Recursive Additive Complement Networks for Cell Membrane Segmentation in Histological Images

Satoshi Yamami, Keita Sugimoto, Masanobu Takahashi, *Member*, *IEEE* and Masayuki Nakano

***Abstract*— A recursive additive complement network (RacNet) is introduced to segment cell membranes in histological images as closed lines. Segmenting cell membranes as closed lines is necessary to calculate cell areas and to estimate N/C ratio, which is useful to diagnose early hepatocellular carcinoma. The RacNet is composed of a complement network and an element-wise maximization (EWM) process and is recursively applied to the network output. The complement network complements the lacking parts of cell membranes. The network, however, has a tendency to mistakenly delete some parts of the segmented cell membranes. The EWM process eliminates this unwanted effect.**

Experiments carried out using unstained hepatic sections showed that the accuracy for segmenting cell membranes as closed lines was significantly improved by using the RacNet.

Three imaging methods, bright-field, dark-field, and phase-contrast, were used, as unstained sections show very low contrast in the bright-field imaging commonly used in pathological diagnosis. These imaging methods are available in optical microscopes used by pathologists. Among the three methods, phase-contrast imaging showed the highest accuracy.

I. Introduction

Histological diagnosis is made by observing histological sections under an optical microscope. The diagnosis by pathologists is discretionary, so it is important to develop a support system that analyzes histological images and provides useful quantitative information.

We previously developed a support system [1] for diagnosing early hepatocellular carcinoma, as the histological atypism of an early hepatocellular carcinoma is so slight that it is very difficult to distinguish it from a non-cancerous one [2]. The support system can automatically visualize the distribution of nuclear density (the number of nuclei per unit area) in a whole slide image, as nuclear density is key information to diagnose early hepatocellular carcinoma [3].

Another piece of useful information is the nuclear / cytoplasmic (N/C) area ratio [4]. The average N/C ratio of a non-cancerous part is about 7.7%, while in the case of liver cancer, it is as high as 12.3%, even at a very early stage [4]. The N/C ratio is calculated using the nuclear area and cytoplasmic area. To calculate the cytoplasmic area, it is necessary to segment the cell membranes surrounding it.

A method for the cell membrane segmentation of C. elegans embryos has been reported [5]. However, this method is for the 3D images of live embryos obtained using a spinning disk confocal unit, and cannot be applied for histological images obtained from thin sections. As for cell membrane segmentation for histological images, methods for segmenting cell membranes in immunostained breast cancer images have been reported [6], [7]. Both methods, however, require special staining, and the target is to evaluate HER2 scoring by segmenting only immunostained cell membranes, rather than segmenting all cell membranes.

We previously reported cell membrane segmentation in histological images for H&E stained sections [8], [9] and unstained sections [10], [11], where segmentation of the cell membrane is difficult due to small differences in color between cell membrane and cytoplasm. Although more than 85% of cell membranes were segmented correctly using deep learning, some parts were not segmented. One reason for this is the presence of artefacts that appear during surgery, fixation, transportation, and tissue processing [12]. If a part of the cell membranes is not segmented, the cell membranes are not segmented as closed lines. As a result, the cytoplasmic area, which is necessary to calculate the N/C ratio, cannot be calculated.

In response to the issues above, we propose a deep learning network, which we call the recursive additive complement network (RacNet), to complement those lacking cell membranes. Experimental results showed that the RacNet can greatly improve the accuracy for segmenting cell membranes as closed lines.

In the experiments, we used unstained sections because cell membrane segmentation would be more difficult than in H&E stained sections. Unstained sections are advantageous because there are no problems concerning uneven dyeing or discoloration, and they can eliminate the unwanted chemical effects on sections. We experimentally compared three imaging methods that are commonly used in an optical microscope and found that the highest accuracy was obtained with phase-contrast imaging.

This work has obtained ethics approval by the Institutional Research Ethics Board of Shibaura Institute of Technology.

II. Methods

A. Cell membrane segmentation

In order to obtain segmentation results as an image, we configured the network for cell membrane segmentation as a segmentation network with image input and image output, which is typified by a fully convolutional network [13].

Satoshi Yamami, Keita Sugimoto and Masanobu Takahashi are with Graduate School of Engineering and Science, Shibaura Institute of Technology, 307, Fukasaku, Minuma-ku, Saitama-city, Saitama, 337-8570 Japan, (mtaka@shibaura-it.ac.jp).

Masayuki Nakano is with Yokohama City University, 3-9, Fukuura, Kanazawa-ku, Yokohama-city, Kanagawa, 236-0004 Japan.

978-1-7281-1990-8/20/$31.00 ©2020 IEEE

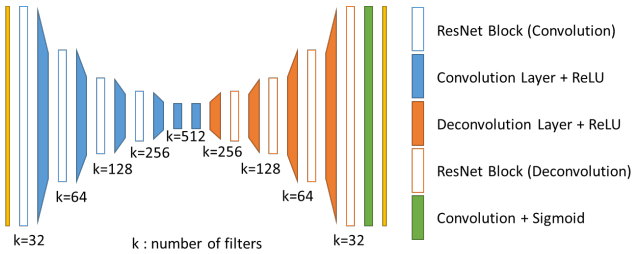


Fig. 1. Cell membrane segmentation network.

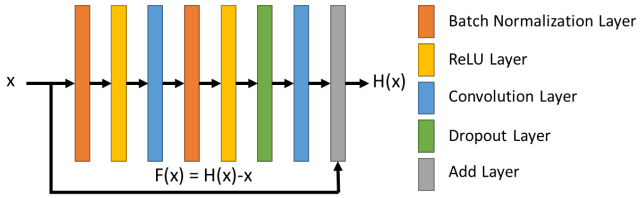
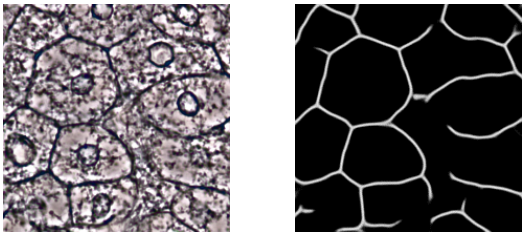


Fig. 2. ResNet block.



(a) Unstained section (b) Network output

Fig. 3. Example images of unstained section and network output.

Fig. 1 shows the network configuration. The network is based on ResNet [14], which enables deeper networks. The ResNet block in Fig. 1 is composed of batch normalization layers, ReLU layers, convolution layers, a dropout layer, and a shortcut connection, as shown in Fig. 2. Two sets of batch normalization, dropout, and convolution layers were connected serially in the ResNet block because the accuracy was higher than the case where two sets were separated into two ResNet blocks.

The network configuration was experimentally determined. An example of network output for an unstained section image (Fig. 3 (a)) is shown in Fig. 3 (b). Although most of the cell membranes were segmented correctly, as shown in Fig. 3 (b), some parts of them were not segmented due to vague pattern or a lack of cell membranes caused by artefacts.

B. Complement Network

If there are defects in the segmented cell membranes, it is impossible to calculate the cytoplasmic area for each cell, and as a result, the N/C ratio cannot be calculated. Therefore, we introduce a network that complements the lacking parts.

Fig. 4 shows the configuration of the complement network. Both input (original) and output images of the cell membrane segmentation network were used as input images, as shown in the figure. Both images are input separately and then concatenated after independent encoding processes.

The network was trained to output cell membrane segmentation results in which lacking parts were

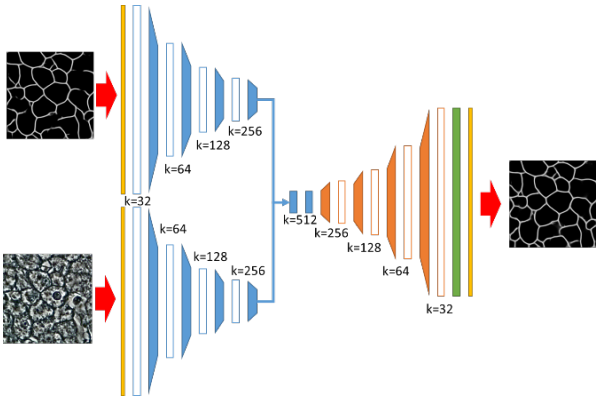


Fig. 4. Complement network.

complemented for the input images (the original image and the output image of the cell membrane segmentation network). In the training, correct cell membrane images with intentional defects, which were added by deleting cell membranes in one or two small random areas, were also used as the output image of the cell membrane segmentation network. The correct cell membrane images were manually determined, including the complemented parts of the lacking cell membranes.

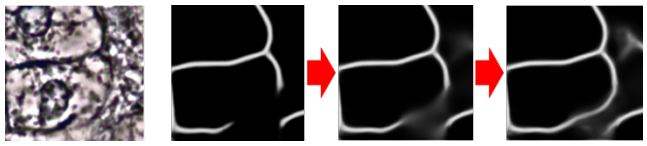
C. Recursive Additive Complement Network

The complement network was applied recursively to enhance the complement effect. Fig. 5 shows an example of the effect of iterative application. The lacking part, which would be made by artefacts, was successfully complemented after four iterations in this case.

The complement network, however, has a tendency to delete some parts of the cell membranes. As a result, some of these parts, which were successfully segmented by the cell membrane segmentation network, were erroneously deleted by applying the complement network, as indicated by the blue circle in Fig. 6 (b).

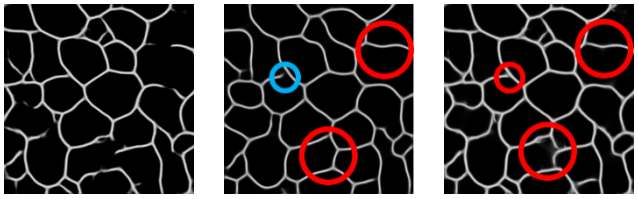
To eliminate this unwanted effect, we added an element-wise maximization (EWM) process to the complement network, as shown in Fig. 7. This network, which we call the recursive additive complement network (RacNet), outputs the maximum value of the output image and the input image (previous output image) for each pixel. The output (the maximum value) is then fed back to the input. As a result, the RacNet can complement cell membranes without deleting any cell membranes, as shown in Fig. 6 (c).

Fig. 8 shows the configuration of a full network composed of the cell membrane extraction network and the RacNet.



(a) Original image (b) N = 0 (c) N = 2 (d) N = 4

Fig. 5. Effect of iterative application of the complement network. N is the number of iterations.



(a) Output of cell membrane segmentation network (b) Without EWM (c) With EWM

Fig. 6. Effect of element-wise maximization (EWM).

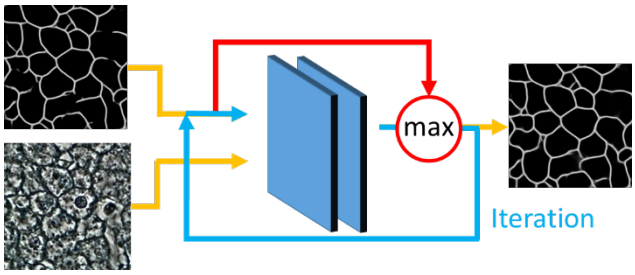


Fig. 7. Recursive additive complement network (RacNet).

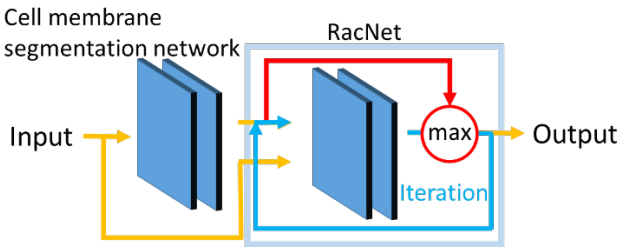


Fig. 8. Full network for cell membrane segmentation composed of the cell membrane segmentation network and the RacNet.

III. Experiment

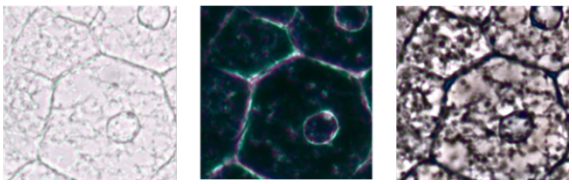
A. Materials

Five unstained sections of liver tissue were used in the experiment. Three imaging methods, bright-field, dark-field, and phase-contrast, were used to capture histological images of unstained sections, as these methods are readily available for general pathologists.

In bright-field imaging, which is commonly used in daily diagnosis, the light transmitted through the section is observed. As such, a bright-field image reflects the distribution of absorption, which is suitable for observing stained sections. However, in the case of unstained sections, the bright-field image shows very low contrast due to little difference in the light absorption rate, and the visibility of cell membranes is very poor (Fig. 9 (a)).

In dark-field imaging, the light reflected or scattered by the sections is observed. As such, the cell membranes are basically observed as bright lines (Fig. 9 (b)).

In phase-contrast imaging, phase differences in light caused by differences in refractive index are observed as brightness changes. The contrast of phase-contrast images is high even for unstained sections (Fig. 9 (c)).



(a) Bright-field image (b) Dark-field image (c) Phase-contrast image

Fig. 9. Example images of unstained section.

In this experiment, we evaluated the accuracy for each of these three imaging methods. Three images (400 × 400 pixels) were captured at two areas for each section. Training images for the cell membrane segmentation network were generated by randomly cropping (128 × 128 pixels), rotating, and changing the brightness and contrast of the image. As a result, 1000 images were created for each image.

Training images for the complement network were generated similarly except for the size of the images (192 × 192 pixels), as wider information would be necessary to complement lacking cell membranes.

B. Results

The output image of the RacNet was binarized and thinned to obtain the center lines of cell membranes. Then, closed regions were extracted as segmented regions. Each segmented region was then compared with the correct cell region to determine if it had been segmented correctly.

In Fig. 10, Sc denotes the correct cell region, Ss denotes the segmented region, and So denotes the overlap region between Sc and Ss. Each segmented cell was determined to be correctly segmented if both equations (1) and (2) were satisfied.

$$\frac{Area\ of\ S_o}{Area\ of\ S_c} \geq 0.9 \tag{1}$$

$$\frac{Area\ of\ S_o}{Area\ of\ S_s} \geq 0.9 \tag{2}$$

The number of correctly segmented cells was then used to calculate the recall rate, as

$$\text{Recall rate} = \frac{Number\ of\ correctly\ segmented\ cells}{Number\ of\ currect\ cells}. \tag{3}$$

The evaluation of the recall rate was performed by 5-fold cross-validation without dividing the images of the same section into training and evaluation.

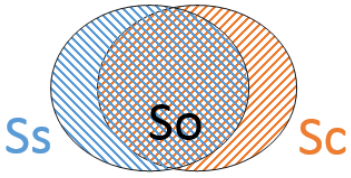


Fig. 10. Regions to determine if segmented region is correctly segmented (Sc: correct cell region, Ss: segmented region, So: overlap region).

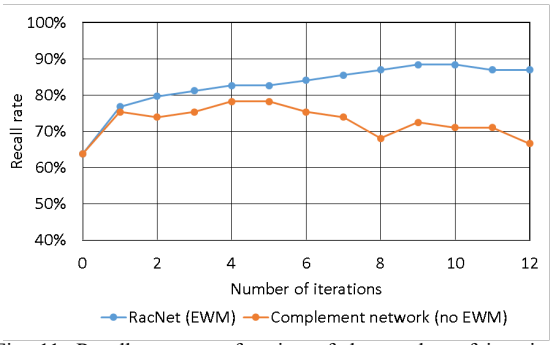
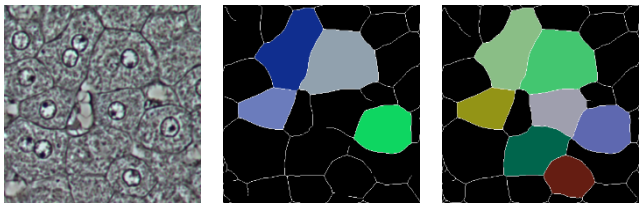


Fig. 11. Recall rate as a function of the number of iterations for phase-contrast images (EWM: Element-wise maximization).

TABLE I. RECALL RATE FOR THREE IMAGING METHODS

Bright-field		**Dark-field**		**Phase-contrast**	
Without RacNet	***With RacNet***	***Without RacNet***	***With RacNet***	***Without RacNet***	***With RacNet***
62.3%	78.3%	47.8%	69.6%	63.8%	88.4%



(a) Input image (b) Without RacNet (c) With RacNet

Fig. 12. Example output images for a phase-contrast image. Colored regions denote correctly segmented cells. The computation time with the RacNet was 1.6 seconds on two NVIDIA GeForce GTX 1080 GPUs.

Fig. 11 shows the recall rate when the phase-contrast image was used as the input. The horizontal axis denotes the number of iterations of the RacNet or the complement network. As shown, in case of the RacNet, the recall rate improved as the number of iterations increased, and became stable. In contrast, in the case of the complement network that does not use the EWM process, the recall rate degraded as the number of iterations increased after a peak at five iterations, showing the effect of using the EWM process. The maximum recall rate of 88.4% was obtained using the RacNet, which is significantly better than the recall rate of 63.8% obtained without using the RacNet (number of iterations = 0).

Table 1 shows the recall rate with and without the RacNet where the number of iterations and the number of epochs were optimized for each of the three imaging methods. The recall rate was improved by using the RacNet for all three. Among the three methods, phase-contrast imaging had the highest recall rate, and bright-field imaging was second best. Fig. 12 shows example output images for a phase-contrast image. In this case, the number of correctly segmented cells was increased to 7 by using the RacNet.

IV. CONCLUSION

We introduced a recursive additive complement network (RacNet) to segment cell membranes in histological images as closed lines. The RacNet is composed of a complement network, which complements the lacking parts of cell membranes, and an element-wise maximization (EWM) process. The EWM process can eliminate the unwanted effect of the complement network, which is to delete some parts of segmented cell membranes.

Experimental results using unstained sections showed that the accuracy for segmenting cell membranes as closed lines was significantly improved by using the RacNet. Three imaging methods, bright-field, dark-field, and phase-contrast, were used in the experiments, as these methods are available in the optical microscopes used by pathologists. Among the three, phase-contrast imaging had the highest accuracy.

Future work will include the estimation of an appropriate number of iterations to prevent a slight degradation of the recall rate due to excessive iterations. Automatic calculation of the N/C ratio by segmenting cell nuclei would also be the target.

REFERENCES

[1] A. Nagase, M. Takahashi, M. Nakano, "Automatic calculation and visualization of nuclear density in whole slide images of hepatic histological sections," *Bio-Med Mater Eng*, vol.26, pp.s1335-s1344, 2015.

[2] M. Nakano, A. Saito, M. Yamamoto, M. Doi, K. Takasaki, "Stromal and blood vessel wall invasion in well-differentiated hepatocellular carcinoma," *Liver*, vol.17, pp.41-46, 1997.

[3] International Consensus Group for Hepatocellular Neoplasia, "Pathologic diagnosis of early hepatocellular carcinoma: a report of the international consensus group for hepatocellular neoplasia," *Hepatology*, vol.49, pp.658–664, 2009.

[4] S. B. Erler, M. H. Truong, S. S. Kim, et al., "A study of hepatocellular carcinoma using morphometric and densitometric image analysis," *American Journal of Clinical Pathology*, vol.100, pp.151-157, 1993.

[5] Y. Azuma, S. Onami, "Biologically constrained optimization based cell membrane segmentation in c. elegans embryos," *Bmc Bioinformatics*, vol.18, pp.307, 2017.

[6] M. Saha, C. Chakraborty, "Her2Net: A deep framework for semantic segmentation and classification of cell membranes and nuclei in breast cancer evaluation," *IEEE Trans Image Process*, vol.27, pp.2189-2200, 2018.

[7] F. D. Khameneh, S. Razavi, M. E. kamasak, "Automated segmentation of cell membranes to evaluate HER2 status in whole slide images using a modified deep learning network," *Computers in Biology and Medicine*, vol.110, pp.164-174, 2019.

[8] N. Matsushita, M. Takahashi, M. Nakano, "Multimodal method for cell membrane extraction in hepatic histological images," in *Proc. 32nd Annual Intl. Conf. of IEEE Engineering in Medicine and Biology Society*, Buenos Aires, 2010, FrBPo07.5.

[9] K. Sugimoto, M. Takahashi, M. Nakano, "Cell membrane extraction in H&E stained histological sections using deep learning," in *Proc.40th Annual Intl. Conf. of IEEE Engineering in Medicine and Biology Society*, Honolulu, 2018, WePoS25.32.

[10] T. Ushikawa, M. Takahashi, M. Nakano, "Multimodal image analysis of unstained histological sections," *6th European Conf. of the Intl. Federation for Medical and Biological Eng*, vol.45, pp.216-219, 2014.

[11] S. Yamami, K. Sugimoto, M. Takahashi, M. Nakano, "Cell membrane extraction in unstained histological sections using deep learning," in *Proc.41st Annual Intl. Conf. of IEEE Engineering in Medicine and Biology Society*, Berlin, 2019, ThPOS-32.2.

[12] S. Chatterjee, "Artefacts in histopathology," *J Oral Maxillofac Pathol*, vol.18, pp.S111–S116, 2014.

[13] J. Long, E. Shelhamer, T. Darrell, "Fully convolutional networks for semantic segmentation," *IEEE T Pattern Anal*, vol.39, pp.640-651, 2017.

[14] K. He, X. Zhang, S. Ren, J. Sun, "Deep residual learning for image recognition," *CVPR2016*, pp.770-778, 2016.