U-RISC: An Ultra-high Resolution EM Dataset Challenging Existing Deep Learning Algorithms

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Abstract Connectomics is a developing filed aiming at reconstructing the connection of neural system at nanometer scale. Computer vision technology, especially deep learning methods used in image processing, has promoted connectomic data analysis to a new era. Even though, the performance of the state-of-the-art methods is still fall behind the demand of researchers. To alleviate this situation, here we first introduce a new annotated Ultra-high Resolution Image Segmentation dataset for the Cell membrane, called U-RISC, which is the largest annotated Electron Microscopy (EM) dataset for the cell membrane with multiple iterative annotations and the resolution of 2.18nm/pixel. Then we reveal the performance of existing deep learning segmentation methods on U-RISC through an open competition. The performance of participants appears to have a huge gap with human-level, however, the results of same methods on ISBI 2012, a smaller EM dataset, are near-human. To further explore the differences between the performance of two datasets, we analyze the neural networks with attribution analysis and uncover the larger decision-making area in the segmentation of U-RISC. Our work provides a new benchmark data for EM cell membrane segmentation and proposes some perspectives in deep learning segmentation algorithms.

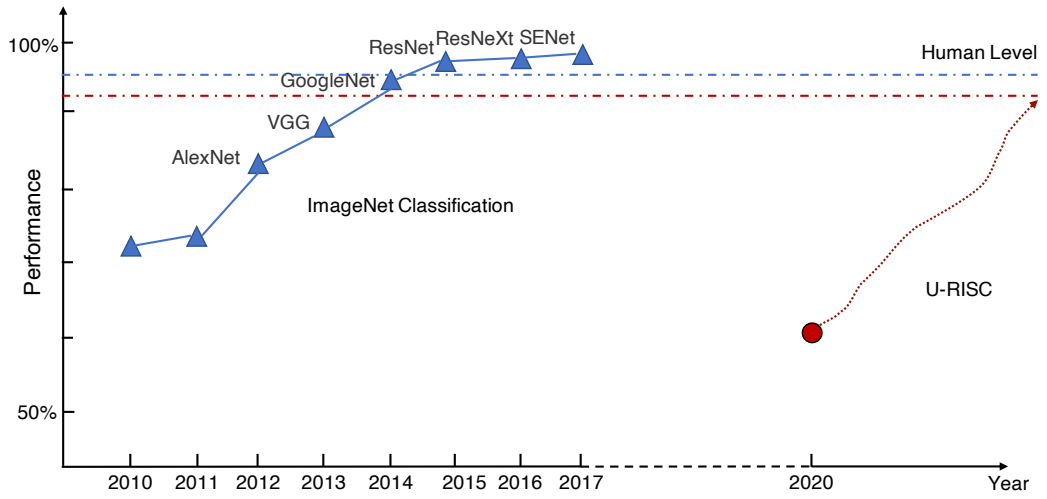

Figure 1. The history of ImageNet.

Introduction

Accurate description of neurons is important to uncover the mystery of brain. By depicting neurons with the help of Golgi-staining method (***Golgi, 1885***), Cajal could propose the classic "Neuron Doctrine" more than a century ago (***y Cajal, 1888***). Nowadays the development of electron microscopy (EM) has enabled us to explore the neural system at higher resolution and larger scale (***Kornfeld and Denk, 2018***; ***Shawn, 2016***), opening up microscale connectomics that aims to reconstruct every single connection in the neural system. One of famous examples is the project of a C.elegans (***White et al., 1986***). However just as it took almost 15 years to complete the connectome of C.elegans, structural reconstruction is becoming more and more daunting with the explosion of connectomic data. Among many bottlenecks, accurate annotation from large amounts of EM images is the first one that has to be solved.

Hopes are higher today because of the rapid development of deep learning methods. The goal can come true, enabling a machine to automatically annotate the connectomic data with near human performance. In order to make the deep learning method work well, there are successful experiences to learn from the community of computer science. It is well known that the success of deep learning methods highly depends on the amount of training data and the quality of annotation. Take the task of image classification for example. ImageNet (***Russakovsky et al., 2014***) has set up a research paradigm of applying deep learning methods for vision tasks. In 2009, by releasing a large-scale accurately annotated dataset, ImageNet first provided a benchmark (72%) for image classification. From 2010 to 2017, a challenge named as "The ImageNet Large Scale Visual Recognition Challenge (ILSVRC)" was organized every year. This challenge greatly boosts the development of deep learning algorithms. Many champions of this challenge have become the milestones of deep learning methods, such as AlexNet (***Krizhevsky et al., 2012***), VGG (***Simonyan and Zisserman, 2014a***), GoogleNet (***Szegedy et al., 2015***) and ResNet (***He et al., 2016***) etc. As shown in Fig. 1, the performance of deep learning on the task of image classification finally exceeded human level (95%) after eight years of development. To summarize, there is a roadmap for the success of ImageNet which includes three key steps. The first step is to establish a large-scale dataset with high quality annotation which is very important for deep learning. Based on the dataset, the second step is organizing a challenge which can evaluate algorithms at large scale and allow researchers to estimate the progress of their algorithms taking advantage of the expensive annotation effort. The third step is the design of new algorithms based on the previous two steps. Each of the three steps is indispensable.

Great progress of EM automatic segmentation was promoted by the 2012 IEEE International Symposium on Biomedical Imaging (ISBI 2012) by organizing the first challenge on EM automatic segmentation with releasing a public available dataset (***Arganda-Carreras et al., 2015***). The state-of-the-art method exhibited unprecedented accuracy in EM cellular segmentation in the dataset of ISBI 2012, of which the image is 512 pixel $\times$ 512 pixel with the resolution of 4 nm/pixel $\times$ 4 nm/pixel. But there are many EM images with higher resolution in connectomics, because enough high resolution is important to unravel the neural structures unambiguously. And 2 nanometer has been suggested as the historic gold standard to identify synapses (***Debello et al., 2014***), in especially gap junctions (***Leitch, 1992***) which are widespread in neural tissues such as retina (***Anderson et al., 2009***). That how is the performance of deep learning on EM images with higher resolution is unclear yet.

Therefore, we annotated the retinal connectomic data, RC1, from rabbit (***Anderson et al., 2011***) and presented a brand new annotated EM dataset named U-RISC (Ultra-high Resolution Image Segmentation dataset for Cell membrane). Compared with ISBI 2012, U-RISC has a higher resolution of 2.18 nm/pixel and larger size of 9958 $\times$ 9959 pixels. The precision of annotation was guaranteed by multi-steps of iterative verification, costing over 10,000 hours in total. Based on U-RISC, a competition of cellular membrane prediction was also organized. Surprisingly, from 448 domestic participants/teams, the top performance of deep learning methods on U-RISC ($\sim$ 0.6, F1-score) was

far below the human-level accuracy (> 0.9), in contrast with near-human performance in ISBI 2012. To investigate the underlying reason for such a big gap in performance, we made comprehensive analysis on the deep learning methods those applied in the competition. Besides, fair comparison was made between ISBI 2012 and U-RISC with same segmentation methods. The comparison results suggested that U-RISC provide new challenges to deep learning methods, including the network model design, training technique, loss function design and optimization. To further explore how these methods work on segmentation tasks, we introduced a gradient-based attribution method, integrated gradient (***Sundararajan et al., 2017***), for the analysis of ISBI 2012 and U-RISC. The result showed that when making a decision on whether a pixel belonged to cell membrane or not, deep learning methods represented by U-Net would refer to a lager attribution region on U-RISC (about 4 times on average) than that on ISBI 2012. It suggests that the deep learning methods might require more background information to decide the segmentation of U-RISC dataset. Finally, we integrated current available advanced methods and provided a benchmark (0.6659), about 10% higher than the leader board, for U-RISC.

Overall, our contribution in this work lies mainly in the following three parts: (1) we provided the community a brand new public available annotated mammalian EM dataset with known highest resolution (∼ 2.18 nm/pixel) and largest image size (9958 pixel × 9959 pixel); (2) we organized a competition and made comprehensive analysis to reveal the challenges of U-RISC for deep learning methods; (3) we improved the benchmark with 10% to the F1-score of 0.6659. In discussion, we proposed further suggestions for the improvement of segmentation methods from perspectives of model design, loss function design, data process etc. We hope our dataset and analysis can help researchers to gain insights into designing more robust methods, which can finally accelerate the speed of untangling brain connectivity.

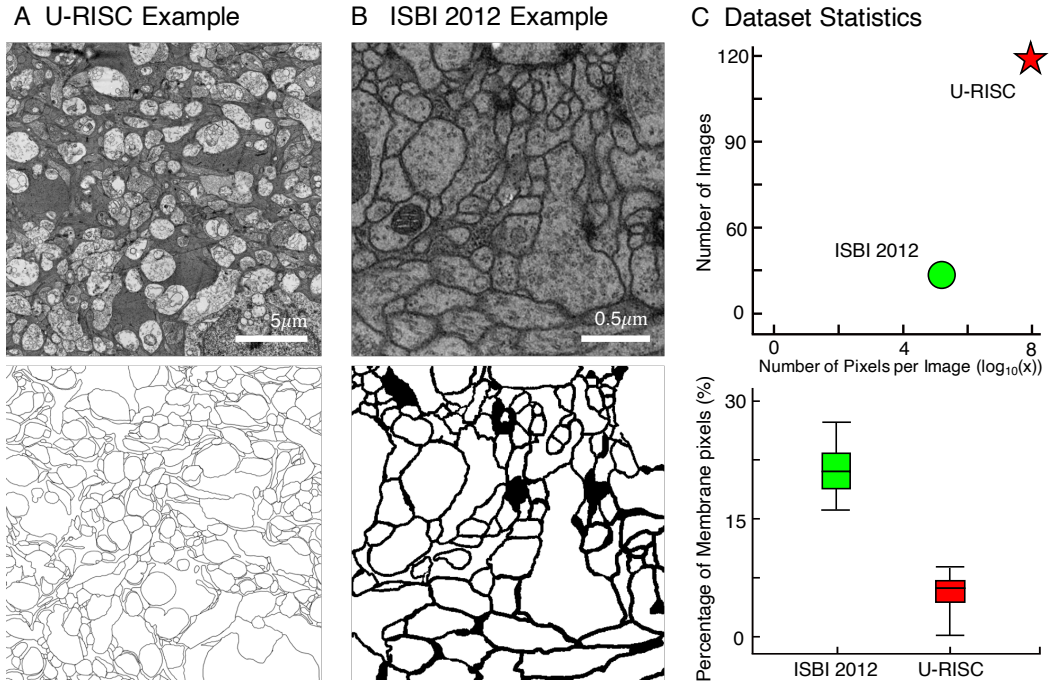

Figure 2. Comparison between U-RISC and ISBI 2012. (A and B) An example of the data in U-RISC and ISBI 2012, including the raw EM image (top) and the corresponding annotation result (bottom). Black pixels in annotation results represent cellular membranes. (C) (Top) Both the number and size of images in U-RISC surpass those in ISBI 2012. (Bottom) Proportion of annotated pixels, 5.10%±0.02 in ISBI 2012 and 21.65%±0.02 in U-RISC, making latter a more imbalanced dataset.

Results

The largest ultra-high resolution EM cell membrane segmentation dataset

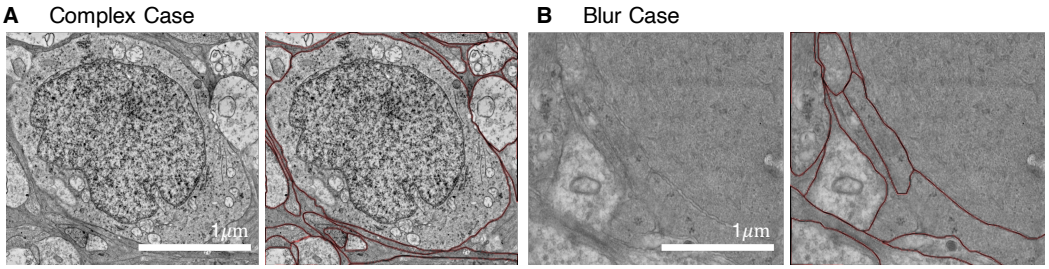

Figure 3. Examples of images with their annotations. (A) Organelle membranes were cautiously avoided to be annotated. (B) More time and patience were needed to annotate the image with low contrast.

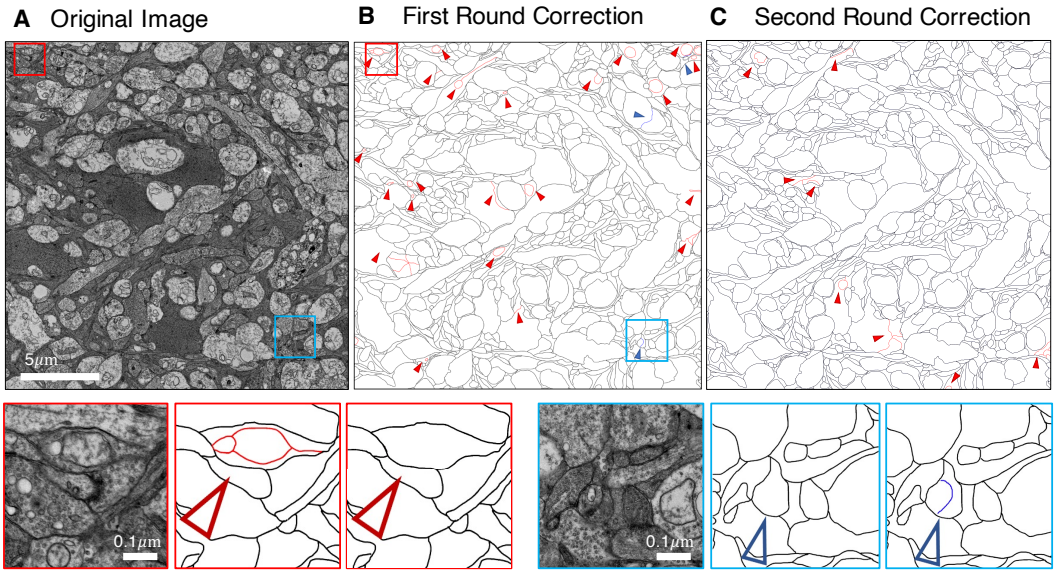

Figure 4. Example of iterative human annotation. (A) Original image to be annotated. (B) Many errors were found out in the first round of annotation. (C) After correction, much fewer errors were detected in the second round of annotation, and the correction results were served as the final annotation. Red small triangles and boxes indicate false positive errors (enlargement in bottom left), blue for false negative errors (enlargement in bottom right).

Along with this paper, we proposed a new EM dataset with cell membrane annotated: Ultra-high Resolution Images Segmentation dataset for Cell membrane (**U-RISC**). To our best knowledge, U-RISC has the highest resolution among the public available annotated EM datasets (see ***Figure 2***(A) as an example). It is annotated upon the rabbit retinal connectomic dataset RC1 (***Anderson et al., 2011***) with the resolution of 2.18 nm/pixel at both x and y axes. The size of individual image and the total number of images in U-RISC both exceed the published dataset by far, taking ISBI 2012 as an example (***Figure 2***(C)) (120 pairs of 9958 (px) × 9959 (px) images in U-RISC and 30 pairs of 512 (px) × 512 (px) images in ISBI 2012). One characteristic of U-RISC is that cell membranes only cover a small area of the images, which makes it an imbalanced dataset for deep learning (an average of 5.10% ± 0.02 in U-RISC compared with 21.65%± 0.02 in ISBI 2012).

To ensure the quality of annotation, an iterative manual annotation procedure was employed. Because of the difficulty of distinguishing cell membrane from organelle membrane, special attention

was paid to exclude organelle membrane from annotation (***Figure 3***(A)). In practical connectomic research, the image quality can be affected by many reasons like insufficient staining, thick section, etc. Considering this, we retained several images with low quality in U-RISC to make the dataset closer to real situation. Annotation on these images costed more time and caution (***Figure 3***(B)). Labeling errors could be detected and then corrected in each round of iteration (***Figure 4***). For scientific research reason, the human labeling process is very valuable for uncovering the human learning process. Therefore, the intermediate annotated results were also reserved for public release at http://120.92.109.254/biodb-rabbit-details.html.

Table 1. Leaderboard of Track 1 and Track 2.

Track 1 (Original)			Track 2 (Downsample)		
Team Name	Institution	F1-score	Team Name	Institution	F1-score
Human 1st		0.92128± 0.012	Human 1st		0.96915± 0.014
Human 2nd		0.99334± 0.008	Human 2nd		0.99891± 0.003
SCP173	Tencent[1]	0.60704± 0.043	horch	UCAS[2]	0.56932± 0.053
yangsenwxy	SCU[3]	0.60701± 0.042	deadline	NJU[4]	0.56213± 0.055
SpongeBobbb	HDU[5]	0.60480± 0.042	SpongeBobbb	HDU[5]	0.56136± 0.049
VIDAR	USTC[6]	0.60303± 0.041	VIDAR	USTC[6]	0.55170± 0.046
deadline	NJU[4]	0.60066± 0.045	archer	THU[7]	0.55107± 0.047
Chasingstar	JLU[8]	0.59647± 0.044	scu_ws	SCU[3]	0.54847± 0.053

Mean and standard error are computed over test images.

Ultra-high resolution EM images segmentation benchmark

To investigate the performance of the deep learning methods on U-RISC and propose a benchmark, a competition about cellular membrane segmentation was organized [1] by BAAI (Beijing Academy of Artificial Intelligence Institution, Beijing, China) and PKU (Peking University, Beijing, China). In total, 448 participants took part in the competition, who were mainly from domestic competitive universities, research organizations, and top IT institutions.

There were two tracks in the competition (***Table 1***): Track 1 used original images with the size of 9958 (px) × 9959 (px) as training and testing datasets. In Track 2, images were downsampled to the size of 1024 (px) × 1024 (px). The purpose of Track 2 was to allow researchers with limited computational resources to participate in the competition. For evaluating the algorithms, the final round of human annotation was used as the ground truth and F1-score was applied as the evaluation metric. More details are described in the section of Methods and Materials.

From the competition(***Table 1***), our first surprising finding was that the top 6 teams in each track gained F1-scores around 0.6 on U-RISC, which was far below the human level (0.92 and 0.99 according to the first and second round of annotation). However previous research has shown that performance of the top teams in ISBI 2012 have already been fairly close to human level (***Arganda-Carreras et al., 2015***). To investigate reasons of the performance gap between the methods and human on U-RISC, a survey about the algorithms from the top 6 teams was

[1] Tencent Holdings Ltd (China)
[2] University of Chinese Academy of Sciences (China)
[3] Sichuan University (China)
[4] Nanjing University (China)
[5] Hangzhou Dianzi University (China)
[6] University of Science and Technology of China
[7] Tsinghua University (China)
[8] Jilin University (China)
[1] https://www.biendata.xyz/competition/urisc/

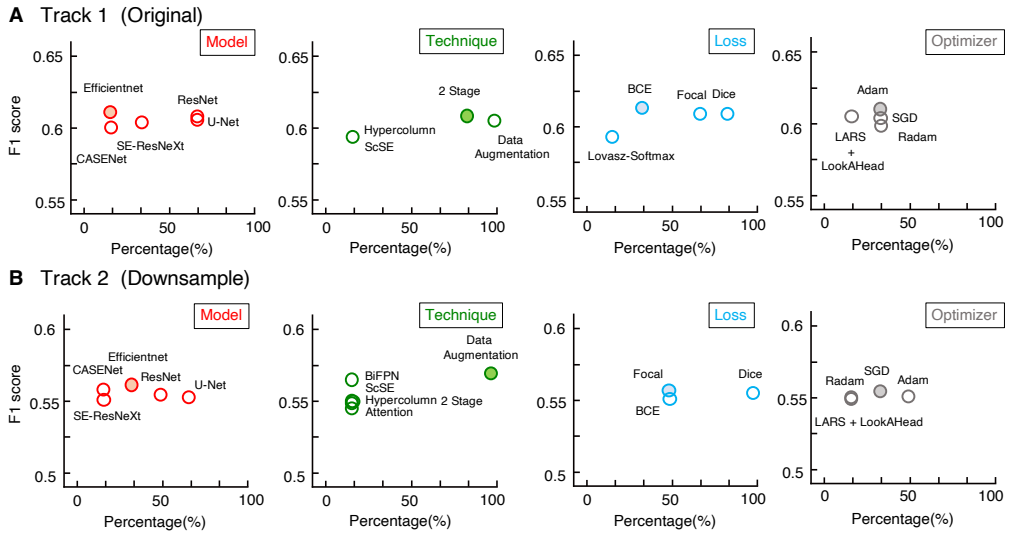

Figure 5. Mean F1-scores of teams with different methods usage. (A and B) The statistics of Track 1 and Track 2 respectively. X-axis represents the proportion of the team with the method. Y-axis represents the average of F1-scores.

carried out. It indicated that a variety of current popular approaches to segmentation had been utilized (***Figure 5***). From the choice of models (***Figure 5***(A)), the participant had used current popular image segmentation networks, such as U-Net (***Ronneberger et al., 2015***), Efficientnet (***Tan and Le, 2019***) and CASENet (***Zhiding Yu et al., 2017***). For backbone selection, ResNet (***He et al., 2016***) and their variants were the most chosen architectures. From the selection of data processing techniques, data augmentation was ubiquitously applied to improve generalization of the models. Hypercolumns (***Hariharan et al., 2015***) was used by 13% of the participants to improve the expressiveness of the model. From the design of loss function, to reduce the effect of sample imbalance, functions which can adjust penalty ratios according to sample distributions were applied, such as Dice loss (***Dice, 1945***), Focal loss (***Lin et al., 2017***), and BCE loss (***Cui et al., 2019***). And Adam (***Kingma and Ba, 2014***) was shown to be the most chosen optimization method.

The analysis suggested that even though participants considered many popular methods, their performance was still not satisfactory and varied only slightly between each other. To identify whether this is because of the challenges of U-RISC or the methods themselves, we picked out three widely used methods: U-Net (***Ronneberger et al., 2015***), LinkNet (***Chaurasia and Culurciello, 2017***), and CASENet (***Zhiding Yu et al., 2017***). Fair comparison was conducted between the performance of each method on U-RISC (Track 1) and ISBI 2012. Results showed that these methods could reach over 0.97 (F1 score) in ISBI 2012, but only around 0.52-0.61 in U-RISC (***Table 2***). It confirmed that the performance gap in competition comes from the challenges of U-RISC. This fully demonstrated that the performance gap in the competition was not caused by the methods, but the specific challenges of U-RISC different previous dataset.

Table 2. F1-scores in U-RISC and ISBI 2012.

Method	U-RISC	ISBI 2012
LinkNet-*	0.60701± 0.063	0.97246± 0.08
CASENet-*	0.60065± 0.053	0.97132± 0.08
U-Net-*	0.57123± 0.049	0.97010± 0.09

To start the discussion about the special challenges of U-RISC to deep learning algorithms, two types of errors were analyzed first: false positive errors which leaded to incorrect membrane predictions and false negative errors which caused continuity of cell membrane. According to our analysis, both false positive errors (pink boxes) and false negative errors (orange boxes) were common in U-RISC, while rare in ISBI 2012 (***Fig-***

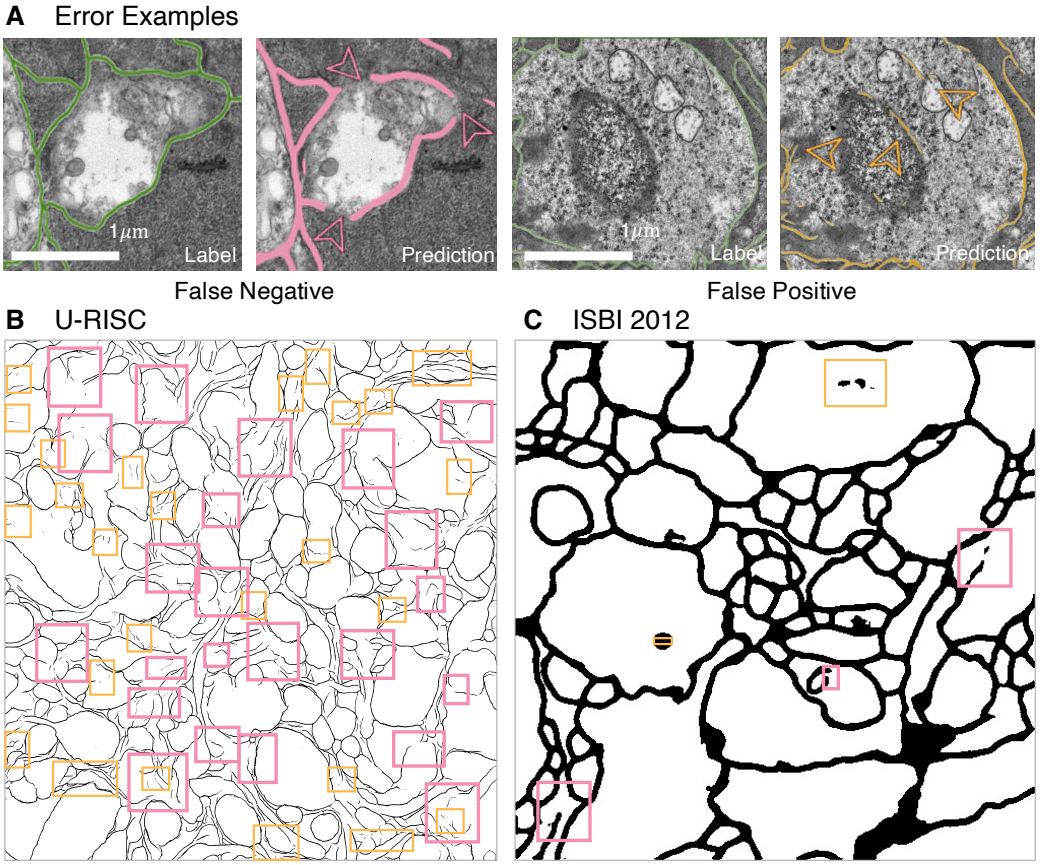

Figure 6. Errors in segmentation predictions of U-RISC and ISBI 2012. (A) The examples of False Positive and False Negative errors. (B and C) The examples of two errors in the segmentations of U-RISC and ISBI 2012. Pink arrows and lines represent false negative errors, and orange represents false positive errors.

Figure 6–Figure supplement 1. Supplements for segmentation predictions of U-RISC.

Figure 6–Figure supplement 2. Supplements for segmentation predictions of ISBI 2012.

ure 6(B) (C)). To explore the reason and find ways to reduce them, further investigations for the networks were required.

Attribution analysis of deep learning method on U-RISC and ISBI 2012

To acquire a deeper understanding of the different performance in U-RISC and ISBI 2012, we performed an attribution analysis (*Ancona et al., 2019*) on the trained U-Net. We selected the gradient-based attribution method, **integral gradient (IG)** (*Sundararajan et al., 2017*), which is widely applied on explainable artificial intelligence, such as understanding feature importance (*Adadi and Berrada, 2018*), identifying data skew (*Clark et al., 2019*), and debugging model performance (*Guidotti et al., 2018*). In brief, IG aims to explain the relationship between predictions and input features based on gradients(*Figure 7*(A)). The output of IG is the **Attribution Field** A, a heatmap in which each pixel was assigned with a normalized value between $[-1, 1]$ to reflect their contribution to the final prediction.

With IG, we analyzed the attribution field of each predicted pixel of U-Net in U-RISC and ISBI 2012. Color and shade were used to represent the normalized contribution values in attribution fields *Figure 7*(B). For fair comparison between U-RISC and ISBI 2012, areas of **Pixel Attribution Field** S_k was converted to physical size according to their respective resolutions. Examples of attribution fields were shown as *Figure 8*, where bounding boxes with different colors represented different classifications of the pixel, green for a correct predicted pixel, orange for a false positive

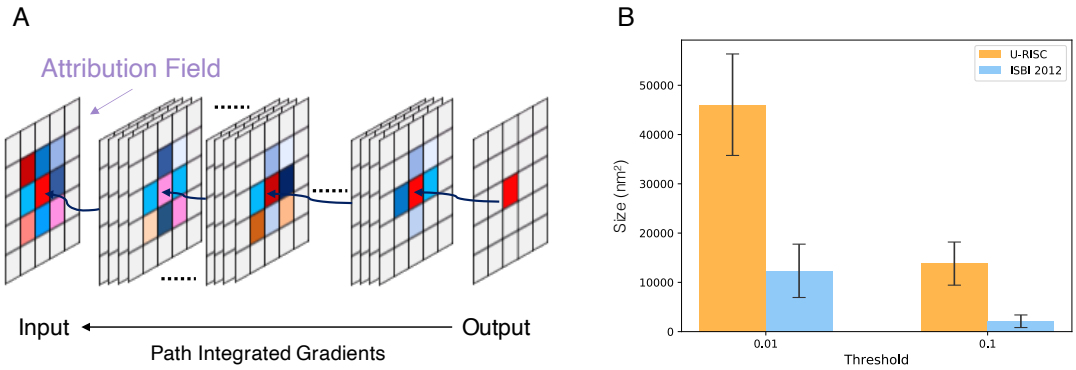

Figure 7. Attribution analysis. (A) Integrated gradients attribution method. (B) Statistics of attribution filed for U-RISC and ISBI 2012.

error, and pink for a false negative error. We noticed that the areas of attribution fields S_k of two datasets were both relatively small to the whole images (***Figure 7***(B)). For example, at the threshold of $k > 0.01$, the S_k of the correct cases (the green bounding box in ***Figure 8*** in U-RISC and ISBI 2012 accounted for only 5.1% and 0.8% relative to the whole image). This suggested that U-Net would focus on local characteristics within small areas of the images when making predictions. In addition, we found that U-Net would refer to larger area when making decisions on U-RISC compared with ISBI 2012. The averaged S_k of each predicted pixel in U-RISC was significantly larger than that in ISBI 2012, specifically 46000 nm^2 in U-RISC and 10300 nm^2 in ISBI 2012. Taken together, U-Net would make the prediction according to local information around the pixel, and the average attribution filed was larger in U-RISC than that of ISBI 2012, which might indicate that more information was required for the segmentation in U-RISC.

U-Net-transfer model achieve the state-of-the-art result on U-RISC benchmark

Take into account the comprehensive analyses of competition and attribution analysis, we integrated outperformed methods together (***Figure 9***(A)) and here provide a new benchmark for U-RISC dataset, which achieved the F1-score of 0.66 (Figure 9B), about 10% higher than the top performance in the competition. For basic segmentation architecture, we chose U-Net due to its better characteristic extraction ability. Many useful techniques were also considered, including cross-crop strategy for saving computational resources and data augmentation for increasing the data diversity. For loss function design, we chose both focal loss and dice loss to deal with the imbalance of samples. Some parameters used for training were also optimized, such as batch-size/GPU (4) and the number of GPUs (8). For more details, please refer to the part of Segmentation Networks in Methods and Materials. Specially, a recent research has shown that transfer learning with domain-specific annotated dataset could be effective to elevate the performance of deep learning models (***Conrad and Narayan, 2021***). Therefore, we introduced a pre-trained model, trained with MoCoV2 (***He et al., 2020b***) on CEM500K (***Conrad and Narayan, 2021***). The result showed that the F1-scores of our method was 10% higher than the leader of competition (0.66 vs 0.61 in ***Table 2***). Thus we provide a new benchmark on the cellular membrane segmentation of U-RISC.

Discussion

In this paper, we first proposed the U-RISC, a cell membrane EM dataset created through intensive and elaborate annotation. The dataset is characterized by the highest resolution and the largest single image size compared with other current public available annotated EM datasets. Next, we organized a segmentation competition on U-RISC and proposed the benchmark. During the competition, we noticed that the performances of popular deep learning methods were far below

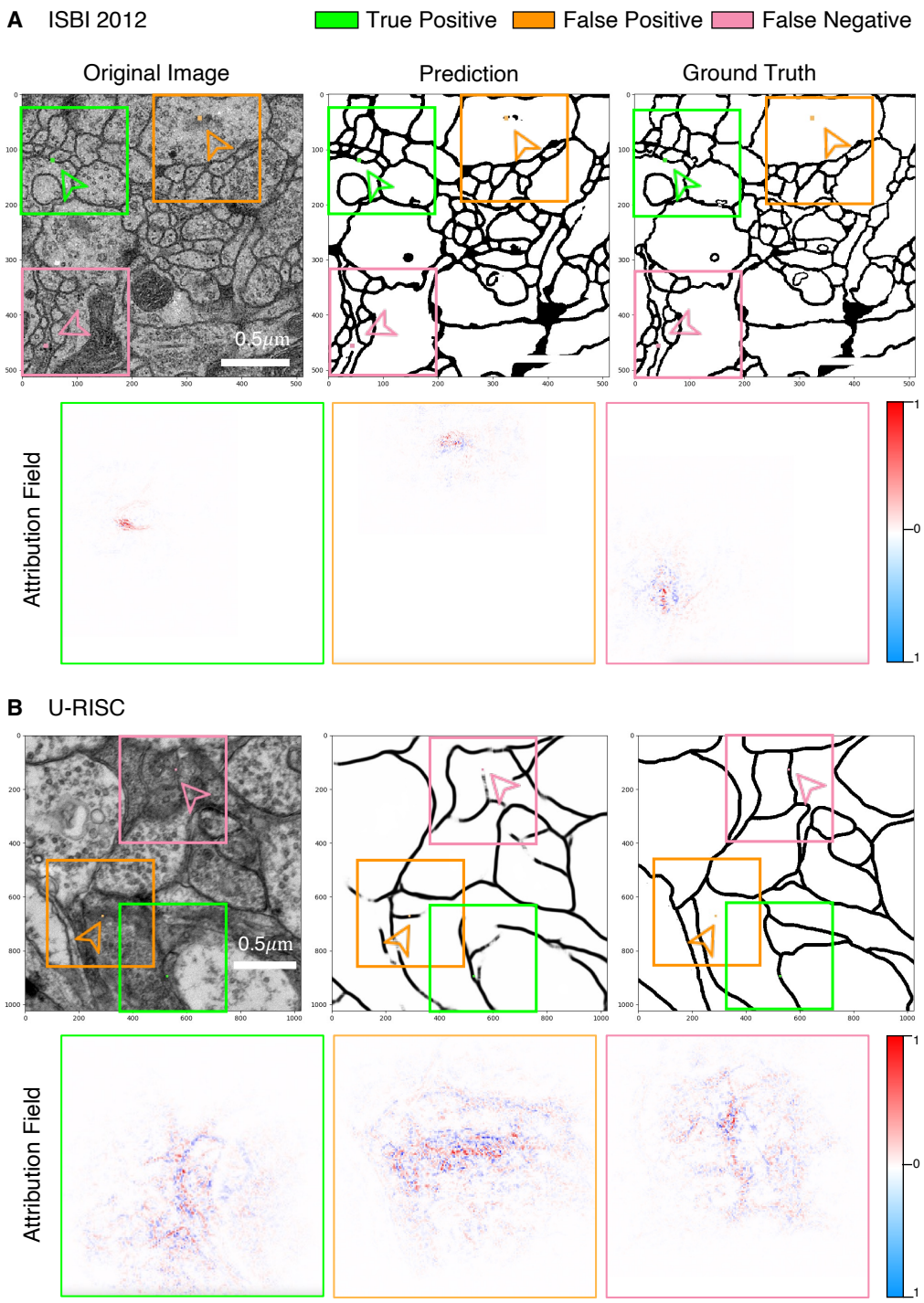

Figure 8. Attribution analysis. (A and B) Attribution fields of ISBI 2012 and U-RISC dataset. The first line represents the original image, network prediction result and annotation respectively. The pixels pointed by green (correct cell membrane pixel), orange (false positive predicted pixel) and pink (false negative predicted pixel) arrows are the prediction points used in attribution method. Images in the three color boxes with the same size in the second line represent the attribution field corresponding to the above three pixels. Blue means the network is likely to predict the pixels as cell membrane, while opposite for red.

Figure 8–Figure supplement 1. Supplements for attribution analysis on ISBI 2012.

Figure 8–Figure supplement 2. Supplements for attribution analysis on U-RISC.

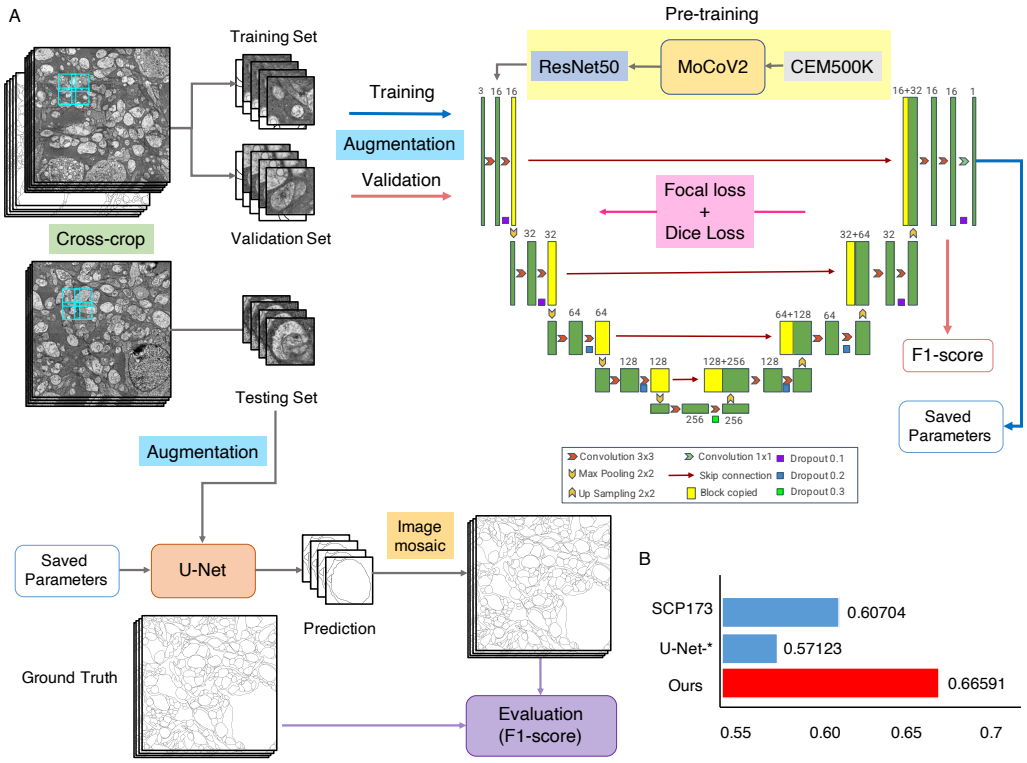

Figure 9. U-Net-transfer method achieve the best performance on U-RISC. (A) The pretraining, training and testing processing for U-Net. (B) The comparison of the F1-scores. "SCP-173" represents the top performance in the competition. U-Net-* represents the performance in ***Table 2***. Ours represents performance of the U-Net-transfer method in this section.

that of human, which motivated us to explore the causes. Thus, we carried out a comprehensive survey on the deep learning methods participants applied in the competition. To our surprise, methods such as U-Net, LinkNet, and CASENet exhibited a significant drop of F1-score on U-RISC compared with ISBI 2012, from 0.9 to 0.6. At last, to explore the mechanisms underlying this discrepant performance, we introduced a gradient-based attribution method, integrated gradient. Through attribution analysis of U-Net, we found the average pixel attribution field of U-RISC is larger than that of ISBI, corresponding to the size of cellular structure, and both of them are relatively small to the whole image size. Based on the analysis in this paper, here we raise some considerations about the challenges for deep learning-based segmentation algorithms brought by U-RISC, and propose several suggestions for the improvement of EM segmentation methods.

Challenges for Deep Learning-based Segmentation

Benchmark showed that the segmentation performance of deep learning algorithms on U-RISC was still far behind human level. U-RISC poses challenges for deep learning-based segmentation in the following aspects: (1) high computational costs needed to deal with images of large size, (2) the extreme sample imbalance caused by low ratio of cellular membrane pixels in the whole image, (3) side effects of common data processing methods.

Deep learning itself is already a computationally intensive method. It would require more computational resources to process the images with much larger size in U-RISC. In practical terms, taking U-Net as an example, processing a 1024 (px) ×1024 (px) image requires a GPU with 12GB memory. This memory is enough to deal with the images in ISBI 2012, of which the size is 512 (px) ×512 (px). But the size of a single image in U-RISC is 9958 (px) ×9959 (px), which is far beyond the processing ability of the commonly used 12 GB memory GPU. Therefore, the additional computational burden brought by U-RISC raises the first challenge for deep learning-based segmentation.

The problem of imbalanced samples widely exists in computational vison tasks (***Alejo et al., 2016***; ***Li et al., 2010***; ***Zhang et al., 2020***), which should be taken into consideration when designing algorithms. Cellular membrane segmentation is a typical situation of sample imbalance, because cellular membrane only occupies a small proportion to the whole cell structure. According to statistics, the pixels belong to cellular membrane account for 21.65% of the entire pixels of ISBI 2012. But the proportion in U-RISC is much smaller, 5.10%, making U-RISC an extreme imbalanced dataset. Pre-existing solutions were mainly proposed from several aspects: loss function design (***Lin et al., 2017***; ***Cui et al., 2019***), data augmentation (***Yoo et al., 2020***), under/over-sampling (***Fernández et al., 2018***; ***Yen and Lee, 2009***), and semantically multi-modal approaches (***Zhu et al., 2020***). While even some of the solutions were used by the participants in the competition, the improvement of segmentation was still limited as we can see from the results. So the imbalanced problem of U-RISC is yet to be solved and becomes another challenge for deep learning based segmentation.

Proper data processing is important and helpful to deep learning algorithms. For example, downsampling process on raw image with enormous size is normally adopted in segmentation tasks (***Marin et al., 2019***; ***Chen et al., 2014***). And in the Track 2 of the competition, we used downsampled dataset to reduce computational consumption as usual. Surprisingly, we found that the F1-score of the same method dropped as well as the overall performace decreased in Track 2 compared with Track 1. We speculated a key reason might be the degradation of image quality from Track 1 to Track 2 and confirmed the quality reduction through 4 representative indexes including Brenner (***Subbarao and Tyan, 1998***), Variance (***Subbarao and Tyan, 1998***), SMD2 (***Thakkinstian et al., 2005***), and Vollath (***Vollath, 2008***) (shown in appendix X). From this point of view, more caution should be paid when using traditional data processing methods and more advanced data processing techniques are expected.

Suggestions for the Improvement of Segmentation Methods

To some degree, increasing computational resources is a possible way to cope with the challenges mentioned above. However, it might be not easy for all the community researchers to get access to

sufficient computational power, therefore innovations in algorithms are still crucial for our future success. To improve the performance of deep learning in EM segmentation, we provide several suggestions for the development of deep learning algorithms from following perspectives: model design, training techniques, data processing, loss function design, and visualization tools.

Model Design. As shown in the attribution analysis, the current models for segmentation such as U-Net (***Ronneberger et al., 2015***), Efficientnet (***Tan and Le, 2019***) , and CASENet (***Zhiding Yu et al., 2017***), are designed to focus on local information to make prediction. However, in a high-resolution image, other structures, like organelle membrane and synaptic vesicles, might share similar features with cellular membrane in local scale, which leads to false negative results. And this constitutes one of the major error types in the competition. Therefore, it might be not enough for the classifiers of a model to make correct decisions with only local features. And studies have shown that models using global information could improve performance greatly (***Liu et al., 2018b***, ***2020***; ***Wang et al., 2019***). Therefore, more global information could also be taken into consideration in future design of segmentation network.

Training Techniques. Skillful training techniques also can be helpful to improve the segmentation performance. According to our survey, a two-stage training strategy could be much better than a single-stage training strategy. Besides that, much experience can be learnt from existing training methods. Hypercolumns module (***Hariharan et al., 2015***) was used to accelerate the convergence of training by combining features at different scales, and the combination of features from different scale can help to bring in global information. ScSE (***Roy et al., 2018***) module introduces attention mechanism into network, thus is another choice to bring in global information. Hybrid architectures can also be considered because of its ability to expand the receptive field (***Goceri, 2019***). In a word, improvement can be made at the phase of training by utilizing advanced training techniques.

Data Processing. Data processing is commonly used in deep learning, while traditional downsampling methods was shown to have side effects in the competition. To alleviate the side effects, some quality enhancing methods for downsampled images could be expected, such as edge and region-based image interpolation algorithms (***Asuni and Giachetti, 2008***; ***Hwang and Lee, 2004***), low bit rate-based approaches (***Wu et al., 2009***; ***Lin and Li Dong, 2006***), and quality assessment researches (***Vu et al., 2018***; ***Wang and Bovik, 2006***; ***Wang et al., 2003***). Meanwhile, other data processing methods can also be taken into account. Take data augmentation as an example, by augmenting the training data randomly, the dependence of the model on specific attributes can be reduced, which can be beneficial in EM segmentation with many imbalanced samples.

Loss Function Design. Loss function design is another important part of deep learning. But many current loss functions have their own disadvantages in our competition. For example, Dice loss (***Dice, 1945***) was designed to optimize F1-score directly, without consideration of data imbalance. Focal loss (***Lin et al., 2017***) and BCE loss (***Cui et al., 2019***) were used in the competition to care more about data imbalance by giving different penalty according to sample difficulty, but the improvement is limited as the results have shown. A better design of loss function should take overall consideration of both sample imbalance and evaluation criteria. While the most common evaluation criterion F1-score, a pixel-based statistical value, is inconsistent with human subjective feeling to some extent. It might be a major cause of the performance gap between human and algorithms. Some other structure-based criteria have appeared with better performance, such as V-Rand and V-info (***Arganda-Carreras et al., 2015***) integrating skeleton information of cell membrane, and ASSD (***Zhang, 1996***) considering the distance of point sets. Therefore, a suitable loss function is meaningful to the development of segmentaion algorithms.

Visualization tools. Visualization tools can help us have a better understanding of the network. In this paper, with IG we could learn the attribution fields of U-Net from the view of gradient, which inspires us to improve deep learning methods by paying more attention to global information. While there are many other visualization tools starting from other characteristics of the network. Layer-wise relevance propagation (LRP) (***Bach et al., 2015***) and deep taylor decomposition (DTD) (***Montavon et al., 2017***) get attribution distribution by modifying propagation rules.

Information-based method IBA (***Schulz et al., 2020***) restricts the flow of information to accomplish attribution fields. Combining different visualization tools can help to promote much more insightful inspiration in improving deep learning methods.

Overall, we provide an annotated EM cellular membrane dataset, U-RISC. It indeed brings many challenges for deep learning and promote the development of deep learning methods for segmentation.

Methods and Materials

Dataset

The U-RISC dataset was annotated upon RC1, a large scale retinal serial section transmission electron microscopic (ssTEM) dataset, publicly available upon request and detailedly described in the work of ***Anderson et al.*** (***2011***). RC1 came from the retina of a light-adapted female Dutch Belted rabbit after invivo excitation mapping. The imaged volume represents the retinal tissue with a diameter of 0.25mm, spanning the inner nuclear, inner plexiform, and ganglion cell layers. Serial EM sections were cut at 70-90nm with a Leica UC6 ultramicrotome and captured at the resolution of 2.18 nm/pixel across both axes using SerialEM (***Mastronarde, 2005***). In RC1, there are in total 341 EM mosaics generated by the NCR Toolset (***Anderson et al., 2009***), and we clipped out 120 images in the size of 9958 (px) $\times$ 9959 (px) from randomly chosen sections.

To annotate cell membrane with high quality on 120 images, we launched an iterative annotation project lasted for 3 months. All the annotators were trained to recognize and annotate cellular membrane in EM images, but only two thirds of all, 53 annotators were finally qualified to participated in the project according to their annotation results. In the iterative annotation procedure, each EM image would undergo 3 continuous rounds of annotation with the guidance of blind review. The final round of annotation was regarded as the "ground truth". While since the first two rounds are valuable for the analyzing human learning process, we also reserved the intermediate results for public release. All of the U-RISC dataset are released on https://www.biendata.xyz/competition/urisc/.

Competition

The goal of the competition was to predict cell membranes in the EM images of U-RISC. Participants were required to return images depicting the boundary of all neurons. F1-score was selected as the evaluation criterion for the accuracy of the results. There are two tracks in the competition, images in Track 1 were kept as the original size (9958 (px) $\times$ 9959(px)), images in Track 2 were downsampled to the size of 1024 (px) $\times$ 1024 (px). 50 images, 30 as the training dataset and 20 as the test dataset, were released in Track 1. And Track 2 contained 70 images in total, 40 training images and 30 testing images. The training dataset included EM images with their corresponding ground truth, while the ground truth of the test dataset was kept private. In both tracks, 10 images from the training dataset served as the validation dataset for the participants to monitor and develop their models. No statistical methods were used to determine the assignment of images in the whole arrangement.

Segmentation Networks

We conducted experiments to compare the performance of same methods on U-RISC (Track2) and ISBI 2012. Three representative deep learning networks were considerred ***Table 2***, U-Net (***Ronneberger et al., 2015***), LinkNet (***Chaurasia and Culurciello, 2017***), and CASENet (***Zhiding Yu et al., 2017***). The three networks are all pixel-based segmentation networks. To be specific, given the input image x, the goal of the networks is to classify the corresponding semantic cell membrane pixel by pixel. For the input image x and classification function $F(x)$, $Y\{p|X,\Theta\} \in [0,1]$ is taken as the output of the network, which represents the edge probability of the semantic category of the pixel p. Θ are the parameters in the network and are optimized in the training processes. Architectures of the three networks are described as follows.

U-Net. U-Net (***Ronneberger et al., 2015***) is a classical fully convolutional network (that is, there is no fully connected operation in the network). The model is composed of two parts: contracting path and expansive path. The contracting path follows the typical architecture of a convolutional network. At each downsampling step, U-Net double the number of feature channels to gain a concatenation with the correspondingly cropped feature map from the contracting path. At the final layer a 1×1 convolution is used to map each 64-component feature vector to the desired number of classes. In total the network has 23 convolutional layers. We use ResNet50 as its encoder.

LinkNet. The model structure of LinkNet (***Chaurasia and Culurciello, 2017***) is almost similar to U-Net, which is a typical encoder-decoder structure. The encoder starts with an initial block which performs convolution on input image with a kernel of size 7×7 and a stride of 2. This block also performs spatial max-pooling in an area of 3×3 with a stride of 2. The later portion of encoder consists of residual blocks and are represented as encoder-block. To reduce parameters, LinkNet uses ResNet18 as its encoder.

CASENet. CASENet (***Zhiding Yu et al., 2017***) is an end-to-end deep semantic edge learning architecture adopting ResNet-101 as backbone. The classification module here consists of a 1×1 convolution and a bilinear interpolation up-sampling layer to generate M active images, each image size is the same as the original image. Each residual block is followed by a classification module to obtain five classification activation graphs. Then, a sliced concatenation layer is used to fuse the M classification activation graphs, and finally a $5M$ channel activation graph is obtained. The activation graphs are used as the input of the fused classification layer to obtain a M-channel activation graph. The fusion classification layer is convolution of M group 1×1.

Transfer learning. The pre-trained model from (***Conrad and Narayan, 2021***) was used in our method, specifically MoCoV2 (***He et al., 2020b***) and CEM500K (***Conrad and Narayan, 2021***) were respectively selected as the pre-training method and dataset.

Training settings. For each dataset, same training and testing data distribution were utilized on the three methods. For U-RISC, during the training, the original images were cut into 1024 × 1024 patches with overlaps, and the patches was randomly split assigned in numbers of according to the ratio of 50,000/20,000 to yield the training set and the validation set, respectively. For ISBI 2012, 20 images were used for training and 10 images were used for testing.

Loss function and optimization. For each algorithm, we use same loss functions and optimization method. Specifically, focal loss and dice loss are chosen. Define y as the ground truth segmentation and y' is the predicted segmentation. The calculations of focal loss and dice loss are:

$$L_{\text{Focal}} = -(1-\hat{y})^{\gamma}\log(\hat{y}) \tag{1}$$

$$L_{\text{Focal}} = -(1-\hat{y})^{\gamma}\log(\hat{y}) \tag{2}$$

And the final loss function is the summation of the two losses with the proportion of $1:\lambda$. That is $L = L_{\text{Focal}} + \lambda L_{\text{Dice}}$. We set $\lambda = 1$ in these experiments. When optimizing the parameters in the network, we chose Adam (***Kingma and Ba, 2014***) as the optimizer.

Parameter settings. The parameters, training techniques, and loss functions were the same in both datasets. Data augmentation (random horizontal/vertical flip, random rotation, random zoom, random cropping, random cropping, random translation, random contrast, and random color jitter) were used. Four Nvidia V100 GPUs were used for training. In the testing stage, the original image was cut into the same size of training image, and the patch was tested. These patches were eventually mosaiced back to the original size for evaluation. The parameters settings are shown in ***Table 3***. Mean value and standard error are computed over testing images of each dataset. The methods with "-*" in the table represent that they are implemented by ourselves.

Table 3. Parameter settings.

Parameters	U-Net-*	CASENet-*	LinkNet-*	U-Net-transfer
Data augmentation	√	√	√	√
Pre-training	-	-	-	√
Learning Rate	1e-3	1e-7	5e-4	2e-5
Batch Size	4	2	1	4
GPUs	4	4	4	8
Epoch	100	300	300	50
Worker	16	16	8	32

Image definition criteria

Four representative image definition criteria **Brenner** (***Subbarao and Tyan, 1998***), **SMD2** (***Thakkinstian et al., 2005***), **Variance** (***Saltelli et al., 2010***), and **Vollath** (***Vollath, 2008***) were used for analyzing the effects of downsampling on EM images. The former two consider the difference and variance of gray values between adjacent pixels, while latter two among pixels of the whole image.

Brenner gradient function is the simplest gradient evaluation function, which simply calculates the square of the gray difference between two adjacent pixels.

$$D(f) = \sum_{y} \sum_{x} |f(x+2, y) - f(x, y)|^2 \tag{3}$$

where: $f(x, y)$ represents the gray value of pixel (x, y) corresponding to image f, and $D(f)$ is the result of image definition calculation (the same below).

SMD2 multiplies two gray variances in each pixel field and then accumulates them one by one.

$$D(f) = \sum_{y} \sum_{x} |f(x, y) - f(x+1, y)||f(x, y) - f(x, y+1)| \tag{4}$$

The variance function is defined as follows:

$$D(f) = \sum_{y} \sum_{x} |f(x, y) - \mu|^2 \tag{5}$$

where: μ is the average gray value of the whole image, which is sensitive to noise. The purer the image, the smaller the function value.

The vollath function is defined as follows:

$$D(f) = \sum_{y} \sum_{x} f(x, y) \cdot f(x+1, y) - M \cdot N \cdot \mu^2 \tag{6}$$

where: μ is the average gray value of the whole image, M and N are the width and height of the image respectively.

Attribution analysis

The purpose of the integral gradient (IG) method is to quantify the contribution of each part of the input feature to the decision. To be specific, for a given input image x and model $F(x)$, the goal of IG is to find out which pixels or features in x have an important influence on the decision-making of the model, and sort pixels according to the importance value of each pixel or feature in x. Such a process is defined as attribution. IG uses the integral value along the whole gradient line from input to output. In the cell membrane segmentation task, for the decision of a pixel of y (predicted as cell membrane or not), we can get the contribution of each pixel of the input image $x_{i,j}$ to it. Put the contribution of each pixel together, we record it as an ***Attribution Field*** A, whose size is the same

as the original image, and the value of each pixel is $w_{i,j}$ represents the contribution decision of pixel $x_{i,j}$ to y. $w_{i,j}$ is normalized to $[-1, 1]$.

In binary segmentation task, for the current input image x, if we know that the output y is a specific value, such as $y = 0$, and the corresponding reference image is x'_i, then we can take a linear interpolation, that is

$$x' + \alpha \left(x - x'\right). \tag{7}$$

If the constant $\alpha = 0$, then the input image is the base image as x'_i. And if $\alpha = 1$, then the input image is the current image, which is x. When $0 \leq \alpha \leq 1$, it can be other images.

For the output of the neural network $F(x)$, the formula of the IG method is shown as below.

$$A_i(F, x, x') = (x_i - x'_i) \times \int_{\alpha=0}^{1} \frac{\delta F\left(x' + \alpha\left(x - x'\right)\right)}{\delta x_i} d\alpha \tag{8}$$

In the formula 8, δx_i on the denominator denotes variation. This design makes the whole partial derivative transform into the form of variation. The variation boundary is the reference image and the current image, and the variation path can be selected arbitrarily. The integral gradient method uses linear interpolation as the variational path. That is,

$$\gamma(\alpha) = x' + \alpha \left(x - x'\right). \tag{9}$$

Here we select the random noise image as the reference image.

As the resolution and image size of U-RISC and ISBI 2012 are different, for fair comparison, we also define the size of ***Pixel Attribution Field*** as S_k, which represents the real size of the actual biological neuron tissue corresponding to the pixel area with fixed contribution value threshold k. The pixels with higher contribution are divided by a selective threshold of k, that is, if the contribution value $w_{i,j}$ is greater than k, the pixel is the one with higher contribution to decision-making. Area of attribution field S_k is obtained by multiplying the area of attribution field value $w_{i,j} > k$ and the real tissue area (resolution h) corresponding to each pixel.

$$S_k = S(A_{w_{i,j}>k}) \times h^2, w_{i,j} \in A. \tag{10}$$

Data analysis

All statistical tests used, including statistic values and sample sizes, are provided in the figure captions, including mean and standard. All analysis were performed using custom software developed using the following tools and softwares: MATLAB (R2018a), Python (3.6), PyTorch (1.6.0), NumPy (1.19.0), SciPy (1.5.1), and matplotlib (2.2.3).

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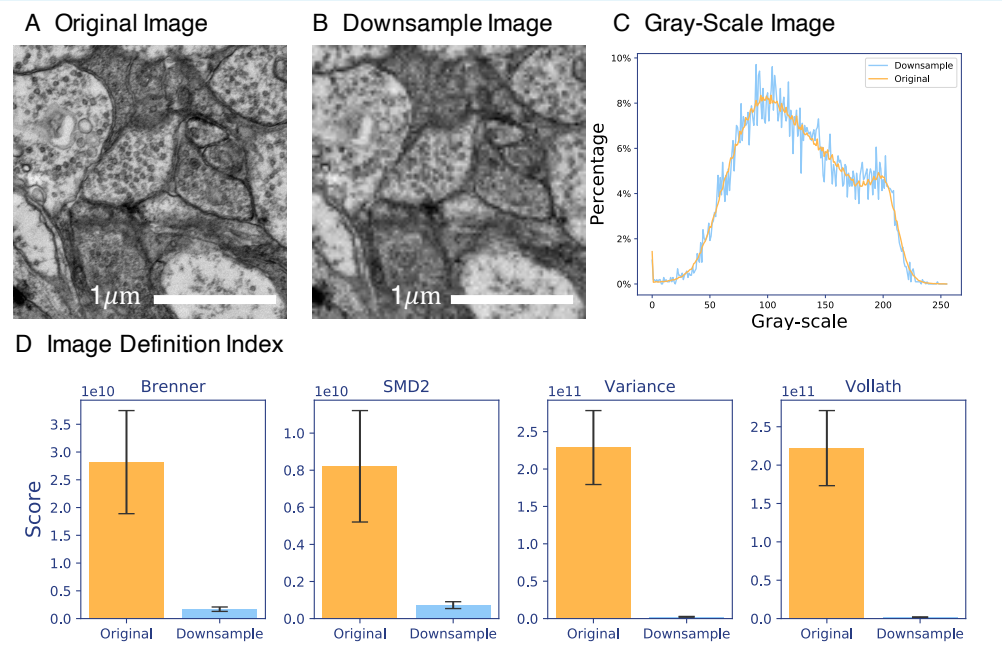
Appendix 1

The Effects of Downsampling on U-RISC Dataset

To our best knowledge, downsampling is an effective approach to process images with enormous size in segmentation tasks (***Marin et al., 2019***; ***Chen et al., 2014***). With the purpose of investigating the effects of downsampling on image definition quality, we analyzed the gray-scale histograms on a group of original and downsampled images in U-RISC, and then calculated the definition values of images in Track 2.

We first found that downsampling produced numerous mutations between adjacent gray value (***Figure 1***(C)). In our analysis, the mutations might engender the texture information in the image to be blurred, and the edges of the cell membrane became blur. For example, the bilayer structure of membrane disappeared after downsampled (***Figure 1*** (A,B)), and some of the cell membranes which were hard to recognizable became obscured. We then found that the definition quality of the images in Track 2 becaome lower after downsampled. Four definition indexes of all the images in Track 2 were calculated. Brenner (***Subbarao and Tyan, 1998***), Variance (***Subbarao and Tyan, 1998***), SMD2 (***Thakkinstian et al., 2005***), and Vollath (***Vollath, 2008***) are common indexes to show the gray value change between adjacent pixels. Results suggested that image indexes were significantly decreased after downsampling (***Figure 1***(D)), and all the four indexes dropped about ten times. Therefore, the analysis indicated that images were heavily blurred after downsampling operation.



Appendix 1 Figure 1. Differences between original image and downsampled image.(A) The crop of original image. (B) The crop of downsampled image at the same position. The Gray-scale Histograms is calculated on A and B. (C) The scores of definition indexes calculated on the whole U-RISC dataset before and after downsampling. Details of indexes are described in Methods and Materials.

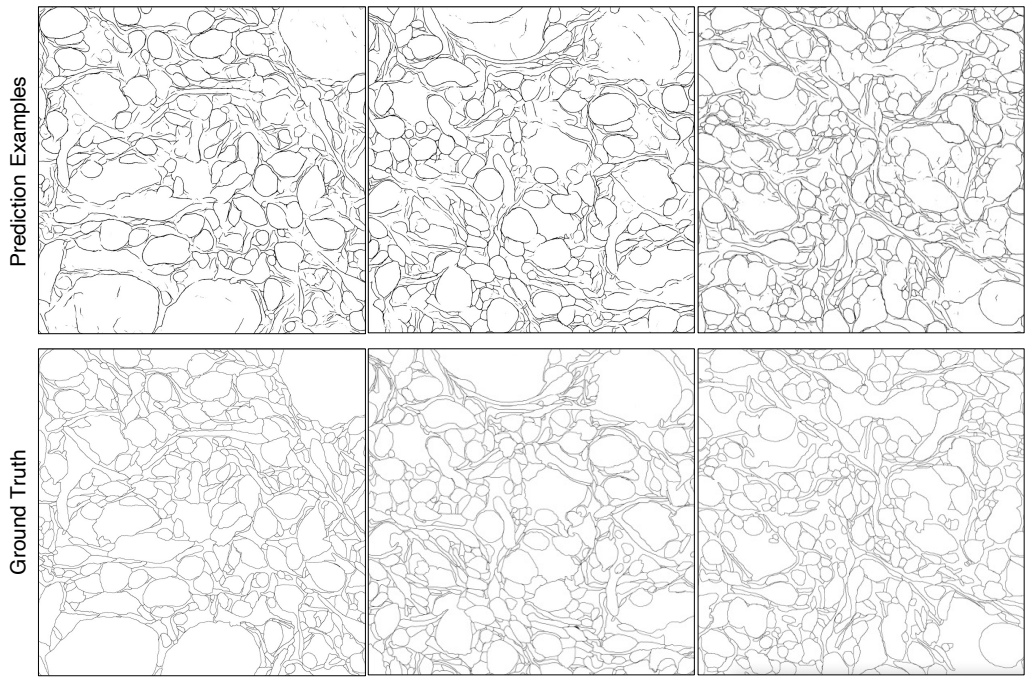


Figure 6–Figure supplement 1. Supplements for segmentation predictions of U-RISC.

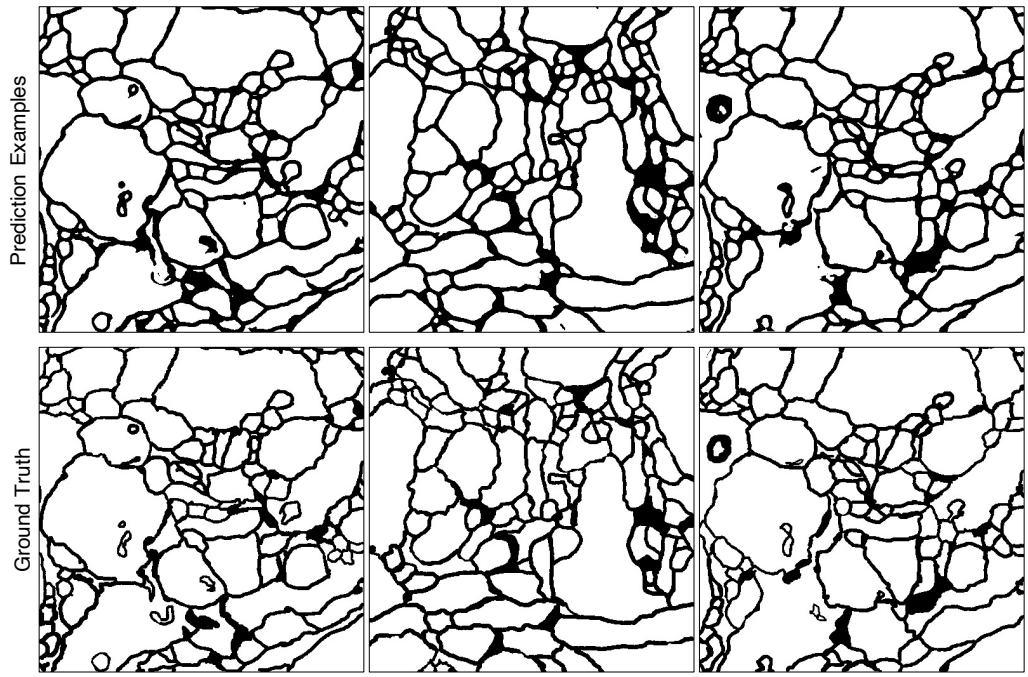


Figure 6–Figure supplement 2. Supplements for segmentation predictions of ISBI 2012.

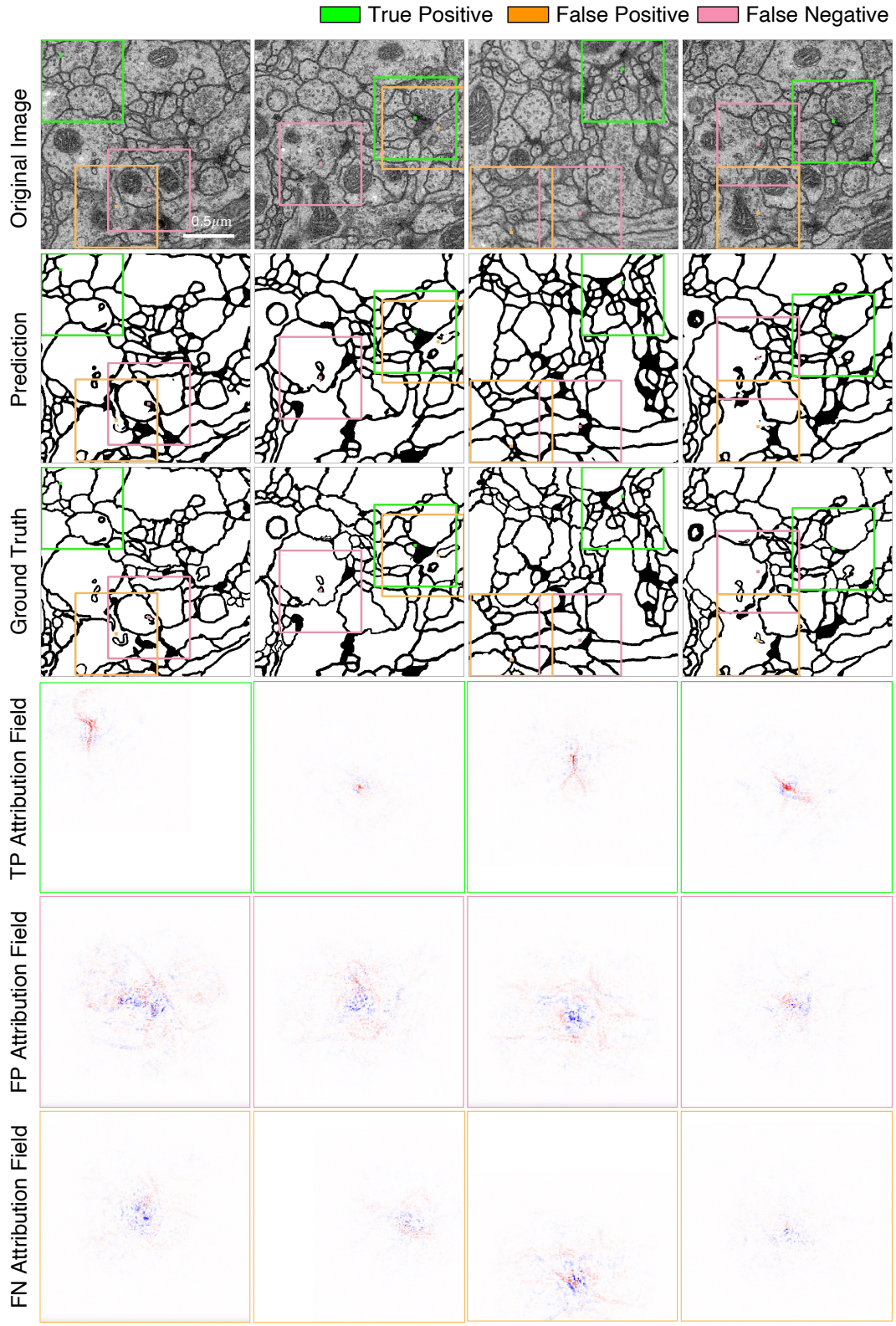


Figure 8–Figure supplement 1. Supplements for attribution analysis on ISBI 2012.

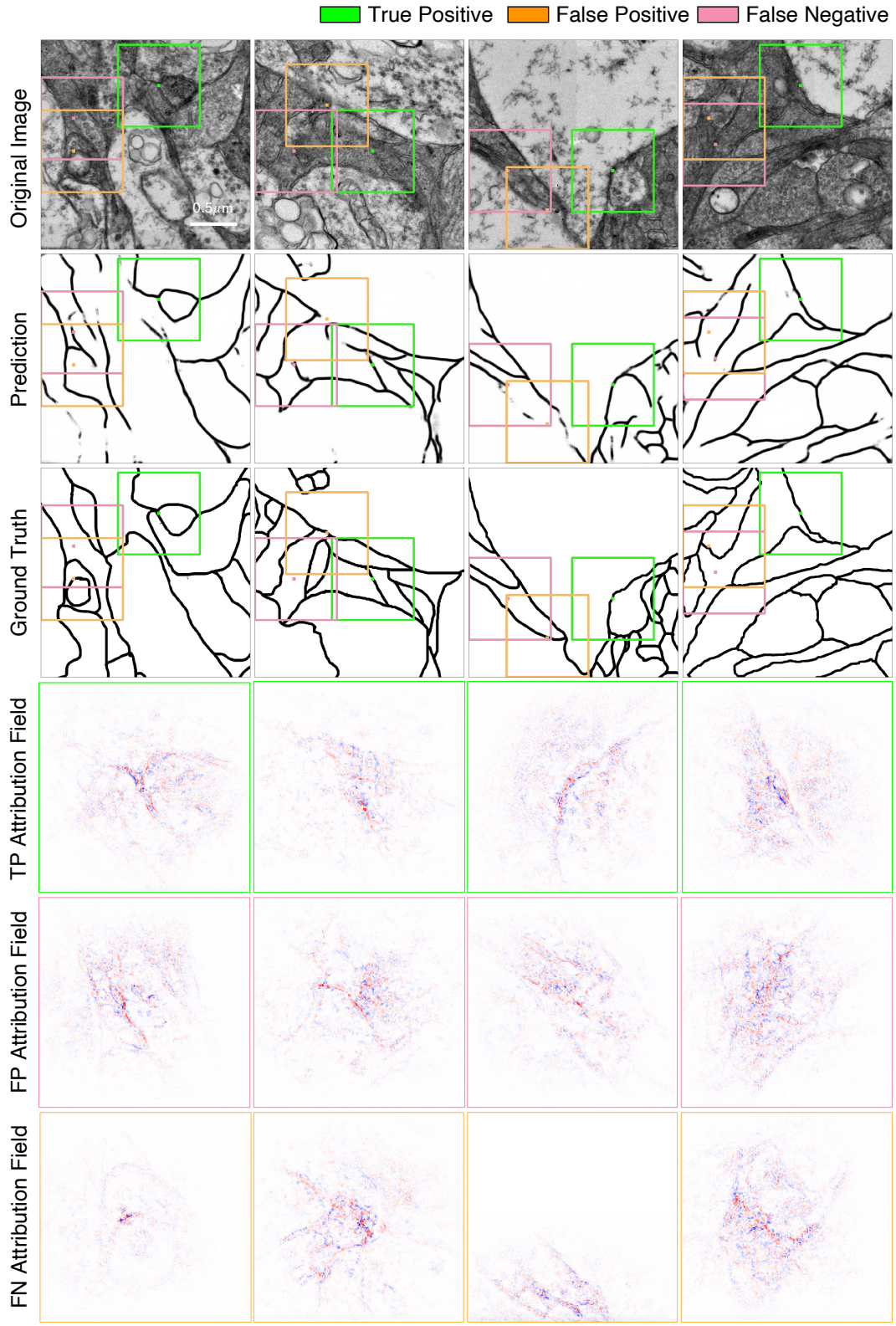


Figure 8–Figure supplement 2. Supplements for attribution analysis on U-RISC.