

Annotation of Microtubule and Membrane in Cell Tomography images

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Abstract—Electron cryo-tomography(cryo-ET) is an important tomography technique used by biologists in determining high resolution 3-D sub-cellular structures. In CryoET, biologists often collect images of crowded cell contexts at relative high electron dose. Although the contrast of subcellular feature such as microtubule and membrane is relatively good for visualization, the reconstruction of these subcellular features relies on accurate and fast feature annotation. Though manual annotation of subcellular features with digital pen is the most accurate method so far, this method no longer suits the need considering the large dataset and fast data collection technique. Here I implemented the algorithm and assessed its performance on cryoET neuron cell tomograms. I found that the algorithm has moderate performance on microtubule segmentation but low performance on membrane segmentation. Considering that several thresholds and parameters need to be tuned for good performance, user interface based on this algorithm would be very useful in the future.

Keywords—cell tomography, annotation, microtubule, membrane, eccentricity.

I. INTRODUCTION

Electron cryo-tomography(cryo-ET) is a popular tomography technique used by biologists in recent years to determine nanometer resolution 3-D sub-cellular structures. In cryo-ET, a set of images (tiltseries) for each specimen area is collected by tilting the specimen stage through a range of angles. Each tiltseries can then be computationally reconstructed into a 3D tomogram representing the 3D structure of the imaged area. The cumulative high electron dose (70 e/A²) is sufficient to resolve cellular organelles and identify large macromolecular complexes[1]. The main method researchers use in reconstructing the 3D volume of the sample starts with manually annotating various features such as cytoskeletal filaments, cell wall elements and internal compartments in each 2D image slice. In particular, lots of efforts are devoted in annotating membranes and the microtubules.

Considering the large number of slices in each 3D volume, low efficiency becomes the biggest challenge in manual annotation. Although remaining as the most accurate method, the concern of this low efficiency becomes more serious when data collection is speeding up. Here I propose an algorithm for microtubule and membrane annotation based on their curvature difference. The goal is to recognize all of the microtubules and membranes in the image and label them in different colors.

II. DATASET

The data is a neuron cell tomogram in size of (442, 960, 960) from Chiu lab at Stanford. This data is acquired at relative high magnification on cryo-electron microscopy. The tomogram has membranes and microtubules as the main subcellular features. I will use a few slices from this tomogram for testing the algorithm. I will implement, test and compare different strategies to identify microtubules and membranes.

III. ALGORITHMS

A. Image Preprocessing.

For convenience of image processing, the grayscale 2D slice images of tomogram are inverted to have features in white and background in dark. Besides microtubule and membrane,

molecules of various size and shape also exist in the crowded cell context. Although the contrast of microtubule and membranes is relatively high due to the high electron dose imaging procedure, the existence of non-target substances introduce lots of interference in tomogram annotation. To speed up the detection of targeted features, these small non-target features are removed with small region removal algorithm (Figure 1).

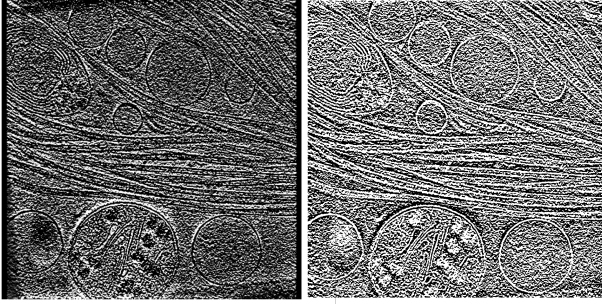


Figure 1. Representative grayscale 2D tomogram slice (left) and its corresponding inverted image (right).

B. Image Binarization and Small Region Removal.

The grayscale image is further binarized with threshold selected to exclude as much noise as possible while keeping most of the target features. To further remove the unnecessary features in the image, image erosion is performed with square structure element of size 3-by-3, followed by removal of small non-target features with thresholding (Figure 2).

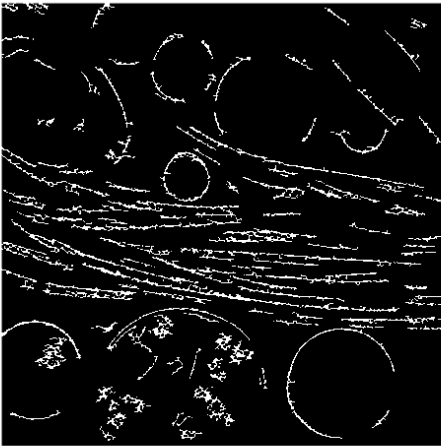


Figure 2. Image after small region removal.

C. Spurious Edge Removal

The major difference between microtubule and membrane lies in their curvatures or eccentricities. Noodle shaped microtubules can be very easily distinguished from circle shaped membranes by human. However, the accuracy of shape

estimation by algorithms for each region with eccentricity or curvature can be dramatically affected by spurious edges on skeletons of each region. To have a more accurate estimation of the major skeleton shape for two features, we removed most of these spurious edges by the following algorithm. First, the branchpoints and endpoints in the image are detected. Second, geodesic distance transform[2] of binary image respective to each endpoint is calculated. Last, spur pixels starting from each endpoint are detected by thresholding the distance to the nearest branchpoint (Figure 3).

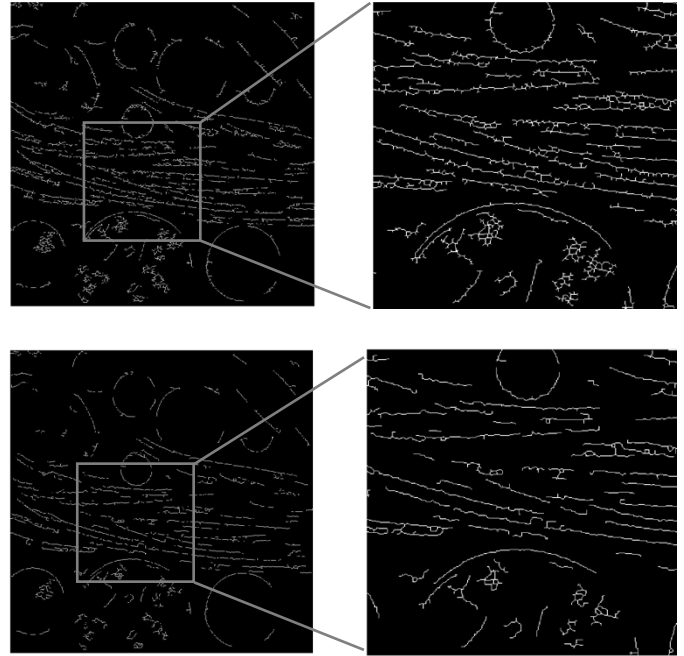


Figure 3. Skeleton features before spurious edge removal (top panel) and after spurious edge removal (bottom panel).

D. Eclipse Fit and Eccentricity Estimation of Regions.

Eccentricity is estimated for each region in the spurious edge removed image with Matlab algorithm `relionprop`, and histogram of the regional eccentricities are generated for feature classification (Figure 5). Based on the eccentricity difference for each region, we classify regions with high eccentricity to membranes and regions with low eccentricities to microtubules. We plot eclipse fit for each region and central pixel of each region as the seed for region growing (Figure 4).

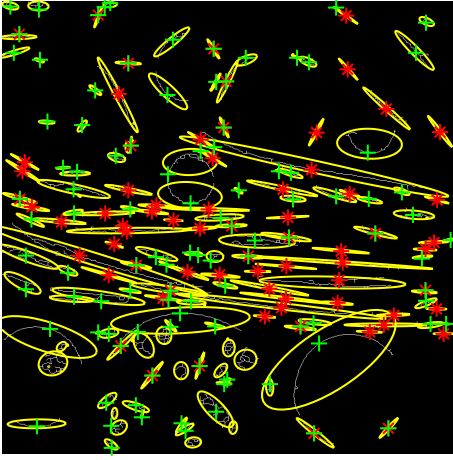


Figure 4. Eclipse fit to each region in the skeleton-featured image (shown in yellow eclipses) and central pixel of each region as the seed for region growing (regions with * are classified as microtubules while regions with + are classified as membranes).

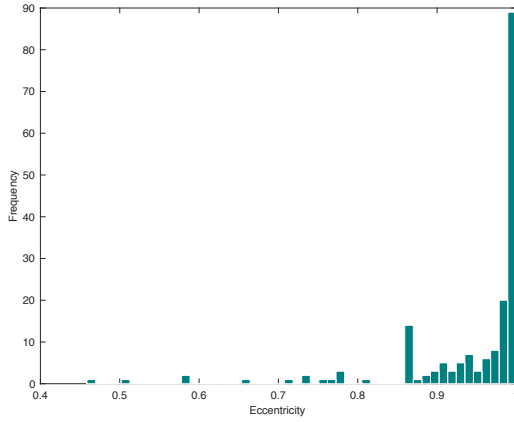


Figure 5. Histogram of eccentricity for each region in the skeleton-featured image.

E. Region growing.

To segment the microtubule and membrane features, region growing from seed method[3] is applied to the image shown in Figure 2. Compared to growing region from the original inverted image (Figure 1), the segmentation accuracy is higher in the noise removed image after image erosion. The seeds for region growing are the central pixels in each region from spurious edge removed image (Figure 3 bottom panel). Regions are recovered by region growing method with 8-connected neighborhood. The recovered regions are classified to microtubules and membranes based on seed source (Figure 6).

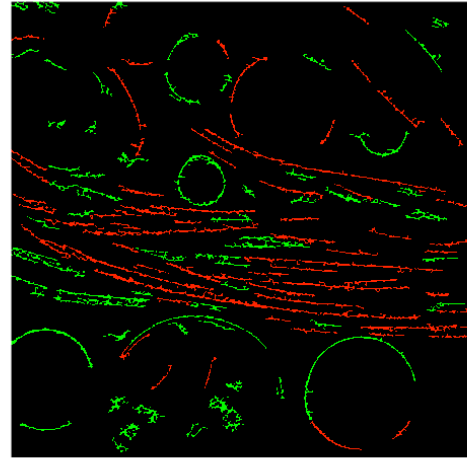


Figure 6. Recovered features from the noise reduced image (Microtubules are shown in red while membranes are in green).

IV. ACCURACY AND PERFORMANCE

A. Comparison to Ground Truth

The ground truth comes from the manually annotated tomograms with digital pens (Figure 7). We compared the performance of our algorithms to the ground truth. From the segmentation outputs of our algorithm, we computed the precision and recall values for each feature (Table I). We performed region growing method both on binary image after image erosion and small region removal (Figure 3) and binary image with a further step of image dilation with same structure element as image erosion. When comparing performance of these two algorithms, we observe an increase of the recall but a decrease of the precision on microtubule segmentation. This performance change could be explained by the fact that the ground truth includes some non-microtubule pixels due to large pen size used in manual annotation. Region growing on binary image after the dilation covers part of these non-microtubule pixels and therefore cause the increase of recall. In contrast, algorithm performance on segmentation of membranes is relatively low. From the segmentation output shown in bottom left of Figure 7, we can see that parts of membranes are misclassified as microtubules. The discontinuity in the membranes from the image is the main reason for this poor performance. In general, the algorithm is less robust compared to human performance in dealing with such noisy images.

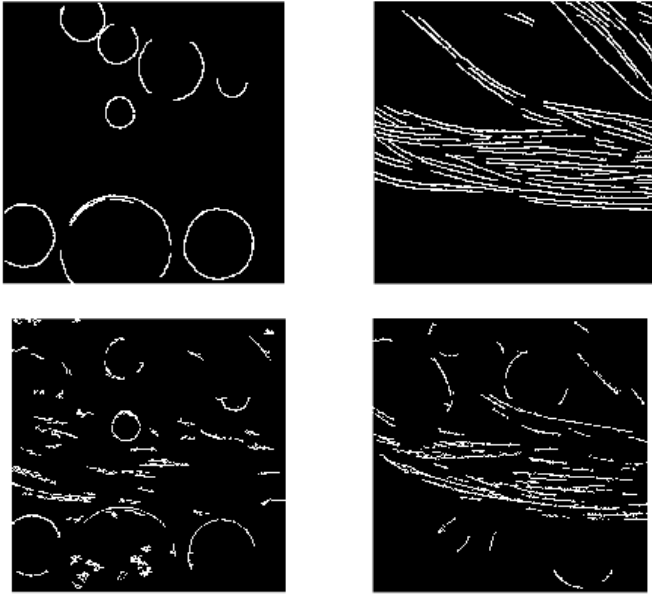


Figure 7. Ground truth for membranes (top left) and microtubules (top right) and segmented membrane (bottom left) and microtubules (bottom right) from the algorithm.

TABLE I. ACCURACY OF ALGORITHM

	<i>membrane</i>		<i>microtubule</i>	
	<i>No image dilation</i>	<i>Image dilation</i>	<i>No image dilation</i>	<i>Image dilation</i>
Recall	0.3106	0.2588	0.2757	0.4062
Precision	0.2106	0.1468	0.5419	0.4277

V. CONCLUSION

I implemented algorithm for microtubule and membrane feature segmentation in Cryo-electron tomography images. I assessed the performance of the algorithm quantitatively pixel by pixel using neuron cell tomograms with manually labeled microtubule and membrane as the ground truth. I found that the

algorithm has low performance on membrane segmentation but have moderate performance on microtubule segmentation. The biggest challenge in this segmentation task is the discontinuity of features in the image, resulting bad performance in estimation of feature shape and further in feature classification. Methods of tracing features from the noisy images is of high significance in improving the performance of this algorithm. Future directions would be to search and implement for better curve tracing algorithms in noisy image, and develop user interface for better performance of the implemented algorithm here.

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