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CALCULATING MYELIN SHEATH THICKNESS WITH WATERSHED ALGORITHM FOR BIOLOGICAL ANALYSIS

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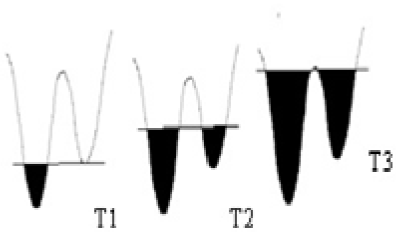
Graphical abstract



Abstract

In this paper, the brief reviews of the significance of the myelin thickness to various root courses of the diseases for a human being, and the similarities between the human and the lab mouse being researched extensively so far are presented. Once we establish the necessity for a biological statistical analysis of the myelin thickness with regard to the particular category of the brain cell, we can then develop a mixed algorithm based on the watershed principle and the binaryzation principle that can be used to automatically process the scanning electron microscope pictures in batches. The initial analysis does indicate that the mixed method improved efficiency of data processing while not overlooking some important medical diagnostic factors.

Keywords: Brain Cell, watershed algorithm, sphingomyelin, image processing

1.0 INTRODUCTION

Twenty-five% of fatty acids, including sphingomyelin, in a human being are located inside the brain; however, the weight of the brain is only 2% of the entire body. The majority of the fat surrounds the neuron, specifically in the form of myelin sheaths [1] that insulate the axon of the neurons while providing structure to the entire brain. Therefore, measuring the myelin thickness (g-ratio) of the axon must have some medical significance related to some, if not all aging diseases.

It is known that several neurological diseases in humans, such as dementia (e.g. Alzheimer's dementia), Multiple Sclerosis, transverse myelitis etc. are all related to the fatty acid level. Similarly, for the mouse and guinea pig, problems like salmonellosis, tularemia, plague, Lymphocytic Chorio-Meningitis (LCM) are also related to fatty acid concentrations. It is common knowledge that a mouse has 20 pairs of chromosomes, and a human has 23 pairs of chromosomes. Between the human and the mouse, 80% of the genes are exactly the same, 19% are very similar, and there is only a 1% difference. Nobel laureate, Dr. James Watson, stated that there are about 30000 genes in both humans and mice.

University of Toronto researchers recently created amyloid vaccinations for Alzheimer's [2]. Their approach is from three angles. Firstly, they evaluate the therapeutic strategies targeting clinical development. Secondly, they consider immunization strategies reliant on a peripheral sink mechanism of action, namely small molecules that have easy access to the brain. Lastly, they propose that the dose will be considered based on age and disease specific changes in order to elicit maximal effectiveness. The researchers have confirmed that furthermore, BACE1 KO (Beta-secretase Knockout) mice demonstrate problems in axon targeting. This may be explained by a developmental issue during adult neurogenesis and neural circuits where the Alzheimer's β-secretase enzyme BACE1 (β-site APP Cleaving Enzyme 1) is required for accurate axon guidance of olfactory sensory neurons and normal glomerulus formation in the olfactory bulb. Since the axon's guidance in this situation is dependent on the axon's size, we will analyze the size of mice axons in the present study. The researchers consider immunization strategies primarily reliant on a peripheral sink mechanism of action, specifically small molecules that enter into the Central Nervous System (CNS) and thus degradation pathways within the brain and lifestyle interventions affect vascular, parenchymal and peripheral degradation pathways. They propose that effective development of Alzheimer's disease therapeutic strategies targeting Ab peptide will require consideration of the age- and disease-specific changes in endogenous Ab clearance mechanisms in order to elicit maximal efficacy. Hence, this will require deep analysis of human axon size for age- and disease-specific changes.

Neurodegenerative dementias like Alzheimer's Disease (AD) [3] are linked to deficits in axonal transport. Furthermore, they are also associated with imbalanced distribution and dysregulation of NTF (Neuro-Trophic Factor). In particular, the Brain-Derived Neurotrophic Factor (BDNF) plays a crucial role in cognition, learning and memory formation by modulating synaptic plasticity and is, therefore, a critical molecule in dementia and neurodegenerative diseases. Again the axonal transport is related to the axon size, by physical electron dynamic law.

In addition, two more references further confirm that the fatty acid concentration level is correlated with brain related illness. Since the human body is a connected complex system, 25% percent of the fat in the brain cannot be completely detached from the rest of the 75% which occupies the majority of the body (98%). Low BMI（Body Mass Index） or a faster decline in BMI in late life may be preclinical indicators of an underlying dementing illness, especially for those who were initially overweight or obese [4].

In both sexes, WC (Waist Circumference) and HOMA (Homeostasis Model Assessment) index were significantly positively correlated [5]. The Optimal Waist Circumference (OWC) cutoff point was 94.5 cm for men and 90.5 cm for women. The high sensitivity (0.80) and specificity (0.84) of WC in males indicate the proportion of IR (Insulin Resistance) which is correctly identified and recognized in all non-IR males. In the regression model only the Triglyceride (TG) level was associated with WC, but the WC is strongly associated with HOMA-IR. While OWC is very likely a good measure to exclude non-IR subjects in the study, determination of optimal WC to identify elderly IR subjects warrants further study in a larger sample of the general population.

The next two references explain where the fat originates from and where the fat dissipates to Fat inside the body will be converted into vitamin D by UV (Ultraviolet Rays), when one is exposed to the sun. Researchers [6] have shown that vitamin D deficiency is associated with a substantially increased risk of all-cause dementia and Alzheimer's disease. This adds to the ongoing debate about the role of vitamin D in non-skeletal disease conditions. Vitamin D may be a neuroprotective factor and "sufficiency" in terms of concentration in the context of dementia risk is about 50 nmol/L. This information is likely to prove useful in improving the design and reducing the cost of randomized controlled trials in investigating whether vitamin D supplements can be used to delay or prevent the onset of dementia and AD in older adults.

Body fact can be generated from vegetable oil. For example, the study shows that total erythrocyte n23 PUFA (Poly-Unsaturated Fatty Acid)[7] concentrations are positively associated with cognitive function, particularly immediate recall, in older people with previous depression. Lower concentrations of n23 PUFAs or ALA (Alanine) in erythrocyte membranes may be good predictors for cognitive impairment in older people with previous recurrent depression.

By now, we have provided enough evidence to support how the amount of fat accumulation can affect the brain cell. There are many different fat molecules involved and they will be described below.

Among those aged 70 or older, high midlife waist-to-hip ratio [8] may increase odds of dementia. However, traditional Cox models do not evidence this relationship. Changing anthropometric parameters in years preceding dementia onset indicate the dynamic nature of this seemingly simple relationship. There are mid-life and late-life implications for dementia prevention, and analytical considerations related to identifying risk factors for dementia.

Lipid dyshomeostasis [9] has been linked to Alzheimer's disease. Lipidomic analyses of brain tissue from AD patients reveal region-specific changes in multiple bio-active lipids, some of which are phenocopied in AD mouse models. Lipid anomalies observed in AD may be linked to pathogenesis, including endolysosomal dysfunction.

Myelin [10] is a unique way to increase conduction speeds and strength along axons of relatively small calibers. It seems to have arisen independently in evolution several times in vertebrates, annelids and crustacea. In conclusion, it is important to understand the relevance and significance of myelin thickness because it contributes to the majority of the fats in the brain.

2.0 THE IMAGE BASED BIOLOGICAL ANALYSIS

With the improvement of living standards, the social problems caused by dementia have caused great concern. Dementia is a progressive degenerative brain disease. From a morphological point of view, we can find the root cause by the various imaging techniques. The researchers are focusing on neurorestoratology, which is done by analyzing the sampling cells, or from dose-color sub-micrometer wave radar imaging, to find the cause of the disease, and cure it as early as possible. The image recognition module of the neurons recognition software is the key to nerve repair process.

In this paper, we design a software called brain neurons recognition software, used to analyze the g-ratio of the samples of the brain neurons. We then compared that with normal healthy brain neurons, or historical data of the same brain section. Based on these results, the medical professionals can make better judgments and give personalized advice to the patients (e.g. adjustment in diet). This paper introduces the dilutive and erosive principles, the process of Matlab design and the combination of the watershed algorithm [11] with the above mentioned traditional binary connectivity algorithm provided by the Matlab itself. The software will recognize the neuron cells by image processing [12], and calculate its g-ratio.

If the neurons can be recognized by brain neurons recognition software, then this can guide the medical professionals in finding solutions to treating dementia (Alzheimer's disease) possibly by comparing g-ratio of abnormal cells with normal cells.

Presently, the medical examination of brain neuron cell slice imaging is still at a relatively early stage, and it is basically done by hand. First, the cell morphology is observed by imaging technology with the naked eye. Then, a large number of observation results are analyzed to obtain the root cause and to develop the corresponding treatment. However, the disadvantages of this method are self-evident. The manual image examination has greatly increased the burden of health care worker, this method is inefficient and cannot guarantee accuracy, and this is not conducive to timely diagnosis and treatment.

In order to deal with such a situation, researchers have attempted to develop an image recognition software, which is designed with excellent performance to help medical personnel diagnose and treat conditions with the aid of a computer. The software involved in image processing technology, image information collection, pre-processing, image segmentation, feature extraction and other knowledge reasoning, especially in how to design a good performance of the image segmentation algorithm has not yet made significant progress. Cell morphology is often diverse, in particular overlapping organelles, fluid between cells and impurities present as challenges to accurate analysis. In order to achieve the intended purpose, an effective mixed image segmentation algorithm is designed to separate the cells from the image into individual cells.

Presently, research on image segmentation mainly includes: threshold segmentation method, region growing method, edge detection method and morphological segmentation method.

Threshold segmentation is one of the most common methods of parallel segmentation and it is based on the gray value of different targets. It is a common segmentation algorithm and the results are dependent on the threshold selection.

The region growing method is a typical example of the serial region segmentation algorithm. The basic idea is that image pixels with similar properties can be formed into a region. In this method, we first select a pixel seed in the region of the split, and then we merge the pixels around the pixel seed in order to establish a merged region of similar pixels . So repeatedly, the new pixels will merge with the growing pixel seed until the end image no longer appears to meet the growing conditions. The region growing method is essentially grouping pixels with similar properties together to form the segmentation region. According to the local spatial information of the image, it can effectively overcome the shortcomings of image segmentation. However, the regional growth method often leads to over segmentation.

Edge detection technology is based on, the use of detecting different regions of gray level discontinuities pixel by pixel to achieve image segmentation. In image segmentation, the characteristics within the region are the same, and the characteristics of different regions are different. Edge detection technology focuses on these differences and detects the location of change in image characteristics. During edge detecting, the edge locating ability contradicts with the ability to resist noise. Even though the ability of edge locating is strong, the ability of resisting noise is poor. The aforementioned methods are already provided by the Matlab software, under the binaryzation principle. Thus, to improve its performance, we further combined them with the watershed algorithm, which will be explained below.

3.0 WATERSHED ALGORITHM

3.1 The Principle

The Watershed algorithm is a segmentation method based on morphological theory. The image is treated like a topographic map, the gray value of each pixel in the image corresponds to the point of the terrain height. High gray values correspond to the peaks and low gray values correspond to the valleys. Water from high to low in the valley forms a water absorption basin, the ridge between different basins becomes a watershed. As an adaptive multi threshold segmentation algorithm, watershed algorithm can effectively avoid the phenomenon that the edge is missing because the threshold is too high, or the false edge is generated by the low threshold. In the process of image segmentation, according to the gray value of the pixel, the different water absorbing basin and watershed are found in the gray image. Eventually, the different water absorbing basins and the threshold are the targets of the division.

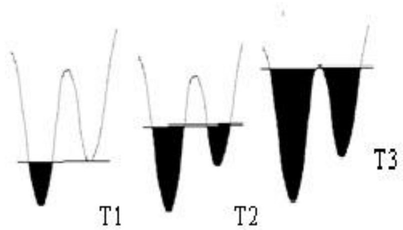


Figure 1 Diagram of the water shed situations

As shown in Figure 1, two low-lying areas are part of the water basin. In particular, the level of the threshold at T1 missed one basin. As the threshold rises, the water level reaches threshold T2, identifying both basins. When the threshold reaches T3, the two basins of water will overflow into each other. Therefore, the threshold can be divided into two regions, namely the watershed and the water absorbing basin. The watershed boundary is the edge of the gray image.

Watershed algorithm is the process of iterative annotation, it mainly consists of two different processes which are sorting and flooding. Firstly, all pixels in the gray scale image are sorted according to the gray level. Then, they are flood in accordance from low to high gray level.

The gray image obtained by the watershed algorithm is related to the image of the basin. Boundary points between adjacent water collecting basins indicate the watershed boundary. The watershed boundary represents the local maximum point of the input image. Therefore, in order to get the edge information from the image, the gradient image is usually used as the input image. That is

$$g(x,y)=grad(f(x,y))=\{[f(x,y)-f(x-1,y)]2[f(x,y)-f(x,y-1)]2\}0.5 \quad (1)$$

From the formula, f (x, y) represents the original image, and grad{.} represents the gradient operation. The watershed algorithm has better adaptability to the weak edge of processing image. If the gray level images occur in fine gray changes, it is possible to produce an over segmentation phenomenon. Therefore, in order to reduce the excessive segmentation of the output image after the watershed algorithm, the threshold value of the gradient function is usually performed, that is

$$g(x,y)=max(grad(f(x,y)),g\theta) \quad (2)$$

In this formula, $g\theta$ represents the threshold value.

By using the threshold to limit the gradient function, we also reduce the possibility of over segmentation. This is done by first obtaining the appropriate area, and then sorting and flooding the edge of these regions based on the gray level. Gradient images can be obtained by the Sobel gradient operator. Briefly, if the threshold value of the gradient image is processed, then it is the key to the image segmentation effect. However, this method has its own shortcoming as well since we cannot expect a "one algorithm fits all images", we can certainly rely on a combination of the algorithms to deal with all images.

3.2 The Design Flow

The design is based on the principle of image processing, which is the combination of binaryzation and morphological image segmentations for final image feature extraction. The mixed algorithm is used to identify brain neuron cells in the slice image, and then the recognition results are processed by the image geometrical feature extraction.

This design is mainly achieved via the Matlab programming for processing slice images as outlined in the following steps:

(1) Read the slice image and convert it to grayscale images.

(2) After the image is filtered by the gradient operator, the image is processed by binaryzation for edge detection.

(3) The image is preprocessed, and the structural elements of the original image are corroded and expanded by using opening and closing operations.

(4) The watershed algorithm in the Matlab program is used to find the image of the watershed transform ridge line in order to achieve the image segmentation of neuron cells.

(5) The geometrical features of the neuron cells obtained by the image feature extraction are used to extract the image features based on both (3) and (4) independently.

4.0 MATLAB PROGRAM

4.1 The Open and Close Binaryzation

When the image background is dark pixels, it is labeled "lobrcbr", and this can be operated by the threshold. The Im2bw function uses the threshold transform method to transform the gray level image into a two value image, where 0 means black and 1 means white. This is called binaryzation.

Matlab code：
```
%Converted to two value images
bw = im2bw(Iobrcbr, graythresh(Iobrcbr));
```

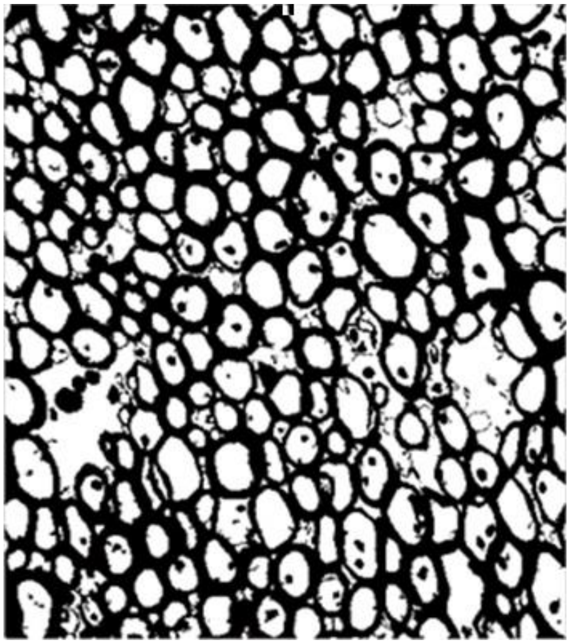


Figure 2 Open close valued image reconstruction

As shown in Figure 2, the gray image is converted to a two value image. In this picture, the contour of the brain neuron cells and the cell apparatus can be clearly seen, which will facilitate the extraction of the cell area.

4.2 Watershed Algorithm

Under ideal conditions, the background pixels in the black region do not have to be close to the edge of the image edge and background. In order to separate foreground from the background, the distance transform is calculated by using the watershed algorithm with ridge DL==0 to achieve the range of the skeleton effect. The value of Euclidean matrix is obtained by using the bwdist command. In addition, the imimposemin can be used to modify the image, to achieve a local minimum only at a specific location, to modify the gradient magnitude image, and to achieve the front and rear labeled pixels that have a local minimum.

Matlab code:
```
% watershed transform and display
D = bwdist (BW);
% calculation value of two Euclidean matrix
DL = watershed (D);
% for the watershed boundary
bgm= DL = = 0;
```

Figure 3 Watershed boundary image

As shown in Figure 3, the white pixel region is the watershed boundary or also known as the edge contour of the brain neuron cells. This will further facilitate the operation of the cell perimeter information. Next we merge back the image as shown below:

Matlab code：
```
% maker the image in the foreground and background
L = watershed(gradmag2);
I4 = I;
% display  the watershed boundary in the image
I4(bgm) = 255;
```

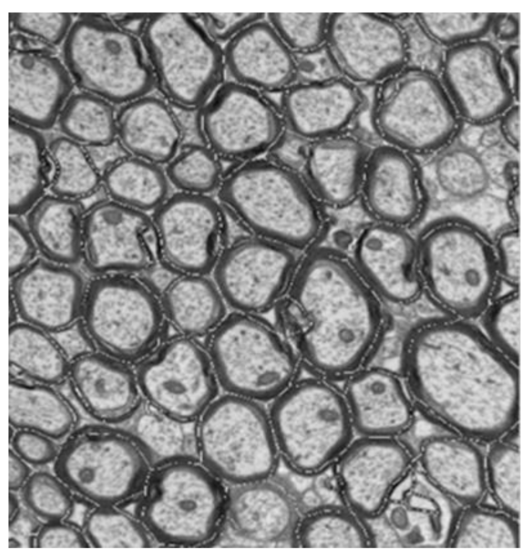


Figure 4 The boundary of the tag and the object

As Shown in Figure 4, the gray images obtained after pre-processing operations are processed, the watershed boundary is depicted by the watershed algorithm, and the contour of the brain neuron cells are also displayed. Compared to the original image, the processed image is clearly separated and the image distortion is also minimized. The process of image processing is relatively simple and the unique advantages of the combined binaryzation and watershed algorithm were highlighted in this image analysis.

4.3 Image Feature Extraction

The image processing process can be summed up as three processes of image acquisition, preprocessing and image segmentation, and finally the image feature extraction. Commonly used image feature extraction are: color feature, shape feature, texture feature and spatial feature. The design will extract the area and perimeter of the cell image, and according to the relationship between the two, further find the average diameter of the cell (i.e. g-ratio) to facilitate analysis of the slice images. The feature extraction is based on a combination of the previously mentioned two algorithms, such that any image can be extracted robustly without problem.

```
Matlab code：
%calculation area
for j= 1:size(bw,2);
%Calculate the number of columns in the binary image
                  sum = sum+1;
                   end
                 end
end
p =l* total;
% Calculate average diameter
r = s/p1;               % average diameter
r1 = s/p1;              % Area and perimeter Division
r2 = s/p2;              % Based on watershed image
r = l*sum/p1;
% Flexibly combined calculation of the diameter
ri = min(r1,r2);     %Inner circle radius
ro = max(r1,r2);     %Outer circle radius
d = (3*ri-ro)/2;
D = (3*ro-ri)/2;
g = d/D;                %Axon diameter ratio
```

Output results of Mouse Splenium Axon Diameter Ratio：

r = 124.5818

g = 0.7450

As shown in the image extraction process, the first method is to calculate the area and perimeter of the specific value, and then divide by the diameter. The second method is to calculate area and circumference of the number of pixels, and then divide by the unit pixel length to obtain the diameter. The same results are obtained with the two methods, but this does not mean that the individual result is perfectly accurate. This is because in the process of seeking the area, the isolated minor gap between cells may also be calculated, hence the result of the area may be slightly larger for one or the other, but the chance that both methods are biased in the same way is low. Secondly, due to the existence of the watershed boundary of the zigzag bending phenomenon, the perimeter may be measured slightly bigger. Thus, the diameter obtained is quite accurate, especially since the above two biases cancel each other out resulting in an average value. Although the results are not accurate enough for each individual cell feature extraction, the advantage of watershed image segmentation improves the g-ratio calculations. To obtain accurate individual cell feature information, this design requires further refinement in terms of individual or group of similar sized cell(s) feature extraction.

5.0 CONCLUSION

With the improvement in living standards, social problems caused by brain degeneration (e.g. Alzheimer's disease) have attracted public attention. Limitations such as the donepezil hydrochloride drug treatment encourages the medical researchers to begin diversifying research directions, and invest in nerve repair using alternative ways to treat Alzheimer's disease. Alzheimer's disease is a progressive brain degenerative disease and cell pathology and morphology can be seen through imaging analysis. This can be done through brain neuronal recognition software for sampling image analysis, image segmentation and image feature extraction technique, using the image with stack connected cells being separated into single cells, and then measuring the geometric characteristics of the cells. It will provide some diagnostic hints by comparing the g-ratio of problematic cell with that of normal nerve cells. Ultimately, this study aims to aid doctors and nurses obtain correct analysis based on individual patients' condition, and put forward an effective personalized treatment plan, in order to achieve the purpose of early detection and early treatment.

In addition, the image recognition software will help nerve repair technology in the treatment of brain degeneration development. The design involved in the image processing principle includes morphological image segmentation technology, image feature extraction technology and the use of morphological watershed algorithm to identify brain neuron cells. Then, the recognition results are processed by the image feature extraction technology, and the geometrical features of the brain neuron cells are obtained. The design of the overall software is conducted through the combination of existing MATLAB binaryzation algorithm and the watershed algorithm in order to deal with the variations of the slice images. If only one algorithm is used, some images may not work out properly. However, with the combination of both, the software becomes much more robust.

In summary, first, the slice image is read and converted into gray image, and then the image is processed by the gradient operator. In the use of watershed algorithm to process the image, which will be already preprocessed by the use of morphological opening and closing operations, the construction of structural elements of the original image is then corroded and expanded. Call the watershed algorithm in the Matlab program and find the image of the watershed transform ridge line, in order to achieve the image segmentation of the neuron cells. The geometrical features of the neuron cells obtained by image segmentation are extracted by image feature extraction. Then the image is converted into binary format, re-calculated for the area of the myelin sheath by averaging the final results based on both algorithms, and finally we relax the requirements for cell clearance between each other on the original image. The complete Matlab code is available on the Matlab server.

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