Visualizing and Modifying Difficult Pixels in Cell Image Segmentation

Daisuke Matsuzuki and Kazuhiro Hotta
Meijo University, 1-501 Shiogamaguchi, Tempaku-ku, Nagoya 468-8502, Japan

Keywords: Cell Image Segmentation, Difficult Pixels, Visualization, Modification, U-net.

Abstract: In this paper, we visualize and modify difficult pixels to recognize for deep learning. In general, an image includes pixels that are easy or difficult to recognize. At the final layer, many deep learning methods use a softmax function to convert the outputs of network to probabilities. Pixels with small maximum probability are often difficult to recognize. We visualize those difficult pixels in a test image using the relationship between confidence and pixel-wise difficulty. By visualizing difficult pixels, we confirm the connection of cell membrane that could not be recognized by conventional method. We can connect the cell membrane by modifying difficult pixels. In experiments, we use cell image of mouse liver dataset including three classes; "cell membrane", "cell nucleus" and "cytoplasm". Our proposed method shows high recall score for "cell membrane". We also confirmed the connection of cell membrane in qualitative evaluation.

1 INTRODUCTION

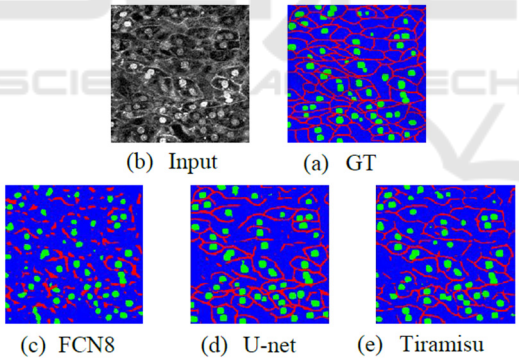


Figure 1: Segmentation results in cell image. (a) represent input image in mouse of cell image dataset, (b) is ground truth and (c), (d), (e) results of segmentation by FCN, U-net and FC-DenseNet (Tiramisu). Red area represents cell membrane, green area is nucleus and blue area show cytoplasm.

In recent years, the amount of usable data has increased in various fields. ImageNet (Russakovsky, 2015) is a big dataset includes a lot of images and classes. Most of segmentation methods use the ImageNet dataset as pre-training, and the accuracy of semantic segmentation was improved. However, the effect of pre-training with ImageNet is small in cell image segmentation because ImageNet does not include cell images. It is still difficult to prepare a large amount of cell data and ground truth. Therefore, cell image segmentation is difficult task yet.

Figure 1 shows the segmentation result by conventional methods. From Figure 1, the most of methods could not connect cell membrane well. Especially, FCN8 (Long, 2015) recognized cell membrane discontinuously. U-net (Ronneberge, 2015) is a famous method in medical image segmentation, and the accuracy is higher than FCN. Tiramisu (Simon, 2017) includes 103 convolution layers, and it is able to extract features effectively. However, it is difficult to connect cell membrane well. It is very important to recognize cell membrane in cell image segmentation. To address this problem, we propose to visualize and modify difficult pixels in segmentation result of a test image. When we predict segmentation result, various methods use a softmax function to convert the network outputs to probabilities. Each pixel in a test image is classified to the class with the maximum probability. Pixels with small maximum probability are often difficult to recognize. We use the relationship between the maximum probability among all classes and pixel-wise difficulty. We visualized difficult pixels according to the maximum probability in test phase. We confirmed that many difficult pixels are misclassified. Thus, we modified the segmentation result for a test image using the difficult pixels, and

Matsuzuki, D. and Hotta, K.
Visualizing and Modifying Difficult Pixels in Cell Image Segmentation.
DOI: 10.5220/0009164203140318
In *Proceedings of the 13th International Joint Conference on Biomedical Engineering Systems and Technologies (BIOSTEC 2020) - Volume 4: BIOSIGNALS*, pages 314-318
ISBN: 978-989-758-398-8; ISSN: 2184-4305

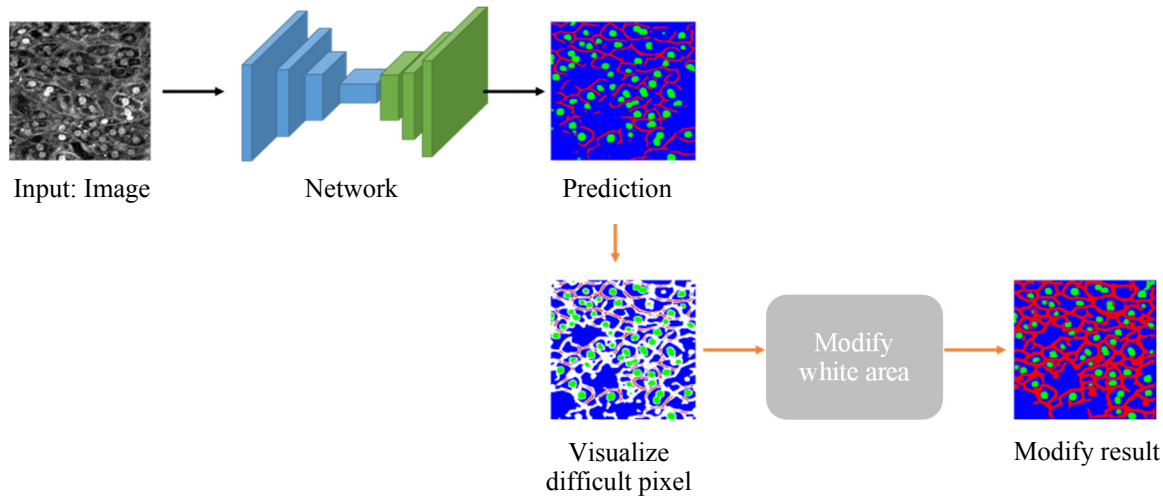


Figure 2: Overview of the proposed methods.

the segmentation result is improved. In experiments on cell dataset, we compared our method with conventional methods. We confirm that the proposed method can connect cell membrane that could not be recognized by conventional methods. Our proposed method can reduce false negative and achieved high recall scores for cell membrane.

This paper is organized as follows. In section 2, we introduce related works. In section 3, we explain the details of the proposed method. Section 4 shows experimental results. We also compare our method with conventional methods. In section 5, we describe conclusion and future works.

2 RELATED WORKS

Semantic Segmentation.
Famous semantic segmentation methods are Fully Convolutional Networks (FCN) (Long, 2015) and encoder- decoder CNN. FCN consists of convolution layers and upsampling layers to recover the spatial information. Famous encoder-decoder CNN is the SegNet (Badrinarayanan, 2017). However, small objects and correct location are vanished in encoder part. Thus, U-net (Ronneberge, 2015) used skip connections between encoder and decoder to compensate for the information.

Cell Image Segmentation.
In the field of cell image segmentation, almost of all methods used the U-net (Ronneberge, 2015). U-net++ (Zhou, 2018) shows high accuracy that introduce deep supervision and Resnet (He, 2016) architecture in backbone network. Those methods show the effectiveness of U-net architecture.

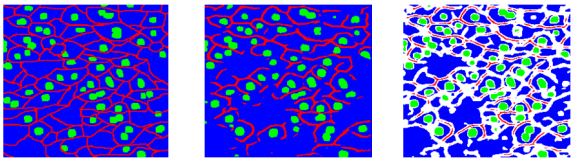
Murata et al. (Murata, 2018) proposed a segmentation method of cell membranes and nucleus by integrating different branches in U-net. Hiramatsu et al. (Hiramatsu, 2018) used a Mixture-of-Experts (Jacobs, 1991) structure with multiple U-nets. Tsuda et al. (Tsuda, 2019) used multiple pix2pix (Isola, 2017) for each class. Those methods improved the accuracy on Intersection over Union but could not get connection of cell membrane in difficult cell image dataset.

In this paper, we visualize the difficult pixels in a test image and improve the accuracy of cell membrane that conventional methods cannot segment well.

3 PROPOSED METHODS

Our goal is to get connection of cell membrane well. To achieve the objective, we modify difficult pixels. Figure 2 shows the overall architecture of our method. First, we predict results using CNN in test phase. This process is shown as a black arrow in Figure 2. We use U-net in this paper. The predicted result is used as a segmentation result in conventional methods. However, as shown in Figure 1, it could not get the connection of cell membrane well.

We would like to modify difficult pixels in the segmentation result. When we predict segmentation result, various methods based on CNN apply a softmax function to convert the outputs of network to probabilities. Then, each pixel is classified to the class with the highest probability. Pixels with small maximum probability among all classes are often difficult to recognize. Therefore, small maximum probability shows low confidence of prediction.



GT Prediction Visualize difficult pixel

Figure 3: Visualization of difficult pixels. In visualized difficult pixels, white area represents difficult pixels according to confidence.

We visualized difficult pixels according to the confidence. Figure 3 shows difficult pixels in prediction result. White pixels represent the pixels below the threshold α. α is defined as

$$\alpha = \frac{1}{y * x} \sum_{0 \le i \le y} \sum_{0 \le j \le x} f(i,j) \quad (1)$$

where $y*x$ is image size and $f(i,j)$ is the confidence map. Each pixel in the confidence map represents the maximum probability among all classes. By calculating the threshold value based on the average value, it is possible to define relatively difficult pixels in a test image. From Figure 3, we confirmed that white pixels on cell membrane are connected well. Therefore, if the white area can be modified, it is possible to get connection of cell membrane.

3.1 Modification of Prediction Results

Figure 4 shows how to modify difficult pixels in a test image by our proposed method. First, we visualized difficult pixels according to the confidence of the network. White pixels represent difficult pixels in the Figure. We use relationship in a cell image. The relationships are as follows.

- Most of cell membranes are connected each other.
- Cell nucleus are not represented by one pixel.

After we visualize difficult pixels in a test image, we modify cell membrane. Most of cell membranes are connected to each other. If white area is adjacent cell membrane, the pixel is defined as cell membrane. The same flow repeats multiple times. In this way, it is possible to connect cell membranes each other. Next, we modify cell nucleus. The cell nucleus is not represented by a little pixel like one pixel. However, the network sometimes recognizes cell nucleus with very small area. From Figure 1, it can be confirmed that conventional methods recognize only part of cell nucleus. To address this problem, if white area is adjacent cell nucleus, we defined that the white pixels are as cell nucleus. In this way, it is possible to obtain the result of the cell nucleus more accurately. Finally, the remaining white pixels are defined as cytoplasm.

4 EXPERIMENTS

4.1 Dataset

We use cell images of mouse liver dataset (Imanishi, 2018). The dataset is fluorescence images of the liver of transgenic mice that expressed fluorescent markers on the cell membrane and nucleus. The size of image is 512×512 and include three classes; cell membrane, cell nucleus and cell cytoplasm. It contains 35/5/10 images for training, validation and test.

4.2 Implementation Details

In this experiment, we use Adam (Kingma, 2014) as optimizer and learning rate is set to 1e-3. We used batch renormalization (Ioffe, 2017) with batchsize 2, and single GPU with GeForce GTX 1080 TITAN. Since various cell image segmentation methods are based on U-net, we also used U-net. We used early stopping according to the mIoU for validation dataset. We use precision score and recall score as an evaluation measure. Calculation of precision and recall are followed as

$$Precision = \frac{TP}{TP + FP} \quad (2)$$

$$Recall = \frac{TP}{TP + FN} \quad (3)$$

where TP represent true positive, FP and FN are false positive and false negative.

4.3 Evaluation

Figure 5 shows segmentation results by our proposed method and conventional methods. From Figure 5, we confirmed that our proposed method can connect membrane well with small number of false positives. However, our method tends to recognize cell membrane with thicker than ground truth. This is because we reconstruct preferentially cell membrane when we modified difficult pixels.

Next, we evaluate precision and recall score. Table 1 shows the recall score of the proposed method and conventional U-net. From Table 1, we confirmed that the proposed method shows very high recall score for cell membrane. This result shows small false negatives. Table 2 shows the precision score of the

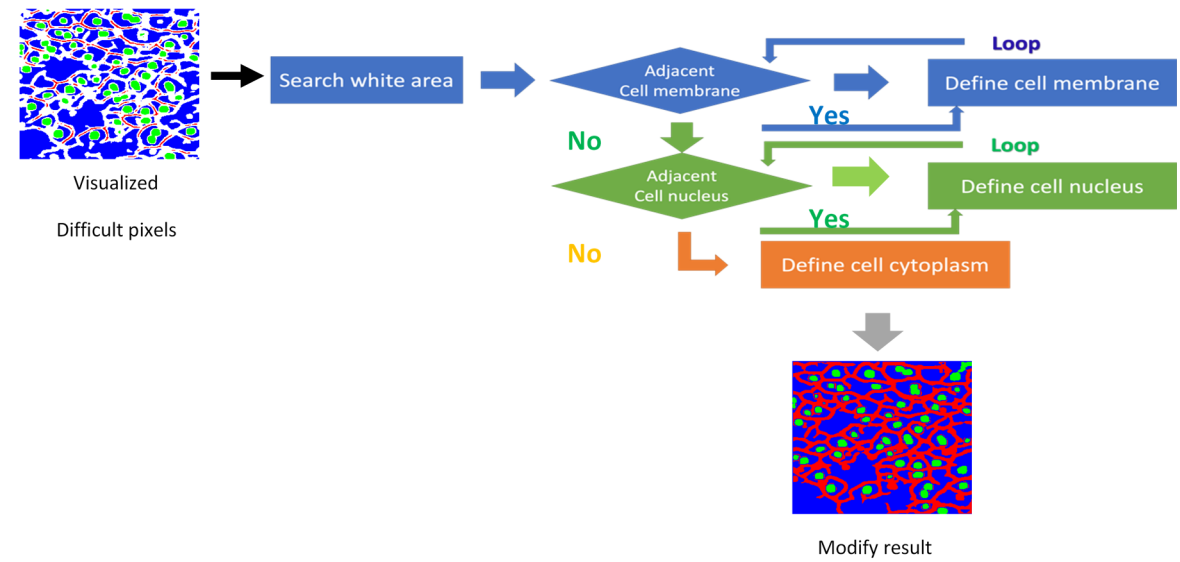


Figure 4: Flow of proposed method.

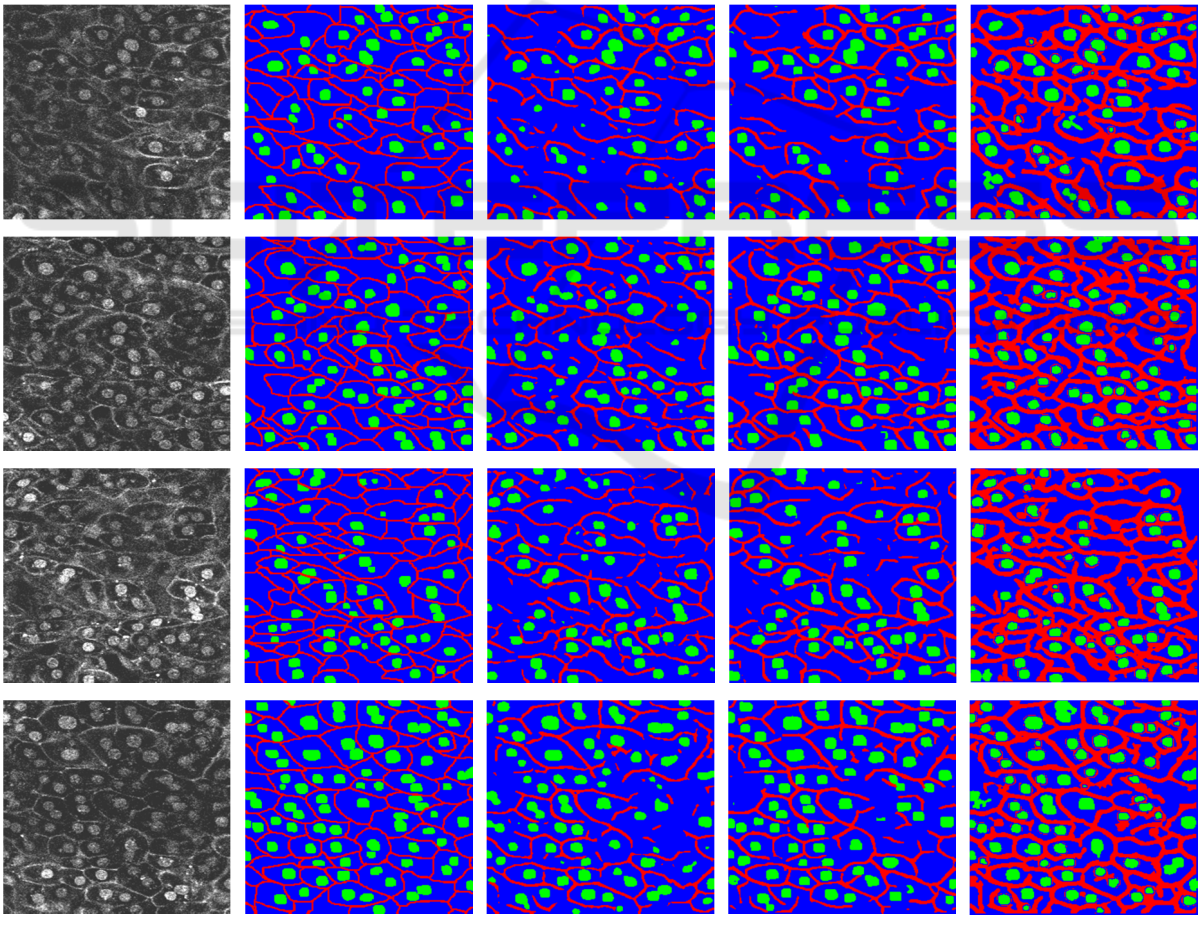


Figure 5: segmentation results. (a) represent input images and (b) are ground truth images. (c) and (d) are the results by conventional Tiramisu103 and U-net and (e) shows the results by our proposed method.

Table 1: Result of recall score.

Recall	Membrane	Nucleus	Cytoplasm
U-net	56.2	**80.9**	**84.1**
Our	**83.0**	72.8	65.4

Table 2: Result of precision score.

Precision	Membrane	Nucleus	Cytoplasm
U-net	**52.6**	78.5	86.0
Our	37.7	**82.5**	**90.3**

proposed method and conventional U-net. From Table 2, we confirmed that cell nucleus is also improved. This result shows that our proposed method can reduce false positive like a noise. On the other hand, U-net shows high precision score for cell membrane. The reason is that our method recognizes the cell membrane thickly. Therefore, our proposed method is high recall score but precision score is low score. However, our goal is to get connection of cell membrane. High recall score is the result we expected.

5 CONCLUSIONS

In this paper, we proposed to visualize and modify difficult pixels according to the confidence of network output. Difficult pixels often show the connection between cell membrane, and we can modify those pixels. We confirmed that the proposed method can get connection of cell membrane well. However, our method tends to that cell membrane is recognized thicker than ground truth. Thus, we would like to study another method for modifying prediction results more accurately.

ACKNOWLEDGEMENTS

This work is partially supported by MEXT/JSPS KAKENHI Grant Number 18H04746 "Resonance Bio" and 18K111382.

REFERENCES

O. Russakovsky, J. Deng, H. Su, J. Krause, S. Satheesh, S. Ma, Z. Huang, A. Karpathy, A. Khosla, M. Bernstein, A. C. Berg, and L. Fei-Fei. Imagenet large scale visual recognition challenge. IJCV, 2015, pp211- 252.

J. Long, E. Shelhamer, and T. Darrell. Fully convolutional networks for semantic segmentation. In CVPR, 2015, pp3431-3440.

O. Ronneberge, F. Philipp, and B. Thomas. U-net: Convolutional networks for biomedical image segmentation. In MICCAI, 2015, 234-241.

J. Simon, et al. The one hundred layers tiramisu: Fully convolutional densenets for semantic segmentation. In CVPR Workshop, 2017, pp11-19.

V. Badrinarayanan, A. Kendall, and R. Cipolla. Segnet: A deep convolutional encoder-decoder architecture for image segmentation. IEEE transactions on pattern analysis and machine intelligence 39.12: 2481-2495, 2017.

Z. Zhou, MMR. Siddiquee, N. Tajbakhsh, et al. Unet++: A nested u-net architecture for medical image segmentation. In Deep Learning in Medical Image Analysis and Multimodal Learning for Clinical Decision Support, Cham, 2018, pp3-11.

K. He, X. Zhang, S. Ren, and J. Sun. Deep residual learning for image recognition. In CVPR, 2016, pp770-778.

T. Murata, K, Hotta., et al. Segmentation of cell membrane and nucleus using branches with different roles in deep neural network. In BIOSIGNAL, 2018, pp256-261.

Y. Hiramatsu, K. Hotta, et al. Cell image segmentation by integrating multiple CNNs. In CVPR Workshops, 2018, pp2205-2211.

R. A. Jacobs, et al. Adaptive mixtures of local experts, Neural Computation, vol.3, Issue.1, pp.79–87, 1991.

H. Tsuda and K. Hotta. K. Cell Image Segmentation by Integrating Pix2pixs for Each Class. In CVPR Workshops, 2019, pp0-0.

P. Isola, JY. Zhu, T. Zhou, and A. A. Efros, Image-to-Image Translation with Conditional Adversarial Networks. In CVPR, 2017, pp. 5967-5976.

A. Imanishi, T. Murata, et al. A Novel Morphological Marker for the Analysis of Molecular Activities at the Single-cell Level. Cell Structure and Function, 43: 129-140, 2018

P. D. Kingma and Jimmy Ba. Adam: A method for stochastic optimization. arXiv preprint arXiv: 1412.6980. 2014

S. Ioffe. Batch renormalization: Towards reducing minibatch dependence in batch-normalized models. In NeurIPS, 2017, pp1945-1953.