Computation of brain neurodegeneration in the Alzheimer's fly

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ABSTRACT

Expression of human Aβ42 peptide in the Drosophila brain induces pathological phenotypes resembling Alzheimer's disease (PNAS 101, 6623-6628). Three-dimensional confocal imaging reveals extensive vacuoles caused by neurodegeneration in the brain of aged but not young Aβ42 flies. Here, we report a three-dimensional computation algorism allowing semi-automatic measurement of numbers and volumes of brain vacuoles. The method employed matched filters, α-shape, and the active-contour techniques. Using this method, a good result depicting the contours of the vacuoles can be obtained. A more accurate algorithm is still under development. Accurate evaluation of brain pathology in Alzheimer's flies may facilitate the understanding of molecular mechanisms underlying Aβ. toxicity and the discovery of novel therapeutic targets for Alzheimer's disease.

Keywords: Confocal microscopic images, Matched filter, α-Shape, Active-contour, Snake

1. INTRODUCTION

Vacuoles in brain tissues are prominent pathological symptoms for neural degeneration in the brain. Many chronicle neurological diseases, such as the Alzheimer's disease, Huntington's disease and Parkinson's disease, to name just a few. An intuitive assessment of the damage in the brain tissue is to direct calculating the volume of vacuoles. Any treatment that may retard or reverse the progress of vacuole formation will be evaluated as an effective remedy. Yet some hurdles that have to be overcome are the accuracy and process automation for huge amount of images collected. Good vacuole evaluation protocol has not been satisfactorily implemented majorly due to poor image quality, scarce tissue samples and ineffective computation algorithms. The brain of fruit flies (Drosophila) has been shown to be a reasonable proper model in brain research, in terms of some function, such as learning, memory and drug responses. Also, whole brian images at the cellular resolution has been successfully acquired from Drosophila recently [8]. Futhermore, it is quite easy to collection large amount of samples under various treatments for Drosophila. Expression of human Aβ42 peptide in the *Drosophila* brain induces pathological phenotypes resembling Alzheimer's disease (PNAS 101, 6623-6628). Three-dimensional confocal imaging reveals extensive vacuoles caused by neurodegeneration in the brain of aged but not young mutant Aβ42 flies. Our purpose is to design and implement a computer method to evaluate the number and volume of vacuoles from the high resolution image of the *Drosophila* brain obtained by confocal microscopy.. The high resolution image is essential for an accurate quantitative estimation of the vacuole.

The vacuoles in slices of image are generally circular shaped dark regions. Based on this property, we design a method consists of a sequence of procedures including matched filtering, α-shape technique, and the snake method to segment the regions. Each region is presented as a closed contour in the image. Examining the overlapped regions of the contours through a user interface, we can find the boundary of the 3D vacuoles from the stacked individual contours. The volume can then be computed.

In this manuscript, we present some preliminaries of the techniques employed in the proposed method, i.e., *α-shape technique, the active-contour,* and *the match filter* in Section 2. The method is also described in this section. We then present the result in Section 3. The experimental results are presented in Section 4.

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2. A SEMI-AUTOMATED METHOD TO EVALUATE THE NUMBER AND VOLUME OF VACUOLES

2.1. Preliminary

Before we describe the method, we briefly present the techniques, including the α-shape, the matched filter, and the snake method, that employed in the proposed method.

2.1.1. Matched filter

Match filter is a frequently used filter to enhance interested features. The details of the match filter can be found in [4].

Given two images f and g, $\max_A |f-g|$ 、 $\iint_A |f-g|$ and $\iint_A (f-g)^2$, are metrics used to evaluate the similarity between the two images. If $\iint_A (f-g)^2$ is the selected metric, the similarity can be divided into two parts as shown in the following equation

$$\iint_A (f-g)^2 = \iint_A f^2 + \iint_A g^2 - 2\iint_A fg \qquad (6)$$

where f and g represent the input image and target image respectively and A is the region of interest. Since f and g are fixed so the similarity is determined by the last term $\iint_A fg$. By Cauchy-Schwartz inequality

$$\iint_A fg \le \sqrt{\iint_A f^2 \iint_A g^2} \qquad (7)$$

and with equality only when $g = cf$, where c is a constant. Applied the concept to the practical usage, if we want to find a feature f on g and determined by $\iint_A fg$, then we can rewrite (7) as

$$\iint_A f(x,y)g(x+u,y+v)dxdy \le \sqrt{\iint_A f^2(x,y)dxdy \iint_A g^2(x+u,y+v)dxdy} \qquad (8)$$

Let $g = f + n$, where n is the noise. Applying the mask h on g, we have $g' = h * g$ and $n' = h * n$ and by the *match filter theorem*, we have the conclusion that in order to reach the best signal-noise-ratio, the mask we choose should be the same as the feature f itself.

2.1.2. α-shape

The concept of alpha-shapes developed by Edelsbrunner and Műcke [2] formalizes the intuitive notion of "shape" for spatial point sets. The alpha-shape is a mathematically well-defined generalization of the convex hull and is a sub-graph of the Delaunay triangulation. Given a finite point set, a family of shapes can be derived from the Delaunay triangulation of the point set: a real parameter positive α controls the desired level of detail.

Given a set of points in 2D or 3D space, the shape of the set of points is defined by the α, Intuitively, if $\alpha = \infty$, the shape of the set of points is the convex hull of the points set. As α decreases, the shape becomes concave or even

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broken. If α =0, the shape is a set of disconnected set of point. The following figure shows the shapes of a set of point in different α value.

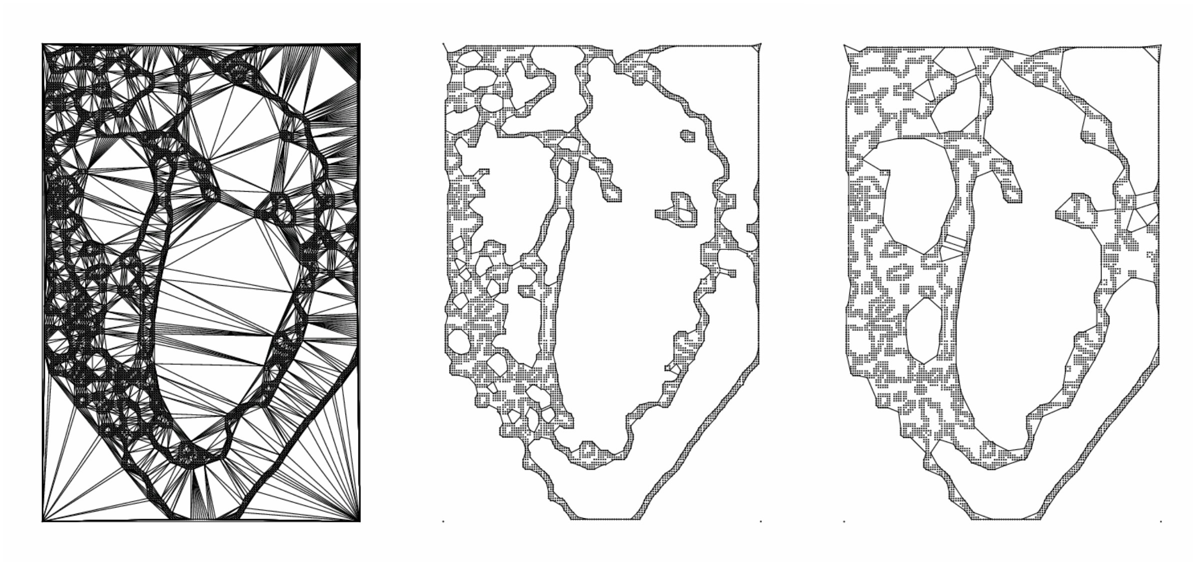

Figure 1(a): $\alpha = 0.0$ Figure 1(b): $\alpha = 5.0$ Figure 1(c): $\alpha = 10.0$

In this work, we used the α-shape technique to connect the set of boundary points to form a closed contour of the region of interested. Given a set points representing the region of interest or a set of boundary points, we compute the Delaunay Triangulation of the set of points. And the α-shape where α was set to x is computed. The α-shape is stored in doubly connected edge list (DCEL) data structure. DCEL data structure supports operations that report the edges around a region in clockwise or counterclockwise direction. We report the region that has the largest number of edges around it as the boundary of the region of interest. Using the α-shape technique to compute the boundary has the advantages that 1. it handles the region of very complex shapes, and 2. it can handles the case where there are broken edges (by given a larger α).

2.1.3. Active-contour

Active-contour method, or the *Snake* method [3], is a method that has curves defined within an image domain that can move under the influence of internal forces coming within the curve itself and external forces computed from the image data. The internal and external forces are defined so that the active-contour will conform to an object boundary or other desired features within an image.

There are two general types of active-contour models: *parametric active contour* [1] and *geometric active contour* [5]-[7]. In this paper we focus on the parametric active-contour. A traditional active-contour is a curve $x(s) = [x(s), y(s)], s \in [0,1]$, the moves through the spatial domain of an image to minimize the energy functional

$$E = \int_0^1 \frac{1}{2}[\alpha \,|\, x'(s)\,|^2 + \beta \,|\, x''(s)\,|^2] + E_{ext}(x(s))ds \qquad (1)$$

where α and β are weighting parameters that control the active-contour's continuity and smoothness (curvature), respectively, and $x'(s)$ and $x''(s)$ denote the first and second derivatives of $x(s)$ with respect to s. The external energy function E_{ext} derived from the image so that it takes on its smaller value at the features of interest, such

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as boundaries. Given a gray-level image $I(x, y)$, it can be viewed as a function of continuous position variables (x, y), typical; external energies designed to lead an active-contour toward step edges [1] are

$$E_{ext}^{(1)}(x, y) = -|\nabla I(x, y)|^2 \quad (2)$$

$$E_{ext}^{(2)}(x, y) = -|\nabla[G_\sigma(x, y) * I(x, y)]|^2 \quad (3)$$

Where $G_\sigma(x, y)$ is a two-dimensional Gaussian function with standard deviation σ and ∇ is the gradient operator. It is easy to see from these definitions that larger σ's will cause the boundaries to become blurry. A large σ is necessary, however, in order to increase the capture range of the active contour.

An active-contour that minimizes E must satisfy the Euler equation

$$\alpha x''(s) - \beta x^{(4)}(s) - \nabla E_{ext} = 0 \quad (4)$$

This can be viewed as a force balance equation

$$F_{\text{int}} + F_{ext}^{(p)} = 0 \quad (5)$$

where $F_{\text{int}} = \alpha x''(s) - \beta x^{(4)}(s) - \nabla E_{ext}$ and $F_{ext}^{(p)} = -\nabla E_{ext}$. The internal force F_{int} discourages stretching and bending while the external potential force $F_{ext}^{(p)}$ pulls the active-contour toward the desired image edges.

2.2. Procedures of the proposed method

The procedures are stated in the following.

2.2.1 Finding the candidates of the vacuoles

Multi-scale Matched filters with appropriate masks were first applied to the volume images. After many experiments, the threshold for the response of the matched filters that presents the vacuoles in the image was determined. The threshold that worked for most of the data sets was a gray level intensity of the top-twenty-percent of the voxels in the volume. After the threshold was applied, regions of connected pixels were obtained. Among these, some were the vacuoles but some of those were noises. In order to have the threshold worked for most of the cases, the threshold was set slightly higher so that the region obtained was a little bit smaller than the true region.

2.2.2 Calculate the contours for the vacuoles

The α-shape technique is applied to find the contours enclosing the candidates obtained in the previous step. To determine whether a contour is the boundary of a vacuole, we applied the knowledge from the experts. A contour encloses a vacuole if 1. the interior has low intensity since the neuron cell is dead, and 2. the neighboring voxels exterior to the contour have bright intensity since vacuoles must be in the interior of the brain. Thus, suppose that we store the contour in counterclockwise direction, the voxels to the left of the contour have low intensity while the voxels to the right of the contour have high intensity. Based on this property, we determine the contours enclosing the vacuoles and eliminate noises. It is not the case that all the contours meet the conditions are the vacuoles. Therefore, user interventions are still needed to eliminate the noises.

2.2.3 Improve the accuracy of contours by using active-contour method

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Since the contours obtained from the connected voxels determined by using the matched filter are smaller than the true regions, the contours could not accurately describe the boundary of the vacuoles. Fortunately, these contours can serve as very good initial contours for the active-contour method. We apply the active contour method to refine the contour so that a accurate contour could be obtained. Once accurate contours are obtained, we can then calculate the accurate volume of the vacuoles.

2.2.4 Calculate the area and volume for the vacuoles

The dimensions of the voxels are knows. Thus the total number of pixels enclosed by a contour reflects the area enclosed by the contour. This number was calculated by a simple region growing method. In order to compute the volume, we have to determine the contours between slices that belong to the boundary of a vacuole. Two contours in the consecutive slices belong to a vacuoles if vertical projection of a contour to another slice intersect the other contour, and the area of the intersection is sufficiently large. This is determined by the both ratios of the area of the intersected region enclosed by the two contours. If the ratio is larger than a gien threshold, the pair of contours belong to the same vacuoles.

3. CONCLUSION

We presented a method to semi-automatically pick out the vacuoles. The experimental results show that the method could achieve good result. The method has an advantage that it requires very few human interventions. We have applied this method to *Drosophila* brain image obtained by using the confocal microscope with *xy* resolution is 1024 by 1024. The algorithm that improves the accuracy of the computed contour is still under development.

4. FUTURE WORKS

Comparing to the resolution in *xy* direction, the resolution on *z*-dimension is low, e.g., the *x*, *y* and *z* ratio of our experiment data is 1:1:6.25. So the volume can only be roughly calculated. To solve the problem and other problems e.g. restoration and reconstruction of the volume data, induced by the low resolution on z-dimension is currently be studied.

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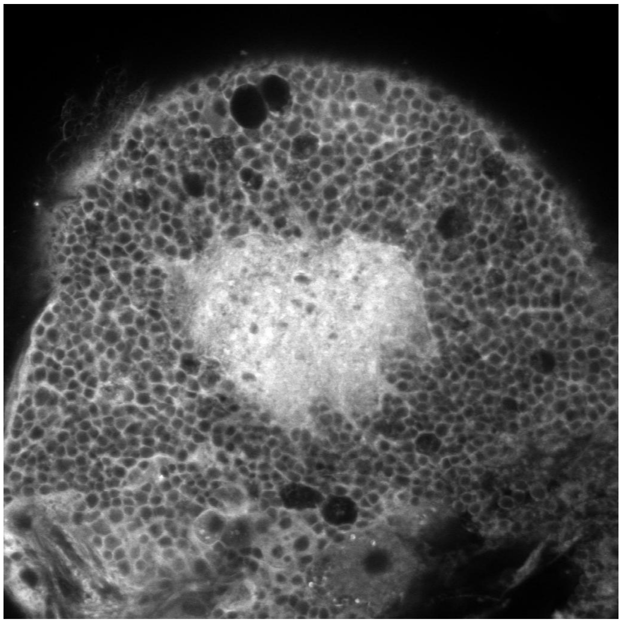

Figure 2(a)

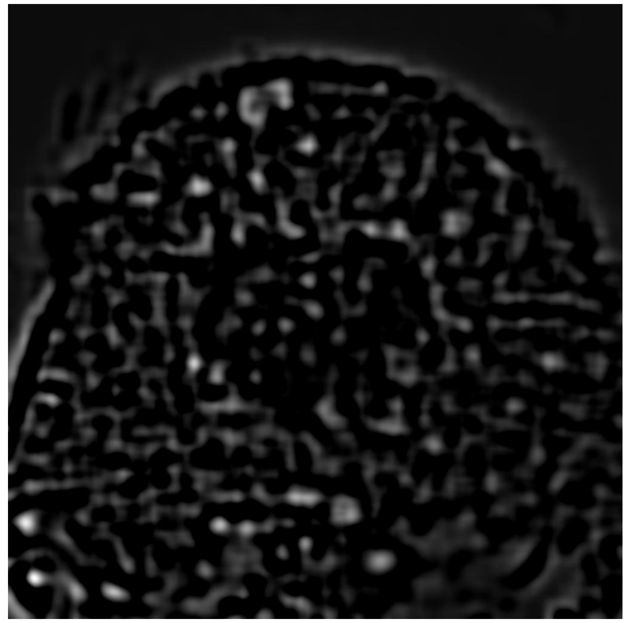

Figure 2(b)

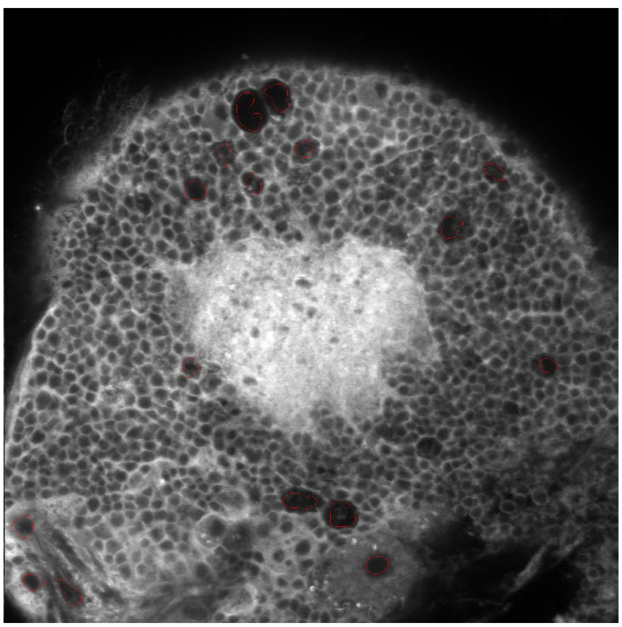

Figure 2(c)

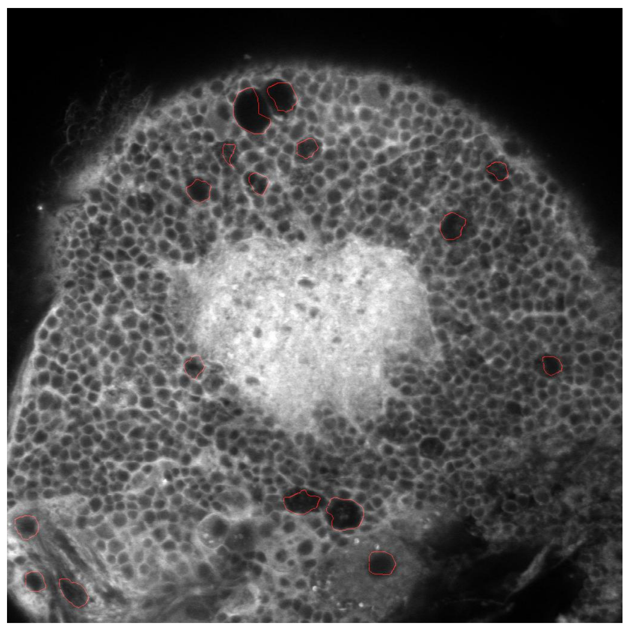

Figure 2(d)

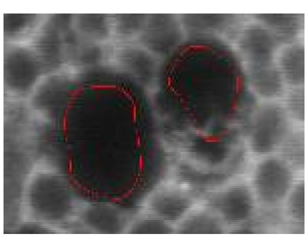

Figure 2(e)

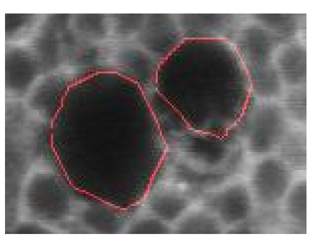

Figure 2(f)

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Figure 2(a) – (f). (a) The original input image. (b) After matched filter applied (c) Initial contour derived by α-shape algorithm (d) Result after contour deformed by active-contour . (e) and (f) Some details about the upper vacuoles initially and after deformation, respectively.

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