EXTRACTION OF 3D MICROTUBULES AXES FROM CELLULAR ELECTRON TOMOGRAPHY IMAGES

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Abstract

Microtubules are structural and motile elements. They are essential in numerous cell processes. Their function will be greatly improved by our understanding of their molecular structure, which requires extracting microtubules from the Cellular Electron Tomography Images. Manual segmentation and measurement of the microtubules from 3D dataset may require several man-hours of work, and the manual approach suffers from inter-observer and intra-observer variabilities.

Localizing microtubules axes is an important step for the successful segmentation. In this paper, a graph searching based technique for extracting central axes of microtubules from three dimensional cellular electron tomography images is presented. A tube enhancement filter is introduced in order to define a distance metric for graph searching. The results are promising, indicating that accurate measurements can be obtained in a semi-automatic fashion.

1 Introduction

All animal and plant cells contain several systems of thin protein filaments, including microtubules. Microtubules are structural and motile elements and are essential in numerous cell processes. Their function will be greatly improved by our understanding of their molecular structure, which requires both extracting microtubules from the kinetochores reconstruction volumes and comparing them in equivalent orientation.

Currently, volume segmentation in electron tomography is almost exclusively a manual operation. As a result, segmentation is often the most time consuming and subjective step in the process. For the kinetochores, it takes about 3-4 hours to segment each microtubule from the reconstruction volume and another 2 hours to trace the connections. Therefore, improving the accuracy and efficiency of volume segmentation will have enormous impact on the precision and throughput of the process.

No mater what kinds of methods we use, localizing tubes' axes is an important step for successful segmentation. Based on the extracted axes, a 3D active surface can be constructed that can be used to fit the volume data and achieve a good segmentation results [9]. Also, an axis may become good seeds for region/volume growing methods [8, 2]. In this paper, we concentrate on 3D axis extraction. Subsequent work will involve extracting the microtubules structures based on the identified axes. However, in cellular electron tomography the reconstruction volumes are usually filled with objects that contain a full range of gray-scale values such that the object of interest does not stand out from its surroundings. This low contrast makes axes extraction a difficult task.

The main objective of this work is to develop an semi-automatic method for microtubules axes extraction using graph searchig. A tube enhancement filter is introduced in order to define a distance metric for graph searching. This filter enhances tubular structures while reducing the effect of other morphologies.

2 Method

The proposed technique consists of two main components: Firstly, a tube enhancement filter is used, which defines tube confidence measurement for each voxel. Then, user manually specifies two voxels, which mark the starting and ending point of the microtubule axis. These two points actually can determine a subvolume. In this subvolume, our method randomly selects some points, which become points of graph. After that, a graph searching algorithm is applied to these points, and the shortest path based on a specific distance metric corresponds to the the central axis of the microtubule.

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2.1 Microtubules Enhancement Filter

Since the low contrast of volume data, it typically needs a preprocessing before segmentation. Microtubules are the tracks along which kinesin and dynein motors move. Their tube structure is specical, and enhancing the tube structure will improve the segmentation accuracy. Tubular structure enhancement in 3D images has been investigated by many researchers[1, 6, 4]. The approach we discuss in this paper is inspired by the work of Sato *et al.*[7] and Frangi *et al.*[4], who use the eigenvalues of the Hessian to determine locally the likelihood that a tube is present. We apply this method to our problem, i.e. microtubules extraction.

The basic idea is that. Let H be the Hessian matrix at a given voxel x.

$$H = \begin{bmatrix} I_{xx} & I_{xy} & I_{xz} \\ I_{yx} & I_{yy} & I_{yz} \\ I_{zx} & I_{zy} & I_{zz} \end{bmatrix}$$

where $I_{\alpha\beta}$ denote derivatives of the volume $I(x)$, which are obtained by convolving the volume with the derivative of the Gaussian kernel at scale σ.

$$I_{\alpha\beta}(x) = \sigma^2 \frac{\partial^2 G(x,\sigma)}{\partial\alpha\partial\beta} * I(x)$$

$$G(x,\sigma) = \frac{1}{\sqrt{(2\pi\sigma^2)^3}} exp(-\frac{\|x\|^2}{2\sigma^2})$$

λ_k is the eigenvalues of Hessian matrix, and let $|\lambda_1| \leq |\lambda_2| \leq |\lambda_3|$. Different eigenvalue combinations correspond to different structures. In particular, a voxel belonging to a tube region will be illustrated by λ_1 being small and λ_2, λ_3 being large and of equal sign. Based on these observations, a filter that enhances tubular structures while reducing the effect of other morphologies was developed[4]. The filter can be expressed as

$$\varphi(x,\sigma) = [1 - exp(-\xi^2)]exp(-\eta^2) \quad (1)$$

where $\xi = |\lambda_2|/|\lambda_3|$,$\eta = |\lambda_1|/\sqrt{|\lambda_2\lambda_3|}$. The property of this filter is that it achieves the maximus at the center of the tube, while the filtered response decays smoothly toward the boundaries. Equation (1) explicitly states that the filter response is a function of the scale at which the Gaussian derivatives are computed. The filter is applied at multiple scales that span the range of expected tube widths. In order to provide a unique filter output for each voxel, the multiple scale outputs undergo a scale selection procedure. This amounts to computing the maximum filter response across scales.

$$\phi(x) = \max_{\sigma_{min} \leq \sigma \leq \sigma_{max}} \varphi(x,\sigma) \quad (2)$$

The output value of the filter is in the range of $[0, 1]$, that in fact is a tube confidence measure. This behavior is desirable since it may help to locate the central axis of a tube.

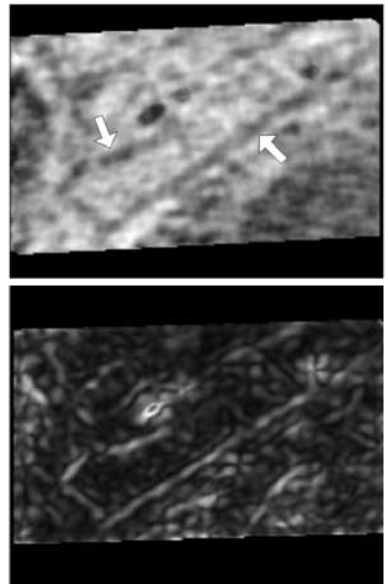

Figure 1. (a) original volume, with microtubules indicated by arrows. (b) enhanced volume

Figure 1 shows the effect of filtering. We can see that, after filtering, the tube structure becomes much clearer, and the contrast with surroundings is sharper.

2.2 Graph Searching

Based on the enhanced volume, we apply Graph searching algorithm to localize the central axis of the tube[3]. The problem of finding a tube axis thus becomes a simple minimal-distance path, graph-searching problem. There are four basic subparts when using graph searching: how to choose starting and ending points; how to construct the graph; how to assign distance; and how to find the minimum-distance path. We will discuss all these issues in details.

2.2.1 Selecting Start/End Points

There are three common ways to select start and end points.

- Use some known special points.
- Find landmark points that have certain special geometric properties.
- Ask the user to locate points

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In this method, we just let users select the starting and ending points, more automatic selection will be done in the future work.

2.2.2 Graph Formulation

With the starting point s and ending point e, we know roughly where the tube lies. We randomly generate 2000 points in this range, and all these points will become points in Graph. The random points generation method is as follows.

First determine 100 basic points, $(x_1, x_2, \ldots, x_{100})$ which uniformly distribute along the line between s and e. Next, for each basic point x_i, we randomly generate 20 points, $(y_1, y_2, \ldots, y_{20})$. y_i conform to the distribution $N(x_i, \bar{\sigma}^2)$, where $\bar{\sigma} = 5$ in our experiments.

The basic principle of how to select points for Graph is that these points should be able to cover the tube, so that the generated microtubule's axis does make sense. Typically, the more points we use, the better the result. However, regarding the computing efficiency, we can not use too many points for the Graph. In the preliminary stage of our project, we just use this simple strategy to construct graph. More sophisticated scheme will be adopted in the future.

2.2.3 Distance Function

The choice of the distance function $c(u, v)$ determines what kind of paths will be found by the graph search algorithm. In our case, the distance metric should be able to reflect the desired property of tube axes. We want to extract the axis that passes as more voxels with high tube confidence as possible, as well keeps as shorter as possible in the sense of Euclidian distance. So, the distance function from point u to point v is like that:

$$c(u, v) = \frac{\|u - v\|}{\phi(u) + \phi(v)} \tag{3}$$

where $\phi(.)$ is the tube-confidence function defined by Equation 2. If both the voxel u and the voxel v are near the tube axis, the confidence $\phi(u)$ and $\phi(v)$ are high, which lead the path between u and v have shorter distance metric.

2.2.4 Graph Searching

Once users have selected a start point and and an end point, and generated all points for the Graph, the shortest path from the start point s to the end point e is computed using Dijkstra's algorithm[3, 5]. This algorithm effectively computes the shortest paths from all points to the start point s. The basic idea of the algorithm is to spread from start point s and label other points in order of their distances from s. Each point u has a label $d(u)$. The label is fixed once we know that it represents the shortest distance from s to u. Firstly, all labels are set to infinity except for the start point which is set to zero. A sorted list L contains all points with non-fixed labels. Initially these are all points. From this list we take a point u with minimal accumulated distances, make its label permanent and check, if it is favourable to reach non-fixed neighbour points v via u.

Before using the Dijkstar's algorithm, we construct a neighborhood graph, that can simplify the computing. Since we use a series of segments to approximate the central axis, which is in fact a smooth curve, so the neighborhood region can not be to wide.

The pseudo-code of Graph Searching algorithm is as follows.

1. Construct neighborhood graph

   Define the Graph G over all data points by connecting points i and j if i is one of the K nearest neighbors of j. Set edge lengths equal to $c(i, j)$ according to Equation 3.

2. Compute shortest path(Dijkstra algorithm)

```
d(s) = 0 and d(u) = ∞ for u ≠ s;
L=all points u;
while L not empty do
    for each edge u → v with v ∈ L do
        if d(v) > d(u) + c(u, v) then
            d(v) = d(u) + c(u, v)
        end
    end
    remove u from L
end
```

The output of the algorithm is an approximation to the central axis of the microtubule.

3 Experiments

3.1 Image Acquisition and Implementation

For our preliminary studies, the method has been tested using cellular electron tomography images of kinetochores. The kinetochores data consists of several microtubule ranging in size from 4 to 7 pixels in diameter. This method is implemented in Matlab.

3.2 Results

Figure 2(a) illustrates the result of an extracted tube axis that was superimposed on a slice image of output of the enhancement filter. Figure 2(b) presents 3D shape of the

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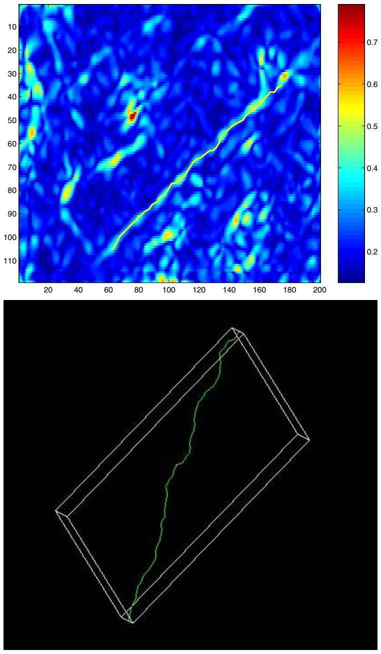

Figure 2. (a) A result of extracted central axis that was superimposed on a slice image of output of enhancement filter. (b)3D view of the extracted central axis.

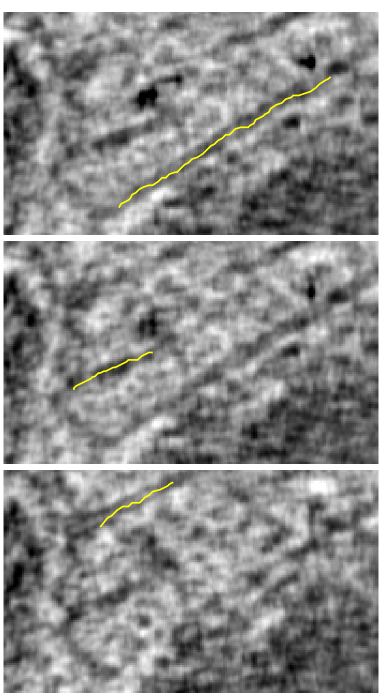

Figure 3. More experimental results of microtubules axes extraction, with identified axes superimposed on the original images

tube axis. In this data, the difficulties arise from a lack of contract between the microbutules and surrounding structures, as shown in Figure 1(a). More experimental results are shown in Figure 3. Our method successfully extracted axes of microtubules, and a visual evaluation, by an expert in the domain, assured us that the extracted axes were of quality.

4 Conclusion

In this work we have presented a graph searching method for extracting microtubules axes from three-dimensional electron tomography images. The contribution of this work is that we introduce a tube enhancement filter, and define a domain specific distance metric for graph searching. The results are promising, indicating that accurate measurements can be obtained in a semi-automatic fashion.

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