Local Contrast Hole Filling Algorithm For Neural Slices Membrane Detection - LCHF

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ABSTRACT

Local Contrast Hole Filling Algorithm for Neural Slices Membrane Detection (LCHF) algorithm is non-learning, simple, easily adopted, and undependable on ground-truth; and it can recognize membrane and eliminates organelles, using a very simple algorithm that consist of short sequences of basic processing steps yet can be relatively competitive. Here, we would like to show the simple processing stages, and the effectiveness of the LCHF algorithm, with other similar neuronal datasets. The performance of the algorithm was measured in terms of Precision, Recall and F1 score. Precision (also known as positive predictive value), and Recall (also known as sensitivity). F1 score (also known as F-score or F-measure). The experiments were performed on data provided by the ISBI 2012 (IEEE International Symposium on Biomedical Imaging). LCHF generously allowed to classify pixels into membrane/non-membrane for these datasets, and took 44 seconds for 30 slices to produce the comparable best result, and recorded average F1 score of more than 71% similarity with the benchmark ground-truth image.

Keywords - **Membrane detection, Non-learning, Segmentation, Image processing**

I. INTRODUCTION

The aim of our research in creating this algorithm is to detect the membrane cell and ignore the organelle or remove the detected organelle from the image output with minimal effort and less processing time with minimal loss of undetected membrane [1].

In this paper we would like to report the usability of our proposed LCHF algorithm with other similar background data-slices. Our concentration would be for neuronal data-slices. The algorithm explanation can be found in Section II. The experiments result can be viewed in Section III of this paper.

In this paper, we would like to highlight that, even with this score we can perform a good membrane detection compared to long hours training with special hardware required. In executing our research, we have divided our research into 3 parts [1]. The aim of the first part, named LCHF, is to select the most effective tuning of a pre-defined processing pipeline. Since the component methods are critically dependent on some parameters, this stage serves also to determine the ranges of effective values of parameters in the processing pipeline for the detection of cell membranes which was simultaneously capable of ignoring organelles. In the Second Part, we try to preserve the simplicity of LCHF whilst improving its accuracy through the incorporation of global stochastic optimization and in the Third Part to enhance the F1 scores we incorporate ensembles techniques to the optimized chains.

In this paper we would only address our first part of research results and effectiveness, which we would like to share with new researcher in the area of medical imaging and computer programming, since this part of our research, consist basic image processing steps and only require minimal knowledge in programming.

II. METHODOLOGY

A. Image Processing Platform Matlab and the Image Processing Toolbox

The LCHF algorithm is based on a sequence of basic image processing steps, most of which we adopted from Matlab's image processing toolbox by MathWorks. This toolbox is useful for the processing, visualization and analysis of images, whilst MatLab is convenient for rapid prototyping.

B. Data

Transmission electron microscopy (TEM) is an important modality for the analysis of cellular structures in neurobiology [2] and it is a main tool for studying connections at the neuronal level [3]. According to Davi et al [4], reliable automated segmentation of neuronal structures in ssTEM stacks so far has been infeasible. Bobby Kasthuri stated in [5] that a solution to this problem however, is essential for any automated pipeline reconstructing and mapping neural connections in 3D. For the testing of the algorithm, we have used many ssTEM images. As for our LCHF algorithm development, we used ISBI 2012 data-slices provided by Albert Cardona and team which are available for public [6].

The training data is a set of 30 sections from a serial section Transmission Electron Microscopy (ssTEM) data set of the Drosophila first instar larva ventral nerve cord (VNC). The

microcube measures 2 x 2 x 1.5 microns approx., with a resolution of 4x4x50 nm/pixel. The corresponding binary labels (ground-truth) are provided in an in-out fashion, i.e. white for the pixels of segmented objects and black for the rest of pixels (which correspond mostly to membranes). [7]

C. Processing Stages

The algorithm as per Figure 1 is divided into several pre-processing, classification and post-processing steps.

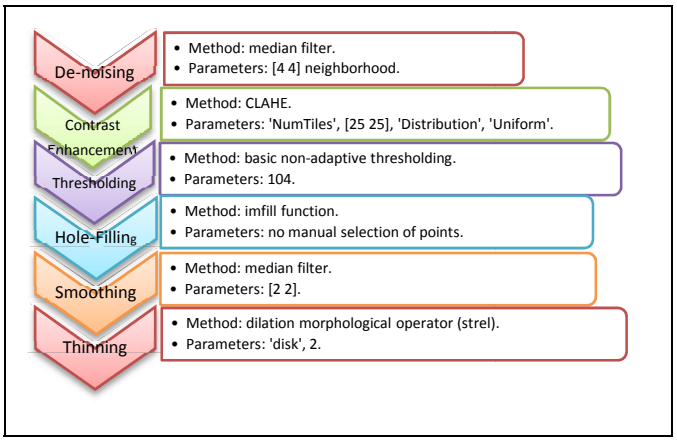


Figure 1: Processing stages of the algorithm

Each processing step has its own parameters which require some data-dependent fine-tuning. Several fine-tuning experiments were carried out, in order to find the most favorable set of parameters in terms of accuracy (i.e. F1 score) and speed [1].

Stage 1: Denoising

Denoising is basically no a need for all types of dataset. As for the Drosophila larva dataset, according to Laptev et.al [8], the dataset is highly anisotropic. The dataset also contains noises which need to be removed. Without doing pre-processing for the image, the output would not resemble as shown in Table 1. We have used Median Filter as our choice in LCHF. We have run several experiments and found that Median Filter work better with the dataset in comparison with Gaussian, Wiener, and Average Filter**.** The results are shown in Table 1 below.

Stage 2: Contrast Enhancement

As for contrast enhancement, Contrast Limited Adaptive Histogram Equalization (CLAHE) is our choice (upon experiments), and it changes the grey value of the pixels with neighboring pixels to improve local contrast [9]-[10].

CLAHE Algorithm

I → Image that needs to be processed for contrast enhancement

T → The output image after the contrast enhancement processed

R→ Window that moved to change the pixels value

a → Contrast Limit (max)

(m,m) → Which determine the height and width of *R*

Firstly, the image *I* need to be pad with (m − 1)/2 pixels on all sides, to prevent it to meet the border. For each and every pixel of *I* image, the window, *R* will move around pixels in image *I*, to change the pixels value with neighboring pixels value according to define window size and type , and this will be done in loop and the output result will be presented as *T*.

Stage 3: Thresholding

Researcher like, Shiying et.al [11], used gray-level thresholding to develop a technique to recognize lungs automatically, Aly, A.Farag et.al [12] applied optimal gray-level thresholding, Binsheng et.al [13] used histogram to calculate threshold, and Michela Antonelli et al [14] used iterative gray level thresholding, to perform segmentation. We too adopt the thresholding method to perform membrane detection. The thresholded (binary) image *g (x,y)* is defined as [15]:

$$g(x,y) = \begin{cases} a \text{ if } f(x,y) > T^1 \\ b \text{ if } f(x,y) \le T^2 \end{cases} \quad \ldots\ldots\ldots (1)$$

Stage 4: Hole Filling

For Hole filling: *I* denote a binary image, marker image *F* to be 0 everywhere except on the image border, where it is set to 1-*I* [15]:

$$f(x,y) = \begin{cases} 1 - I(x,y) \text{if } (x,y) \text{is on the border of } I \\ 0 \qquad\qquad\qquad\qquad \text{otherwise} \end{cases}$$

then, $H = [\mathrm{R}_{I^c}(F)]^c$ …….. (2)

is a binary image equal to *I* with all holes filled.

Stage 5: Smoothing and Thinning

In our experiments, this stage is an optional stage, and only been carried out for better visual inspection approach.

D. Performance Measures

The performance of the algorithm was measured in terms of Precision, Recall and F1 score. Precision (also known as positive predictive value), and Recall (also known as sensitivity). F1 score (also known as F-score or F-measure).

$$Precision = \frac{\mathrm{tp}}{(\mathrm{tp}+\mathrm{fp})} \quad [16] \ldots\ldots\ldots (1)$$

Where tp denotes true positives ((i.e. the number of pixels correctly labeled as belonging to the positive class) and fp denotes false positives (i.e.which are pixels incorrectly labeled as belonging to the class)

$$Recall = \frac{\mathrm{tp}}{(\mathrm{tp}+\mathrm{fn})} \quad [16] \ldots\ldots\ldots (2)$$

Where tp denotes true positives and fn denotes false negatives (i.e. which are pixels which were not labeled as belonging to the positive class but should have been)

$$F1 = 2\left(\frac{\mathrm{Precision} * \mathrm{Recall}}{\mathrm{Precision}+\mathrm{Recall}}\right) \quad [17] \ldots\ldots\ldots (3)$$

F1 is a measure of a test's accuracy. The F1 score can be interpreted as a weighted average of the precision and recall where an F1 score reaches its best value at 1 and worst score at 0. For each slice, a confusion matrix was computed followed by

corresponding precision(1), recall(2) and F1 scores(3). The final performance values were averaged from the results corresponding to each one of the 30 slices.

III. EXPERIMENT AND RESULTS

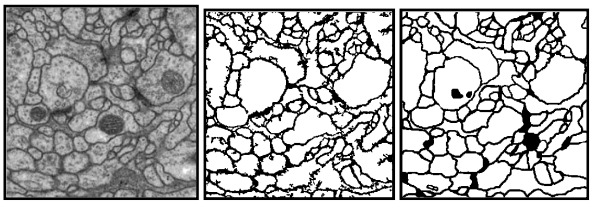


Figure 2: An image with segmentation result using Local Contrast Hole Filling (LCHF) (second from left) compared to existing ground-truth (benchmark) (first from right)

The Figure 2 image shows a randomly picked image from 30 stacks of images, with its corresponding output using LCHF method, which shows detection of membrane cell and the elimination of organelle cell. Our method is easy to use and easily can be adopted by beginners in the field of Medical Imaging. The output result using LCHF is promising and the recorded average F1 score of more than 71% similarity with the benchmark ground-truth image, with average processing time of 44 seconds for 30 slices [1].

A. Experiment Result to Choose the Best Function and Parameter for LCHF algorithm

i) Experiment for Denoising Function

Measures	Median	Gaussian	Wiener	Average	Laplacian
Average F1	0.6569	0.6501	0.6592	0.6503	0.3588
Average Precision	0.6265	0.6333	0.6367	0.6324	0.2194
Average Recall	0.7281	0.7092	0.7232	0.7073	0.9925

Table 1: Shows experiment result using different denoising filter.

Table 1 shows the result of applying different types of filters, namely the median and Wiener filters. In Table 1 we compare the accuracy resulting from five different denoising algorithms when incorporated into the following sequence of three steps: denoising, thresholding and hole-filling. When using this particular sequence, the Wiener filter is the best denoising method, with a resulting F1 score of 0.6592. However when we expand the sequence of steps by incorporating a local contrast enhancement step after denoising, the resulting overall F1 score from using the median filter (i.e. 0.7107) is better than the one resulting from using the Wiener filter (i.e. 0.7091). Based on experiments, we choose Median filters to be adopted in our algorithms. The Gaussian, Average and Laplacian scored low in the experiments.

ii) Experiment for Contrast Enhancement Function

Measures	Using Global Contrast – Histogram Equalization after De-noising	Using MatLab's imadjust after de-noising	Using Local Contrast-CLAHE after De-noising
Average F1	0.6778	0.6861	0.7107
Average Precision	0.5515	0.6301	0.6429
Average Recall	0.8838	0.7660	0.7974
Elapsed Time (second)	14.6197	15.0907	21.0894

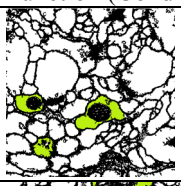
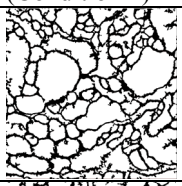
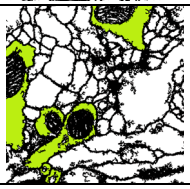
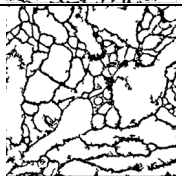
Table 2: Shows Average performance values after using different contrast enhancement techniques.

Table 2 shows the average performance values comparing global and local contrast enhancement methods. The scores shows that CLAHE (local contrast enhancement) perform better than Histeq and Imadjust (global contrast enhancement). From our experiments we encounter that by using histogram equalization method organelles are still being falsely detected, and when we adjust the image intensity values using Matlab 'imadjust' method the membranes are erroneously eliminated. But with CLAHE, there is no major elimination of membranes and no significant false detection of organelles. Because of this, CLAHE was chosen as the contrast enhancement algorithm for LCHF.

iii) Experiment for Thresholding Function

From our experiments with thresholding function, we discovered that different threshold values affect membrane detection and organelle detection. We notice that as the threshold value increases, precision scores decreases, recall scores increases and F1 scores initially improve but then deteriorate. For our experiments we used an exhaustive search procedure (using F1 scores), and it was found that a threshold of 104 was the best choice for the Drosophila dataset.

iv) Experiment for Hole Filling Function

Image Slices	Outputs using only Matlab Thresholding and Hole Filling Function (Condition 1)	Output using LCHF Algorithm (Condition 2)
Slice 3		
Slice 15		

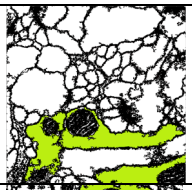
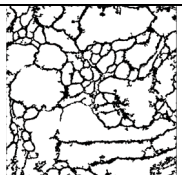
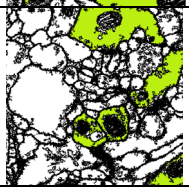
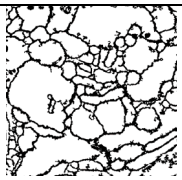
Slice 22		
Slice 29		

Table 3: Shows result of using only thresholding and hole-filling (condition a), and result of using hole-filling with all of the LCHF pre and post-processing stages (condition b)

Table 3 is depicting 4 slices out of 30 slices for illustration purposes. Here in Table 3 shows how by just incorporating thresholding and hole-filling, organelles being erroneously detected, highlighted by a green background. But as for Condition b, when supplemented with LCHF pre-processing and post-processing stages, much better result are shown in term of F1 scores (Table 4) and visual inspection approach (Table 3).

Measures	Denoising +Thresholding + Hole-Filling	Using LCHF (complete algorithm)
Average F1	0.6569	0.7107
Average Precision	0.6265	0.6429
Average Recall	0.7281	0.7974
Elapsed Time (sec)	14.4165	44.4232

Table 4: Shows the measure values for base Hole Filling vs. LCHF

B. Comparison with Edge Detection Method

Figure 3 shows a microscopic image of neuronal structures (left) followed by outputs generated by different edge detection methods (i.e. Canny, Laplacian and Sobel) and the LCHF method. From this figure it is clear that standard edge detection methods clearly do not only detect membranes but also detect other intracellular structures, and therefore are not suitable for solving the membrane detection problem.

Original Image Using Canny Using Laplacian Using Sobel Using LCHF

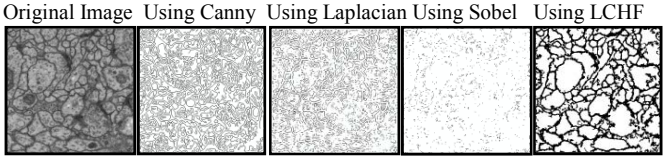
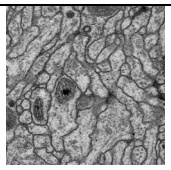
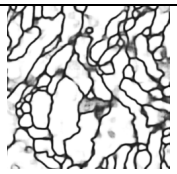
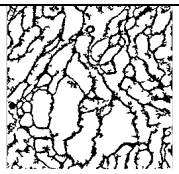
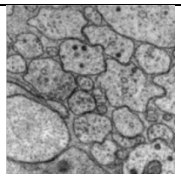
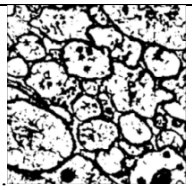
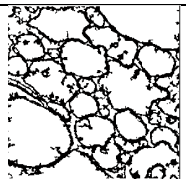
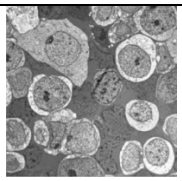
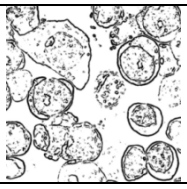
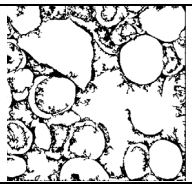


Figure 3: Simple comparison of different edge detection methods and LCHF

C. Comparison of LCHF algorithm with other Dataset

The Table 5 shows an experiment result using LCHF with other datasets and their associated methods. We tested our algorithm and present in the table below the comparison of our algorithm output with output resulted from other methods (please refer to comparison method row in the table below). Our algorithm proves that it can also be used for other neuronal dataset for membrane detection, with some parameter tuning.

Image Type	**Original Images**	**Comparison Method**	**Output using other method**	**Output using LCHF Algorithm**
Drosophila Test Image 16 [3]		Deep Neural Network (DNN) used as a pixel classifier by Dan Ciresan et.al [3]		
C.Elegans [18]		Thresholding and Anistropic Smooth by Elizabeth Jurrus et.al [18]		
Rabbit Retina [18]		Thresholding with Gradient Magnitude by Elizabeth Jurrus et.al [18]		

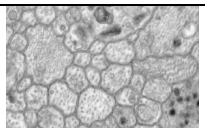
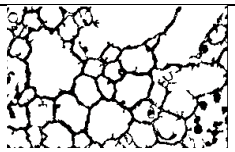
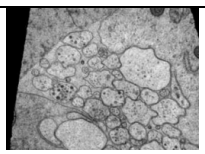
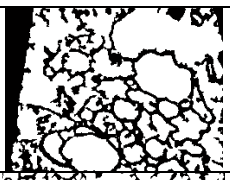
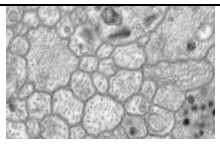
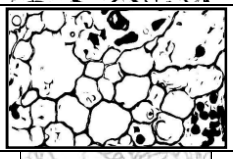
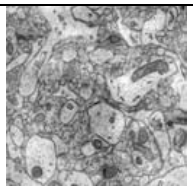
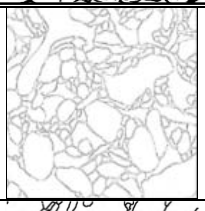
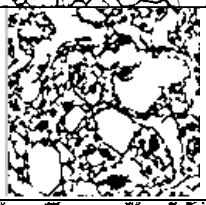
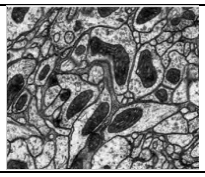
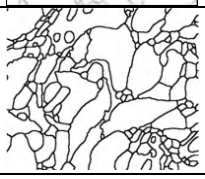
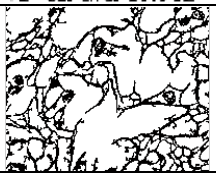
TEM image of Rabbit Retina [19]		Perona Malik's Weickert Partial Differential Equation (PDE)[19]		
C.Elegans [10]		Supervised Learning Approach by Venkataraju. [10]		
TEM image of Rabbit Retina [20]		Tolga Tasdizen's Hessian based diffusion with modification to Weickert PDE[20]		
EM image of mouse cortical Neurons[21]		Human Annotation reported by Viren Jain et.al [21]		
Lamina and medulla neuropiles of optic lobe[22]		Automated Segmentation reported by Dmitri et. al [22]		

Table 5: Shows an experiment result using LCHF with other datasets and their associated methods.

IV. CONCLUSION

The overall message of this algorithm is:

"Even a very simple algorithm consisting of a short sequence of basic processing steps can be relatively competitive".

The LCHF method not only highlights membrane boundaries but also removes internal structures (eliminate organelles) successfully. LCHF is fast in tuning, easy to deploy and use with less cost. Although the best F1 score so far is approximately 71% the algorithm does indeed do a reasonably good job at distinguishing membranes and organelles thus satisfying our original goal. As for future enhancement, one particular artifact that needs to be addressed is the presence of 'squiggly lines' jutting out from the membranes, as can be seen for example in Figure 2.

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