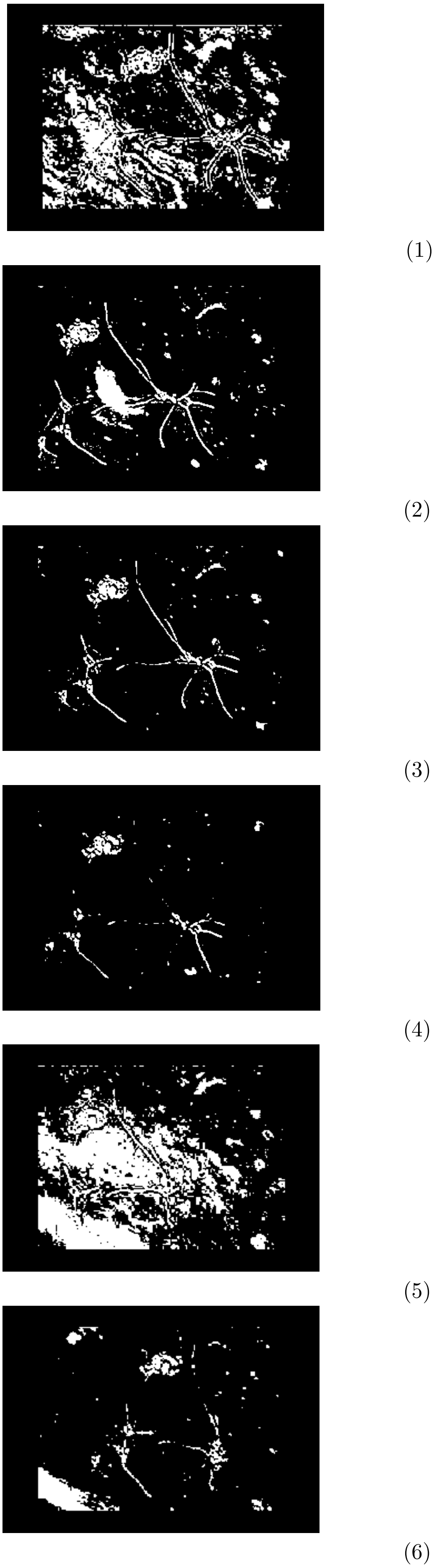


Figure 4: Segmentation results of images in Figs. 1(1-6) by FCM method.

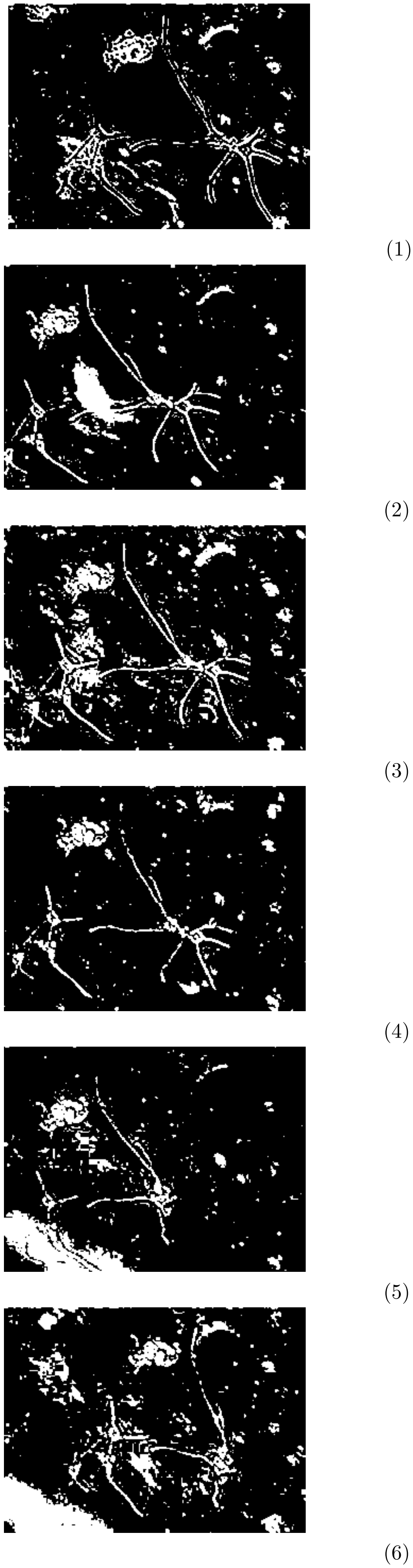


Figure 5: Segmentation results of images in Figs. 1(1-6) by unimodal thresholding method.

$lp(k)$ of region $(2W+1)^2$ is

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

where if k=0, $x = i$ and $y = j+1$; k=1, $x = i-1$ and $y = j+1$; k=2, $x = i-1$ and $y = j$; k=3, $x = i-1$ and $y = j-1$; k=4, $x = i$ and $y = j-1$; k=5, $x = i+1$ and $y = j-1$; k=6, $x = i+1$ and $y = j$; k=7, $x = i+1$ and $y = j+1$. Therefore grey region difference ($llp(k)$) between $lp(k)$ and $f(i,j)$ can be found

$$llp(k) = lp(k) - f(i,j) \geq T \quad k = 0, 1, ..., 7 \quad (2)$$

where "T" is predetermined parameter.
The logical level technique is

$$\text{b(i,j)}=\begin{cases} 1 & \text{if } \{(llp(0)\bigwedge llp(4))\bigvee \\ & (llp(2)\bigwedge llp(6))\bigvee \\ & (llp(1)\bigwedge llp(5))\bigvee \\ & (llp(3)\bigwedge llp(7))\ \} \quad \text{is true} \\ 0 & \text{otherwise} \end{cases} \quad (3)$$

where "1" represents object and "0" to represent background in the resulting binary image.

3.2 Innovative logical logical level technique with difference analysis of grey region and filtering window with contained condition

We can find that logical level technique need two key parameters, window "W" and threshold "T". However their predetermination is difficult, and no efficient method can be found from logical level technique. In fact, the essence of image segmentation is the determination of grey regions. Therefore, we approach new method to determine parameters, window "W" and threshold "T" automatically based on the analysis grey regions. Grey region can be defined as the region between each pair of neighbor grey peak and valley points in horizontal and vertical direction. Mathematically, the peak and valley points of image grey histogram are the points which make the first order derivative of image grey function equal to zero.
For each row the peak point set, P_h, can be found as follows.
If point is starting point of a row and each row has *col* points, the peak points in horizontal direction can be found as follows:
(1) if $f(i,1) > f(i,2)$, then the point $P_h(i,1)$ is a peak point.
(2) if $f(i,1),...,f(i,n) > f(i,n+1)$ and $f(i,1) = ... = f(i,n)$ $(n > 1)$, then the point $P_h(i,1)$ is a peak point.
If point is last point of a row, the peak points in horizontal direction based on two cases:
(3) if $f(i,col-1) < f(i,col)$, then the point $P_h(i,col)$ is a peak point.
(4) if $f(i,col-n-1) < f(i,col)$ and $f(i,col-1) = ... = f(i,col-n)$ $(n > 1)$, then the point $P_h(i,col)$ is a peak point.

In other cases, the peak points in horizontal direction based on two cases:
(5) if $f(i,j-1) < f(i,j)$ and $f(i,j) > f(i,j+1)$, then the point $P_h(i,j)$ is a peak point.
(6) if $f(i,j-1) < f(i,j),...,f(i,j+n)$ and $f(i,j)...f(i,j+n) > f(i,j+n+1)$ where $f(i,j) = ... = f(i,j+n)$ $(n > 1)$, then the point $P_h(i,j)$ is a peak point.

Similarly, the valley point set, V_h can be found for each row of image. Similarly, the peak and valley point sets, P_v and V_v, of each collum can be found. Grey regions can be calculated based on found peak and valley point sets, P_h, V_h, P_v and V_v. For each grey region two parameters are calculated. One is grey difference between each pair of neighbor peak and valley points, which can be represented as $H_g(m), m = 1, 2, ...k$, where k is region number for all rows of an image. Another one is distance difference between each pair of peak and valley points, which can be represented as $H_d(m), m = 1, 2, ...k$. Furthermore, one new data set of grey region in which the number of points that have same grey difference and distance diffrence is found. It can be represented with $H_{dg}(m), m = 1, 2, ...kn$ (k_n being the number of groups). Sort $H_{dg}(m), m = 1, 2, ...kn$ based on $H_{dg}(m)$ get a decreasing data set, $H_{dgd}(m), m = 1, 2, ...kn$. Therefore $H_{dgd}(0)$ is the first number of grey regions with same grey and distance difference, and it is largest. If first tk groups are summed

$$S_{tk} = \sum_{m=1}^{tk} H_{dgd}(m). \quad (4)$$

Parameter tk is selected to meet $(S_{tk}/k) \geq 0.7$, where k is region number for all rows of an image. For example, for the image in Fig. 1(6) tk=81, kn=931, (S_{tk}=8397 and k=11961. We can see only 81 groups of grey region contain 8397 grey regions which is approximately equal to 70% of k=11961. Therefore, here 81 groups of grey region represent major property of region distribution of the image in horizontal direction. Based on this idea, window parameter "W" and threshold "T" can be determined. "W" and "T" are selected as mean region distance and region grey difference of tk groups of grey region respectively. That is

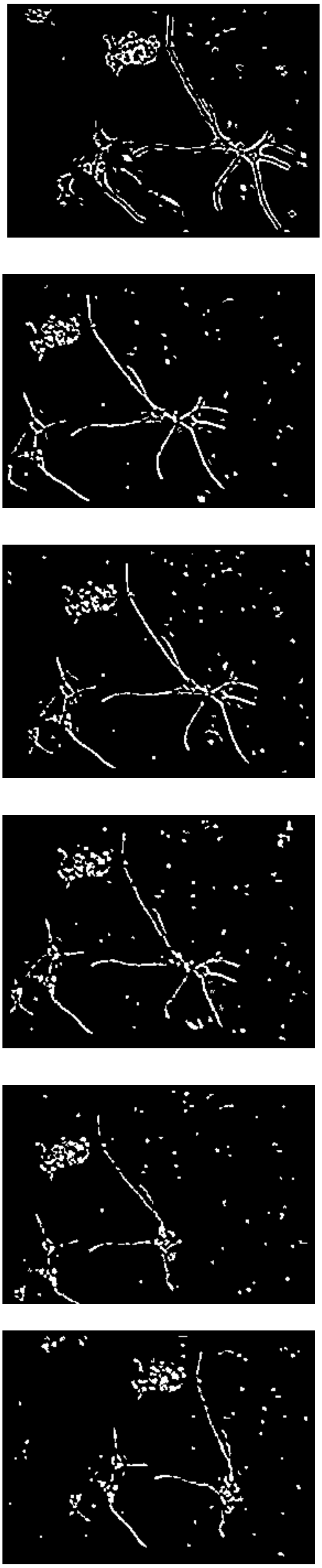
$$W_h = \frac{\sum_{m=1}^{tk} H_{dd}(m)}{tk}. \quad (5)$$

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

where $H_{dd}(m)$ and $H_{gd}(m)$ is region distance and grey difference of each group of tk groups in horizontal direction respectively.
Similarly, the peak and valley points, related analysis parameters, W_v (window parameter in vertical direction) and T_v (thresholding parameter in vertical direction) in vertical direction of images can be found. The final window parameter is $W = (W_h + W_v)/2$, and 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 based the above algorithm. For example, $W = 5$ and $T = 6.95$ for the image in Fig. 1(1), and $W = 4$ and $T = 6.5$ for the image in Fig. 1(6). Based on the found parameters, "W", "T" and logical thresholding algorithm, the images in Fig. 1 can be extracted, and shown in Fig. 6.

3.3 Filtering window with contained condition

As average smoothed grey and grey difference information of image window is used, the algorithm can binarized poor quality grayscale image which has variable background intensity, smear or smudge and low contrast. However, if a region meets the conditions of binarization, it can be selected as an object image. For example, some spurious regions are made in Fig. 6. Usually valuable object image has big size,



(1)

(2)

(3)

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(5)

(6)

Figure 6: Segmentation results of cell images in Figs. 1-6 by innovative logical thresholding.

and one part of object image is close to its neighbor parts. Spurious region is usually isolated little region. For example, neuron images belong to such a case. Therefore, we can detect and remove some spurious region based above idea.
The algorithm can be described as follows:

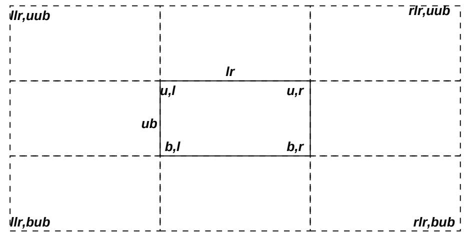


Figure 7: Filtering window with one object region.

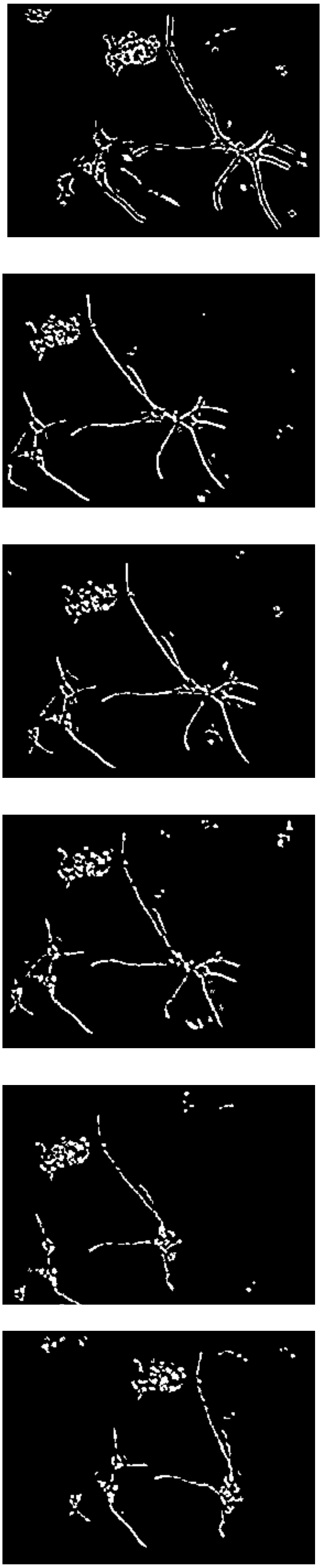
(1) Find all regions of binarization image, which can be represented as $R(k), k = 1, ...rn$, where rn is the number of regions and $R(k)$ is k-th region's area size.
(2) Sort $R(k), k = 1, ...rn$ based their area size in increasing order, and it is represented as $SR(k), k = 1, ...rn$.
(3) From the 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, keep the processed region if yes. Detection region is shown in Fig. 7, 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 $bub = b + ub$.
Based on the above algorithm, the images in Fig. 6 can be processed and shown in Fig. 8.

In above algorithm, filtering window only contains one object region. Hierarchical processing can be done based on new filtering window which consists of two neighbor object regions. The processing method can be described similar to above algorithm. Based on the above procedure, the images in Fig. 8 can be processed and shown in Fig. 9.

Furthermore, filtering window with big size can be selected. However, we should consider whether selected filtering window is reasonable based on the prior knowledge and result of binarization image. Such contained conditions should be considered to select the size of filtering window. For example, if the size of valuable object image is little and the number of object image is not large enough, little size of filtering window should be selected.

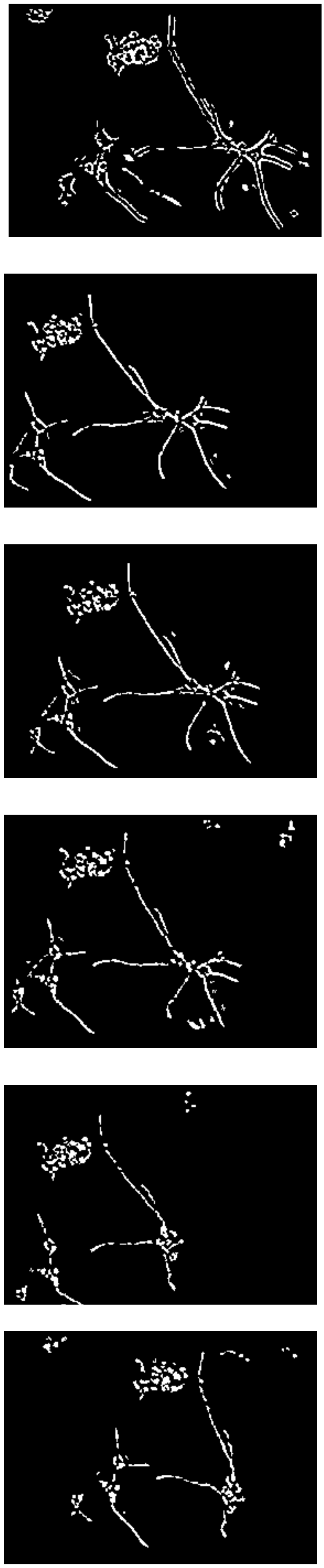
4 Experimental results

We have tested some neuron cell images which taken from screenings of neuron cell images based on our innovative algorithm. For example, six neuron cell images subjected to 350 $\mu M H_2O_2$ are shown in Fig. 1 which are taken in different time (t=5, 15, 30, 60, 120 and 180 mins) respectively. We can see that the poor quality images of neuron cells are with shadows, inhomogeneous backgrounds, complex background patterns which may have a histograms that contains a single peak. Here, histograms of six neuron cell images are single peak. Based on our method, the neuron cell images are processed by innovative logical thresholding with grey difference analysis, and extracted neuron cell images are shown in Fig. 6 firstly. We can see that cellular organelles are well extracted,



(1) (2) (3) (4) (5) (6)

Figure 8: The processed results of segmentation results in Fig. 6 by filtering window with one object image.



(1) (2) (3) (4) (5) (6)

Figure 9: The processed results of segmentation results in Fig. 8 by filtering window with two object images.

but there are some spurious regions in Fig. 6. This is because our method is thresholding based on grey difference analysis. If thresholding conditions ("W" and "T") are meet for some regions which are not belong to neuron cell regions, the spurious regions are formed. However, most spurious regions are isolated because of thresholding with grey difference. Therefore, extracted images in Fig. 6 can further be processed by using hierarchical filtering window secondly. Here two sizes of filtering windows are used to process the neuron cell images in Fig. 6, and the extracted neuron cell images are shown in Fig. 8 and Fig. 9 respectively. We can see that cellular organelles are extracted, and some spurious regions are removed.
For some neuron cell images in Figs. 1(1-6), Ostu's method (Ostu 1978), FCM method (Chi et al. 1996) and unimodel thresholding (Rosin 2001) are used to extract neuron cell images, and the processed results are shown in Figs. 3, 4, 5 respectively. Threshold of all three methods (Ostu 1978), (Chi et al. 1996) and (Rosin 2001) is determined from the measure that best separates the levels corresponding to the peaks of the histogram, each of which corresponds to image pixels of a different part like background or objects in the image. The threshold is an intensity value which is used as the boundary between two classes of a binary segmented image. If there are inhomogeneous backgrounds and complex background patterns in neuron cell images such as Figs. 1(1-6), shadows may become object, and some parts of objects may become background, which are caused by nonuniform illumination, shadow, smear or smudge and low contrast. In these cases no good segmentation results can been got by the intensity thresholding. It is clear our algorithm is more efficient by comparing the result of proposed method (see Fig. 9) with those of other three methods (see Figs. 3, 4 and 5).

5 Conclusion

In this paper, segmentation algorithm of screening neuronal cell images based on logical thresholding of grey difference have been developed. Thresholding parameter can be selected automatically based on analysis of grey difference region. Our method can effectively segment gray scale images such as screening neuron cell images which have variable background intensity caused by nonuniform illumination, shadow, smear or smudge and low contrast. Proposed filtering window can be used to remove spurious regions of binarization images of neuron cell images. Experiment and comparison results show the efficient of our algorithms.

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