Practical method of cell segmentation in electron microscope image stack using deep convolutional neural network

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Electron microscopy is an indispensable method to observe cells and tissues since it provides extremely high resolution images. Although electron microscopy provides valuable information, manual annotations for cell segmentation, which are time-consuming and laborious, are often required to analyze huge amount of sequential images, especially for three-dimensional analysis. Deep convolutional neural network (CNN) works well for detecting a membrane of neural structure [1,2], however, one major bottleneck of segmentation using deep learning remains: to create a large volume of training dataset.

In order to solve this issue, especially for sparse segmentation, we tried to use CNN with minimal training dataset, although manual corrections after applying CNN increases. Mouse Cerebellum cortex was fixed by glutar aldehyde and osmium tetraoxide, and sequential slices were scooped on silicon substrates cut by an ultramicrotome. Then, we automatically captured three-dimensional image stack of mouse Cerebellum cortex stained with uranyl acetate and lead citrate by Array Tomography [3]. Two scanning electron microscopes (SEMs), JSM-7800F and JSM-7900F, were used. Segmentation target is a Purkinje cell in Celebellum cortex. We created a ground truth dataset of cell membrane ten times smaller than that of conventional approach [1,2]. U-Net [2] was used as CNN for membrane detection. A GPU (NVIDIA GeForce GTX 1080) was used to speed up the processing. We proofread false merge and split of calculated results using homemade GUI tool. The time required for each process was compared to that for conventional one.

Figure 1 shows an example of an SEM image and its membrane probability map calculated from our trained model. Most part of the probability map is correct, however, some are falsely merged and split. Orange boxes inside the Figure 1 show an example of the region requiring correction (Figure 2). Figure 3 shows our segmentation result of the Purkinje cell after proofreading. In our method, creating the ground truth took 2.5 hours, learning/applying the model took 0.3 hours and proofreading took 6.5 hours. The total time was less than two working days much smaller than that for conventional method [1,2], although proofreading time is increased because of fewer training data size in our method. We conclude that we can reduce the total labor time for the sparse segmentation by reducing training dataset.

References

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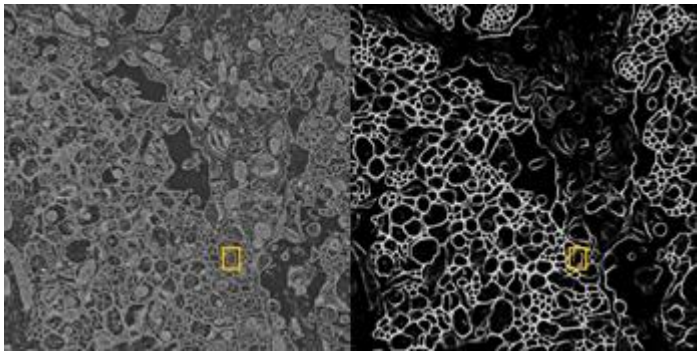

Figure 1. An example of an SEM image (left) and its membrane probability map (right). An orange box inside each figure shows a region of false merge and split.

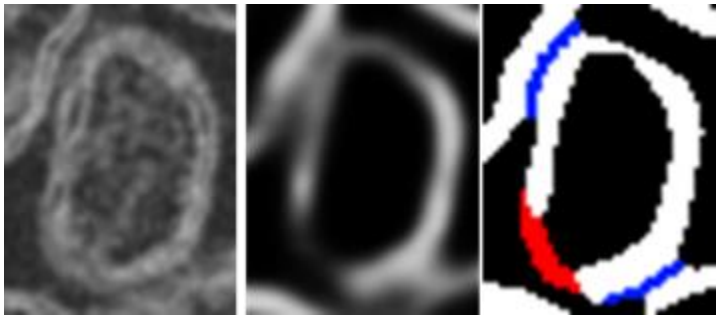

Figure 2: An example of proofreading: a magnified image of the orange box region in Figure 1 (left), membrane probability map (middle) and proofreading (right). The black, white, blue and red color in the right figure indicate true negative background, true positive foreground, false positive merge and false negative split region respectively.

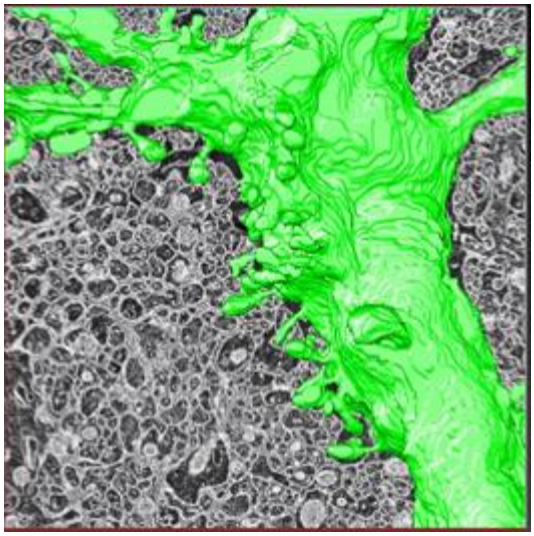

Figure 3: A segmentation result of Purkinjje cell in cerebellar cortex.