Detection and Tracking of Axons is Neuronal Images

Internship Advancement Report

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1 Introduction

The goal of this internship is the analysis and classification of neurons based on their axonal patterns. In particular, the project is focused on the study of normal and mutated Drosophilia fly's neurons. The mutated flies are obtained by suppressing certain genes on the normal ones. These genes are related to neurological diseases in humans.

For this purpose, biologists have acquired two types of image sequences that need to be processed and analyzed:

- Static 3D images of mature neuron axons using confocal microscopy.
- Dynamic 3D+t image sequences of neurons developing within intact fly's brains using fluorescent bi-photon microscope.

Due to the high volume of generated image data and the tortuous nature of the axons, manual processing is infeasible. Therefore, it is necessary to develop techniques for the automatic extraction and analysis of the neuronal structures. However, since both types of images present different characteristics, two different methodologies need to be developed.

The long-term goal of the project is to, given that the axon's morphology is clearly linked with the number of partners a neuron can have, derive and explain some functional behavior of each of the considered classes of neurons. This would allow us to gain a greater insight into certain neuronal diseases such as the Fragile X syndrome.

2 Current Progress

2.1 Static Images

For the static case, a first approach for extracting the axonal tree was proposed in [1]. It consists in denoising the images before applying a binarization and skeletonization step. A gap filling step based on a tensor voting approach is later performed, giving a graph description of the axon. Finally, the end and bifurcation points are detected. Moreover, in order to provide greater accessibility to non-expert users (such as biologist), the image processing algorithms were integrated into an easy to use software (implemented on Matlab).

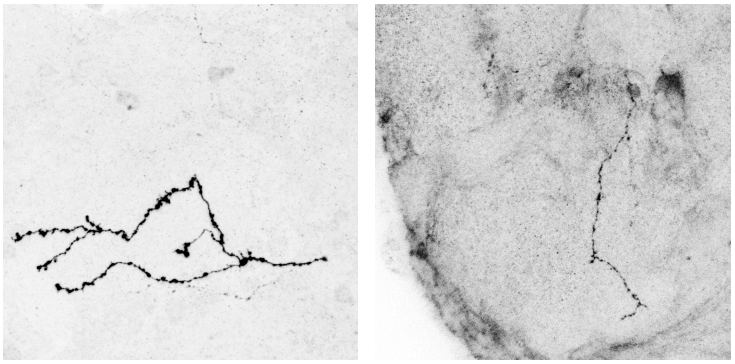

Figure 1: Axonal tree or normal (left) and mutant (right) neurons. One can appreciate the differences between the two.

Although the previously proposed solution obtained good results, its main drawback is its inability to process 3D data. This restricts its usefulness in performing morphological analysis of axons since a 2D analysis does not give crucial information on the axon morphometry and topology. In particular, some of the branching points detected in the 2D images might not correspond to actual branching points of the axon. Moreover, the distance between branching points is not accessible on 2D projections.

Therefore, we concentrated part of our efforts in extending this software to support 3D images.

At the moment, all except the end and bifurcation point detection algorithms have been extended to the 3D case. Preliminary results suggest the superiority of the 3D method.

2.2 Dynamic Images

In the dynamic case, we work on a sequence of 3D volumes containing a population of axons in the process of growing. In these images, axonal extremities appears as bright spheres which must be automatically detected.

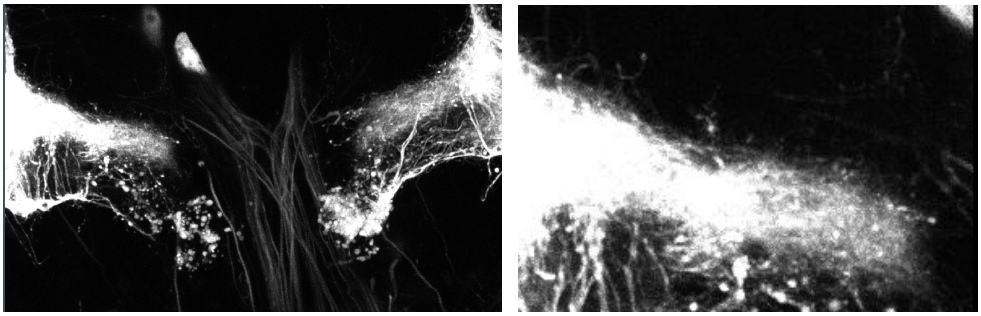

Figure 2: Maximum intensity image for one frame of a 3D+t sequence. Original image (left) and zoom (right) on a section containing the axonal extremities.

The selected detection method is Marked Point Process [2], a statistical framework which has been applied to the detection of objects in different image processing problems. Its main advantages are that the number of objects to detect can be unknown, and that geometric constraints on the objects can be modeled.

A point process Y in P is a random variable whose realizations are random configurations of points, where $P = [0, I_{max}] \times [0, J_{max}]$ is given by the image support. Moreover, a marked point process living in $S = P \times K$ is a point process where some marks in K are added to the position of the points in P. The marks are some parameters that fully describe the objects. In particular, spheres are fully described by the position of their center and their radii. Under this approach, the images are modeled as composed of axonal extremities (spheres) whose positions and attributes are some realization of a marked point process X.

The probability distribution P_X of the marked point process can be expressed using a Gibs energy formulation:

$$P_X(x) = \frac{1}{Z} exp(-U(x)) \tag{1}$$

where $U(x)$ is the energy of configuration x and Z is the normalizing constant. The objective is to design this energy in a way such that it is minimized by the axonal extremities extraction. To accomplish this, the energy is divided into two terms: a data term, fitting the objects (spheres) onto the image, and a prior term constraining the configuration by, for example,

forbidding overlaps between objects.

$$U(x) = \alpha U_p(x) + \beta U_d(x) \tag{2}$$

Different data terms can be used. In particular, we are using a modified Bhattacharya distance between each sphere of radius r (each object of the realization) and an extended crown of radius between 1 and $r + \rho$.

As a prior term, an overlap coefficient between objects is used. This term penalizes (but does not forbid) the overlap of spheres.

Due to the intricate nature of $U(x)$, usual minimization algorithms cannot be applied . Therefore, the solution will be obtained by optimizing the model with a recently proposed algorithm called multiple births and deaths dynamics [3]. In this approach, multiple objects are added to the current configuration with a given probability (referred to as birth probability). Later on, a new configuration is obtained by removing (killing) some of the objects with a different probability (death probability). This process is repeated iteratively until convergence. In addition, the birth and death process is embedded in a simulated annealing scheme to improve its performance.

This algorithm has been proven to solve the object extraction problem and to outperform the convergence speed of other methods such as RJMCMC (Reversible Jump Markov Chain Monte Carlo). Moreover, the combination of these two approaches (marked point process and multiple birth and death) has already been successfully applied to the detection of different objects such as flamingos in aerial images [4].

At the moment, we have implemented the algorithm for the case of spheres. Moreover, we have tested its performance on 3D synthetic images with different levels of noise by calculating the number of missed objects (MO), the number of false detections (FD) and the mean and standard deviation of the Jaccard Similarity Coefficient [1] between the real and the detected spheres (MJ and STDJ respectively).

[1] $J(A, B) = \frac{|A \cap B|}{|A \cup B|}$

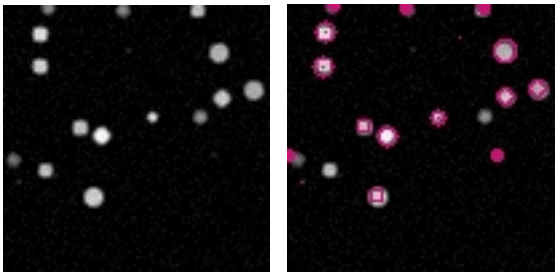

Figure 3: Slice from the original (left) and resulting (right) images. The original image contains additive Gaussian white noise with zero mean and variance equal to 0.001.

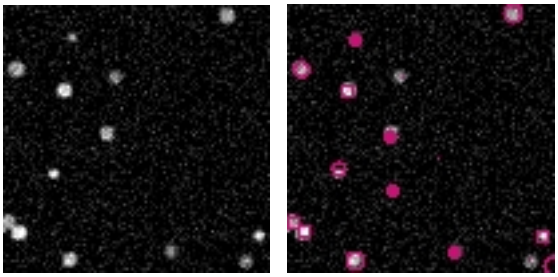

Figure 4: Slice from the original (left) and resulting (right) images. The original image contains additive Gaussian white noise with zero mean and variance equal to 0.01.

Noise Variance	NM	FD	MJ	STDJ
0.001	0	2	0.46	0.19
0.01	3	3	0.38	0.24

Table 1: Evaluation results for the two images

These preliminary results suggest the potential use of this method in the detection of the axonal extremities.

3 Future Work

3.1 Static Images

As mentioned before, only the end and bifurcation point detection algorithms remain to be extended to the 3D case. In addition, the new implementations of the algorithms should be integrated into the existing software along with an improved visualization system to allow for 3D images.

Once the new software is complete, it could be used to process a large number of images in the hopes of finding relevant statistical differences between the two groups of neurons.

3.2 Dynamic Images

Although at the moment the data term of the total energy is calculated using the Bhattacharya distance, alternative methods could be used. In particular, we plan on testing the performance obtain by performing t-tests between the spheres and the crowns. Another related alternative would be to use the A-Contrario framework [5]. Finally, we could extend the work by Graziani et al. [6] on thin object extraction from the 2D to the 3D case.

In addition, the prior term could also be improved. The first step would be to extend it to allow for the detection of ellipsoids. This could improve the results. Moreover, more complex models could be used, such as including the tail of the axons in the model.

Once the static problem (detection of spheres in each frame of the video) has been solved, the solution should be embedded into a tracking algorithm to obtain an estimation of the axon's trajectory during the growing stage.

Due to time constraints, it is unclear if we will be able to complete all the required steps to solve the tracking problem. However, we are confident that, given the time left, we will be able to reasonably solve the static case by the end of this internship.

References

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