NIBLACK METHOD BASED SEGMENTATION FOR MICROSCOPIC IMAGERY

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ABSTRACT

The main objective of Niblack image segmentation is to extract and characterize anatomical structures with respect to some input features or expert knowledge. In this paper, we present a sliding window based local thresholding technique 'Niblack' and given a detailed comparison of some existing thresholding algorithms with this method. The Niblack thresholding method aims at achieving better results, specifically, for microscopic images. It is a local thresholding algorithm that adapts the threshold according to the local mean and the local standard deviation over a specific window size around each pixel location. It exhibits its robustness and effectiveness when evaluated on microscopic images.

Keywords: Thresholding, Niblack, Otsu, Iterative Triclass Segmentation

I. INTRODUCTION

The gray levels of pixels belonging to the object are entirely different from the gray levels of the pixels belonging to the background, in many applications of image processing. Thresholding becomes then a simple but effective tool to separate those foreground objects from the background. We can divide the pixels in the image into two major groups, according to their gray-level. These gray levels may serve as "detectors" to distinguish between background and objects is considering as foreground in the image [1]. Select a gray-level between those two major gray-level groups, which will serve as a threshold to distinguish the two groups (objects and background). Image segmentation is performed by such as boundary detection or region dependent techniques. But the thresholding techniques are more perfect, simple and widely used [2]. Different binarization methods have been performed to evaluate for different types of data. The locally adaptive binarization method is used in gray scale images with low contrast, Varity of background intensity and presence of noise. Niblack's method was found for better thresholding in gray scale image [3].

In this work the input image is segmented using Niblack thresholding algorithm later we are applying edge detection and morphological operations to improve segmentation.

II. THRESHOLDING

Simply the basic function [5] for thresholding creates the binary image from gray level ones by turning all pixels below some threshold to zero and all pixels above that threshold to one [1],[5]. If *g(x, y)* is a threshold version of *f(x, y)* at some global threshold *T*. g is equal to 1 if *f(x, y) T* and zero otherwise [1].

$$g(x,y) = \begin{cases} 0 & if\ f(x,y) < T \\ 1 & if\ f(x,y) \geq T \end{cases}$$

Thresholding techniques can be classified generally into two categories like Global thresholding and Local thresholding. Global thresholding methods consider a single intensity threshold value. Local thresholding methods compute a threshold for each pixel in the image on the basis of the content in its neighborhood. It considers presences of all intensity level in the image. So the local thresholding methods generally perform better for low quality images [3]. We categorize the thresholding methods in groups according to the information they are exploiting. Histogram shape-based methods, this method used the peaks, valleys and curvatures of the smoothed histogram are analyzed. Clustering-based methods perform where the gray-level samples are clustered in two parts as background and foreground (object). Entropy-based methods result in algorithms that use the cross-entropy between the original and binarized image, the entropy of the foreground and background regions [3], [4]. Object attribute-based methods; search a similarity measure between the gray-level and the binarized images, such as edge coincidence, fuzzy shape similarity. The spatial methods use correlation between pixels and/or higher-order probability distribution. Local methods adapt the threshold value on each pixel to the local image characteristics [4].

III. OTSU'S THRESHOLDING METHOD

Otsu's method is used to automatically perform clustering-based image thresholding or the reduction of a gray level image to a binary image. The algorithm assumes that the image to be threshold contains two classes of pixels or bi-modal histogram (e.g. foreground and background) then calculates the optimum threshold separating those two classes so that their combined spread (intra-class variance) is minimal.

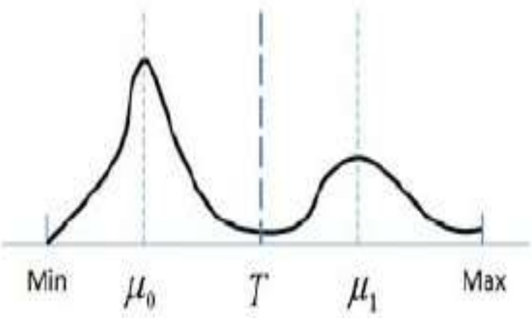


Fig 1: Otsu's Method Binarizes an Image to Two Classes Based on Threshold *T* by Minimizing the Within-Class Variances

The threshold is that which minimizes the weighted within-class variance which in turns out to be the same as maximizing the between-class variance Operates directly on the gray level histogram. Some of the Otsu's assumptions which can be defined are Histogram (and the image) is bimodal. No use of spatial coherence, or any other notion of object structure. This Assumes stationary statistics, but can be modified to be locally adaptive. One more Assumption is uniform illumination so the bimodal brightness behavior arises from object appearance differences only. The weighted within-class variance is

$$\sigma_w^2(t) = q_1(t)\sigma_1^2(t) + q_2(t)\sigma_2^2(t)$$

Where the class probabilities are estimated as:

$$q_1(t) = \sum_{i=1}^{t} P(i) \qquad q_2(t) = \sum_{i=t+1}^{I} P(i)$$

And the class means are given by:

$$\mu_1(t) = \sum_{i=1}^{t} \frac{iP(i)}{q_1(t)} \qquad \mu_2(t) = \sum_{i=t+1}^{I} \frac{iP(i)}{q_2(t)}$$

Finally, the individual class variances are:

$$\sigma_1^2(t) = \sum_{i=1}^{t} [i - \mu_1(t)]^2 \frac{P(i)}{q_1(t)}$$

$$\sigma_2^2(t) = \sum_{i=t+1}^{I} [i - \mu_2(t)]^2 \frac{P(i)}{q_2(t)}$$

All it is need to do is just run through the full range of values [1,256] and pick the value that minimizes. But the relationship between the within-class and between-class variances can be exploited to generate recursion relation that permits a much faster calculation.

Total variance is

$$\sigma^2 = \sigma_w^2(t) + q_1(t)[1 - q_1(t)][\mu_1(t) - \mu_2(t)]^2$$

The basic idea is that the total variance does not depend on threshold (obviously). For any given threshold, the total variance is the sum of the within-class variances (weighted) and the between class variance, which is the sum of weighted squared distances between the class means and the grand mean. After some algebra, we can express the total variance as since the total is constant and independent of t, the effect of changing the threshold is merely to move the contributions of the two terms back and forth. So, minimizing the within-class variance is the same as maximizing the between-class variance. The nice thing about this is that we can compute the quantities in recursively as we run through the range of t values.

IV. ITERATIVE TRICLASS METHOD

The idea of dividing an image's histogram iteratively into three classes is illustrated at the bottom of Fig. 2.

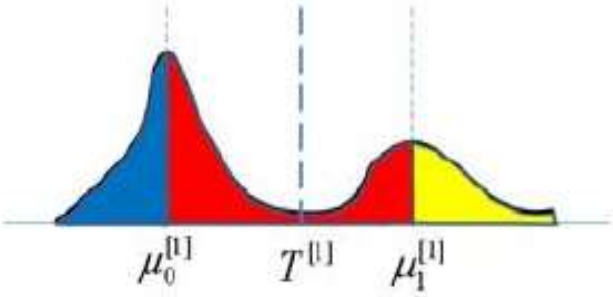


Fig 2: Iterative method we classify the histogram into three classes, namely the foreground region with pixel values greater than μ_1 (shown in yellow), the background region with pixel values less than μ_0 in blue, and the third region, called TBD, in red. The superscript denotes the number of iteration in our new algorithm.

For an image u, at the first iteration, Otsu's method is applied to find a threshold $T^{[1]}$ where the superscript denotes the number of iteration. We then find and denote the means of the two classes separated by $T^{[1]}$ as $\mu_0^{[1]}$ and $\mu_1^{[1]}$ for the background and foreground, respectively. Then we classify regions whose pixel values are greater than $\mu_1^{[1]}$ as foreground $F^{[1]}$ and regions whose pixel values are less than $\mu_0^{[1]}$ as background $B^{[1]}$. For the remaining pixels u(x, y) such that $\mu_0^{[1]} \le u(x, y) \le \mu_1^{[1]}$ we denote them as the TBD class $\Omega^{[1]}$. So our iterative process assumes that the pixels that are greater than the mean of the "tentatively" determined foreground are the true foreground. Similarly, pixels with values less than μ_0 are for certain the background. But the pixels in the TBD class, which are the ones that typically cause miss-classifications in the standard Otsu's method, are not decided at once and will be further processed. By our definition, we have

$$U = F^{[1]} \cup B^{[1]} \cup \Omega^{[1]}$$

Where ∪ is the logical union operation. At the second iteration, we apply Otsu's method to find threshold $T^{[2]}$ on region $\Omega^{[1]}$ only. We then calculate the two class means in $\Omega^{[1]}$ separated by $T^{[2]}$ as $\mu_0^{[2]}$ and $\mu_1^{[2]}$. Similarly, the second iteration will generate a new $F^{[2]}$, $B^{[2]}$, and $\Omega^{[2]}$ such that

$$\Omega^{[1]} = F^{[2]} \cup B^{[2]} \cup \Omega^{[2]}$$

where $F^{[2]}$ is defined as the region in $\Omega^{[1]}$ with pixel values greater than $\mu_1^{[2]}$, $B^{[2]}$ as the region in $\Omega^{[1]}$ with pixel values less than $\mu_0^{[2]}$, and Ω [2] are the new TBD region. The iteration stops when the difference between two consecutive threshold $|T^{[n+1]} - T^{[n]}|$ is less than a preset threshold. At the last iteration [n +1], $\Omega^{[n+1]}$ is separated into two instead of three classes, i.e., foreground $F^{[n+1]}$ is defined as the region of $\Omega^{[n]}$ that is greater than $T^{[n+1]}$ instead of $\mu_1^{[n+1]}$ and background $B^{[n+1]}$ is defined as the regions with pixel value less than $T^{[n+1]}$.

Segmented image=$F^{[1]} \cup F^{[2]} \ldots \cup F^{[n+1]} \cup B^{[1]} \cup B^{[2]} \ldots \cup B^{[n+1]} \cup \Omega^{[1]} \cup \Omega^{[2]} \ldots \cup \Omega^{[n]}$

The method is to iteratively define the TBD regions to gain a high distance ratio, which will result in better segmentation by applying Otsu's method. But it will take more time because iterations depend on type of image.

V. LOCAL ADAPTIVE THERSHOLDING

The local thresholding method is partitioned the original image into smaller sub images and a threshold value is determined for each of the sub images [6], [3]. This yields some discontinuities in gray level due to a different gray level of two different sub images. The threshold of a region can be calculated by the point-dependent method or the region-dependent method. A smoothing technique is then applied to eliminate the discontinuities of gray level between the sub images [6]. A threshold value is calculated at each pixel, which depends on some local statistics like variance, range, or surface-fitting parameters of the pixel neighborhood [4]. The threshold value is indicated as a function *T (i, j)* and the coordinates *(i, j)* at each pixel. If this is not possible, the object / background decisions are indicated by the logical variable *B (i, j)* [4]. Niblack and Sauvola methods are used the local image property variance and standard deviation values. The neighborhood size should be small, it enough to preserve local details, but at the same time large enough to suppress noise [3].

5.1. Smoothing

The local thresholding method is partition the original image into smaller group of pixels or sub images. A threshold value is determined for each of the sub images [6].

This yields some discontinuities in gray level due to a different gray level of two different sub images [3], [9]. The threshold of a region can be calculated by the point dependent method or the region-dependent method. A smoothing technique is then applied to eliminate the discontinuities of gray level between the sub images [6].

5.2. Niblack Thresholding Algorithm

Niblack's algorithm determines a threshold value to each pixel-wise by sliding a rectangular window over the gray level image [7]. The size of the rectangle window may differ. The threshold is calculated based on the local mean m and the standard deviation S of all the pixels in the window and is given by the following derivation [7], [8].

$$T_{Niblack} = m + k * s$$

$$T_{\text{Niblack}} = m + k\sqrt{\frac{1}{NP}\sum(p_i - m)^2}$$

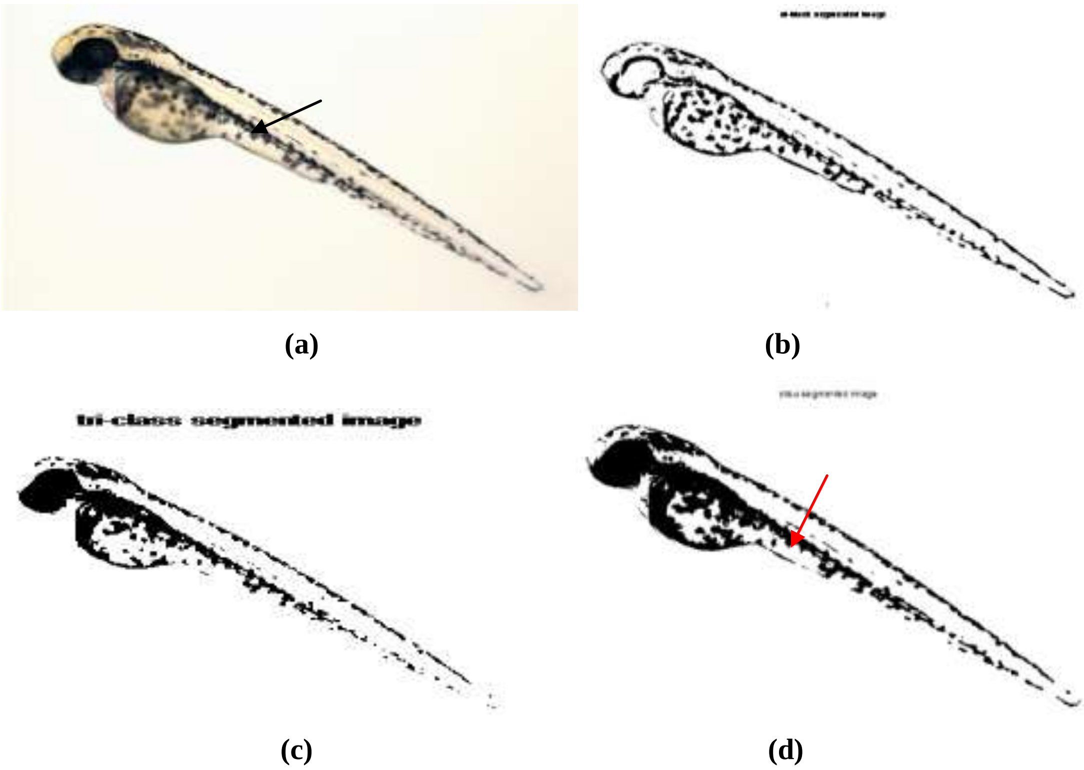
$$= m + k\sqrt{\frac{\sum p_i^2}{NP} - m^2} = m + k\sqrt{B}$$

Where NP is the total number of pixels presents in the gray image [7], [8], [9], *T* represent the threshold value, *m* is the average value of the pixels *pi*, and *k* is fixed depends upon the noise still live on the background it may be -0.1 or -0.2 [9].

VI. EXPERIMENTAL RESULTS

The improved Niblack segmentation shows better performance compared with different thresholding techniques like Otsu and iterative triclass thresholding techniques .The zebra fish embryo (fig.3) is first converted into normalized gray level values then we apply Otsu, Iterative and Niblack method.

In this we have taken two test cases, we applied the modified Niblack method on real microscopic images. For the first type of images we applied the Niblack method on *in vivo* zebra fish images acquired by a bright-field microscope. Fig. 3(a) shows a raw image of a zebra fish embryo. Because zebra fish embryos are transparent we can directly observe many anatomic structures without fixing and staining. For example the spinal cord of the embryo is visible in Fig. 3(a). The segmentation result of Otsu's method is shown in Fig. 3(b). Though the standard Otsu's method can segment the major structure of the embryo it misses detailed anatomic structure such as the spinal cord.



(a) **(b)** **(c)** **(d)**

Fig. 3 Experiments on a zebra fish microscopic image. (a) A raw zebra fish embryo image acquired by a bright-field microscope. Its spinal cord is pointed by the arrow. (b) The result given by Otsu's method. (c)The result given by the triclass iterative method (d) In the final result, the spinal cord of the zebra fish embryo, pointed by the red arrow, is fully segmented by Niblack method.

For comparison, Fig. 3(c) show the result generated by the iterative triclass method a. We can observe that some weak objects are missing. In particularly, the Niblack algorithm is able to accurately segment the spinal cord (pointed by the arrow), as shown in Fig. 3(d).

As the second example, we tested the iterative method on zebra fish images obtained in a different experiment where zebra fish embryos developed pericardial edema.

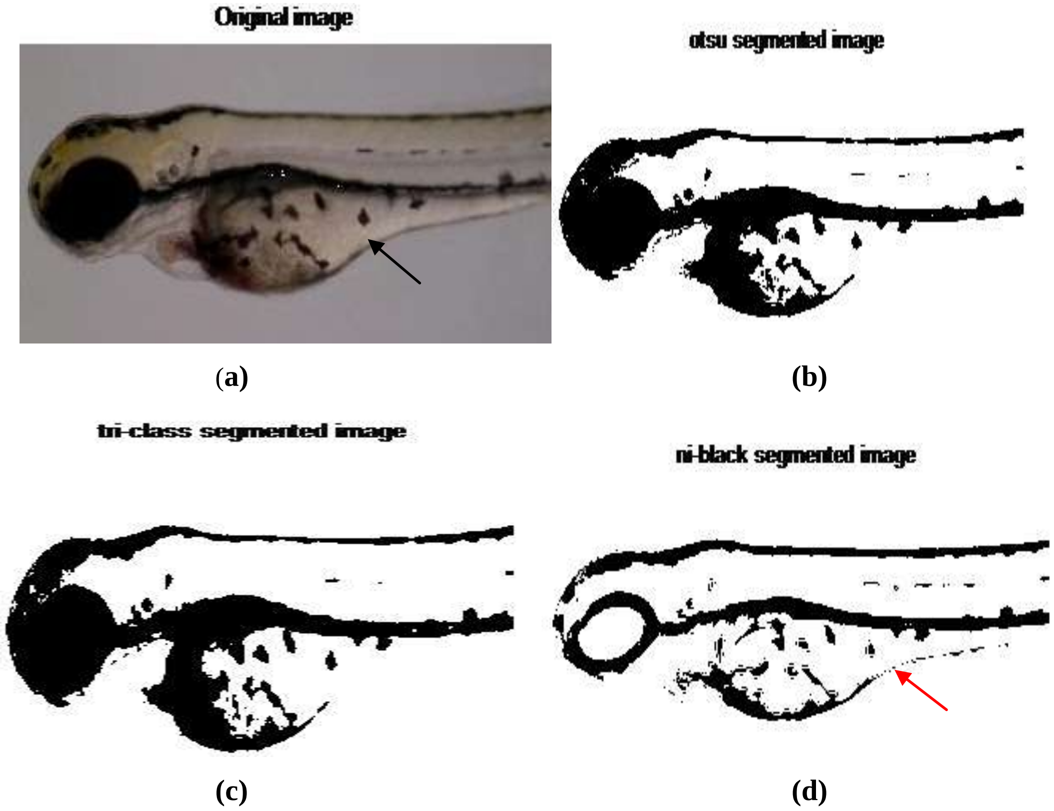


Fig. 4 (a) A test image showing a zebra fish embryo acquired by a bright field microscope. The arrow points to pericardial edema. (b) The result given by the standard Otsu's method. (c) The result given by the triclass iterative method. (d) The result of the Niblack method, which detects the spherical boundary of the pericardial edema (pointed by the arrow) while it is missed by the standard Otsu's method and iterative method.

An original image is shown in Fig. 4(a) and its standard Otsu's result and iterative triclass result is shown in Fig. 4(b) and 4(c) respectively, which does not segment the half spherical boundary of the edema. The results of applying the Niblack method are shown in Fig. 4(d). From the result we can observe that the algorithm is able to segment the half spherical boundary of the pericardial edema.

6.1 Statistical Results

Table: Comparison Between Iterative and Niblack Method

IMAGE	ITERATIVE TRICLASS METHOD			NIBLACK METHOD		
parameter	**MSE**	**PSNR(dB)**	**EXECUTION TIME(Sec)**	**MSE**	**PSNR(dB)**	**EXECUTION TIME(Sec)**
IMAGE 1	2.2680e+05	30.0347	12.315902	0.0408	62.0207	6.759054
IMAGE 2	5.8414e+04	24.1434	11.967807	2.1683	44.7697	6.475221
IMAGE 3	1.5684e+05	28.4328	13.571967	0.1232	57.2236	6.030193

VII. CONCLUSION

As Otsu's method is widely used as a pre-processing step to segment images for further processing, it is important to achieve a high accuracy. However, since Otsu's threshold is biased towards the class with a large variance, it tends to miss weak objects or fine details in images. Though the iterative method is give better result than Otsu but it is also missing some weak objects or fine structures. For example in biomedical images, nuclei and axons may be imaged with very different intensities due to uneven staining or imperfect lightening conditions, raising difficulty for algorithms like Otsu's method to successfully segment them. Without a robust segmentation results, more sophisticated processing such as tracking and feature analysis become highly challenging.

In order to overcome the limitations of Otsu and iterative triclass we used Niblack thresholding technique for segmentation later we applied edge detection and morphological operations for better segmentation. In this the threshold values are spatially varied and determined based on the local content of the target image. In comparison with global techniques, local thresholding techniques have better performance against noise and error especially when dealing with information near texts or objects. Testing results show that the Niblack method can achieve better performance in challenging cases. Though targeted here for microscopic image analysis only, it will be a good candidate for other kinds of applications as well like MRI image processing, scene processing and image segmentation.

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