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Patch-Based Semantic Segmentation for Detecting Arterioles and Venules in Epifluorescence Imagery

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Abstract

Segmentation and quantification of microvasculature structures are the main steps toward studying microvasculature remodeling. The proposed patch based semantic architecture enables accurate segmentation for the challenging epifluorescence microscopy images. Our pixel-based fast semantic network trained on random patches from different epifluorescence images to learn how to discriminate between vessels versus nonvessels pixels. The proposed semantic vessel network (SVNet) relies on understanding the morphological structure of the thin vessels in the patches rather than considering the whole image as input to speed up the training process and to maintain the clarity of thin structures. Experimental results on our ovariectomized - ovary removed (OVX) - mice dura mater epifluorescence microscopy images shows promising results in both arteriole and venule part. We compared our results with different segmentation methods such as local, global thresholding, matched based filter approaches and related state of the art deep learning networks. Our overall accuracy (> 98%) outperforms all the methods including our previous work (VNet). [1].

1. Introduction

Vessel segmentation is important for studying and understanding the morphological attributes for microvasculature remodeling under different experiments and conditions such as the effect of some diseases, hormones, environmental changes or removing some parts of the body such as ovary in our case. Manual annotation is tedious and time consuming. Automated and computerized algorithms helps human in such strenuous tasks. Many methods and algorithms have been introduced in the literature to solve various problems in

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biomedical application [2], [3], [4], [5], [6], [7], [8], [9], [10], [11], however, those methods may fail with challenged and complicated structures because they are either matched filter based approaches or rely on hand crafted features. Recently deep learning architecture shows the state of the art results in both classification and segmentation, however, segmentation for biomedical images still not that popular due to the limitation of annotated data compared to other problems. Regardless of that, different ideas and architecture [12], [13], [14], [15], [16], [17], [18], [19], [20], [21] have been proposed toward achieving better accuracy and less time for training and testing.

Our ovariectomized - ovary removed (OVX) - mice dura mater epifluorescence microscopy images characterized by several challenges such as contrast variations, different foreground and background configurations, depth occlusion, stain diffusion with no distinct boundaries for the venule part. Our previous work [1] characterized by good segmentation for the arteriole part, however it did not work well with venule part (not introduced in the paper). Our proposed algorithm works well for both of them with less time needed for the training and testing. Our network accepts image patches, learn the semantic features and then apply the learned parameters to test the full image.

The rest of the paper is organized as follows. Section 2 provides the details of our CNN architecture and the mechanism of training and testing, Section 3 describes the data set and the evaluation methods. Section 4 provides experimental results on epifluorescence microscopy images and comparisons with other methods. Finally, Section 5 concludes the paper.

2. Semantic patch based segmentation

Semantic segmentation provides the state of the art results in biomedical images [22], however, those architectures usually consists of deep layers to understand the coherency between the pixels in the image and to learn how to classify pixels corresponding to the objects similar to them. Our architecture follows the same idea, however, we apply small semantic network that consists of 9 layers only to the (32×32) patches rather than the whole images. Applying patches takes advantage from the similarity of vessel segments and to maintain the clarity of thin structures after max-pooling operation. This provides us with a fast network that can learn the relations between pixels through the patches and then apply this learning to predict the full image.

2.1. The proposed architecture

The proposed semantic vessel network (SVNet) consists of 9 layers: 3 convolution layers, 2 ReLU, 1 transposed convolution, 1 pooling layer and 1 softmax layer at the end to discriminate between classes see figure 1 for better visualization. Convolution is a simple procedure in which different random filters convolved with the input layers and feature maps to produce features that characterize the data set without human supervision, ReLU (Rectified linear Unit) is necessary to add some non-linearity to the output and it eliminates the gradient vanishing problem caused by other functions such as sigmoid. Pooling performs down sampling with considering either the max, min, or average of the values to summarize the region and choose the best feature values. Then by up sampling, the image can return to

it's original size with learning the semantic and the relations between pixels whether they are corresponds to the foreground (vessels in our case) or background (non-vessel). Softmax layer is responsible upon that decision depending upon the probability map produced by the network. Cross entropy with stochastic gradient descent algorithm are responsible to learn the parameters. Our proposed architecture built on top of Matlab2017b using the neural network toolbox.

2.2. Training and testing

Any deep learning architecture needs to be trained first with some data, then use the trained model with the learned parameters to test different data and perform classification either by image, patch or pixel level. In our work, the trained model discriminates pixel-wise between vessel and non-vessel pixels. Our architecture accepts patches with size 32 × 32 and predict pixel wise the whole image. This architecture has two advantages compared to our previous work [1]:

- The training doesn't need any condition for the patches to be considered as foreground. In our previous work [1], the patch is considered as foreground if the vessel passes through the center of the patch, otherwise it is a background. In this architecture, there is no need for any condition as the network will learn the morphological features from the patches themselves using our semantic network. All the patches go through our CNN and then use the trained model to predict the whole image.
- Our new architecture is faster to converge compared to [1] since it learns pixels attributes and their relations rather than classifying patches. See figure 2, the top part contains two sets of patches that considered as input for both networks (VNet [1]) and our proposed (SVNet), however, the training is different between the two. In A, the vessel is considered a vessel/foreground if the the vessel passes through the center of the patch otherwise, it is a non-vesel/background patch. In B, SVNet will learn the coherency between pixels through the annotated ground truth. For this reason, in (A), the net needs time to learn this discrimination, whereas in our new architecture, learning the attributes is fast as it considers the relations between pixels. Further, our previous work [1] was computationally expensive, testing needs about 20 minutes to predict every image since it considers the prediction of overlapping patches. Currently, it needs just seconds to predict all the images. See section 4 for more details.

3. Data set and evaluation

The experiments were performed on high resolution epifluorescence images of mice dura mater acquired using a video microscopy system (Laborlux 8 microscope from Leitz Wetzlar, Germany) equipped with 75 watt xenon lamp and QICAM high performance digital CCD camera (Quantitative Imaging Corporation, Burnaby, Canada) at 0.56 micron per pixel resolution. In our experiments, we utilize 20 epifluorescence microscopy images taken form the dura mater layer in ovariectomized (OVX) mice. 10 images are from knock-out mice (KO) and other 10 are from wildtype mice. The big goal behind the work is to quantify the

segmented regions to detect the difference in remodeling between arteries and veins in different conditions. We've evaluated our work through 4 evaluation methods: Dice, Sensitivity(sens), Specificity(spec) and accuracy(acc).

$$Dice(P,Q) = \frac{2|P \cap Q|}{|P| + |Q|},$$

where P and Q are the pixel level automatic and ground-truth (GT) segmentations; values closer to one indicate better performance compared to the physiologist expert verified gold standard.

$$Sensitivity = \frac{TP}{TP + FN},$$
$$Specificity = \frac{TN}{TN + FP},$$
$$Accuracy = \frac{TP + TN}{TP + FP + FN + TN}$$

where TP stands for true positive, TN for true negative, FP for false positive and FN for false negative.

4. Experimental results and discussion

Table 1 shows the validation results for 20 OVX epifluorescence microscopy images for both arteriole and venule parts. We compared our results with 4 methods: Otsu (global thresholding) [23], Multi scale line detector [24], MNIST (CNN optimized to discriminate between digits) built on top of MatConvNet [25] and retrained with our vessel images, VNet (vessel net) [1] a CNN network which reconstructs the image through testing it's overlapping patches and our proposed SVNet (semantic vessel network). In terms of Dice, it can be observed from our results a difference of 3% between arteriole part (87.96) and the whole vascular network (arteries and veins) which is about (84.53). The prediction of venule part is usually worse than the arteriole part as it doesn't have any distinct boundaries causing stain leakage to randomly diffused around the vein that lead sometimes to cover the vessel itself. Usually Dice metric can give us a real estimation about the quality of segmentation since it considers the intersection (overlapping) between the prediction and the ground truth, however, we also consider other evaluation metrics to give us better view about the prediction. It appears that our results are better than all other methods (Dice, Sensitivity, and Accuracy) except a small difference in specificity with our previous work [1] due to some false positive predictions. Figure 4 shows visually the difference between various methods. Otsu [23] in the third column suffers from false positive depicted by the blue color which indicates that fast thresholding techniques may fail with these challenged images. MS-line [24] which is a type of match filters approaches that depends upon a line detector oriented in different orientations to find a response for each pixel, it also has a lower value compared to our work. CNN-mnist, that originally built to identify linear structures (digits), didn't recognize very well the whole network. Further, VNet [1] has good values, however, it is computationally expensive. Finally, our prediction by SVNet is better by 2% in terms of arteriole part and by 3% in terms of the whole network compared to [1]. Our semantic patch based network has been trained on 32 × 32 patches from 20 different microscopy

epifluorescence images. The dimension of each image is 1036×1360. The total number of patches are 120,499 selected randomly which is even less than the training patches in VNet [1] that use 880,600 patches. It has been trained for the same number of epochs (60 epochs) as shown in figure 3, however in our case, it takes only 112 minutes to converge whereas the training in VNet [1] took 10 hours for every 10 epochs and it took 20 minutes for testing the full image since the full image reconstructed from predicting all the overlapping patches. In addition, the figure shows that training accuracy is over 90 with the first 10 epochs which doesn't need more than 20 minutes with 8 core CPU.

5. Conclusions

Our proposed semnatic patch based network (SVNet) tested on epifluorescence microscopy images shows promising results compared to other methods. Our architecture accepts image patches and predict pixel wise segmentation results. Our work characterized by its fast convergence through training, further, it has fast prediction with testing. It has been tested on a very challenged data set for OVX mice. Those images suffers from contrast variations, depth occlusion, clustered diffusion and no distinct boundaries for the venule part. The experimental section shows better results in Dice 84.53% and overall accuracy is above 98%.

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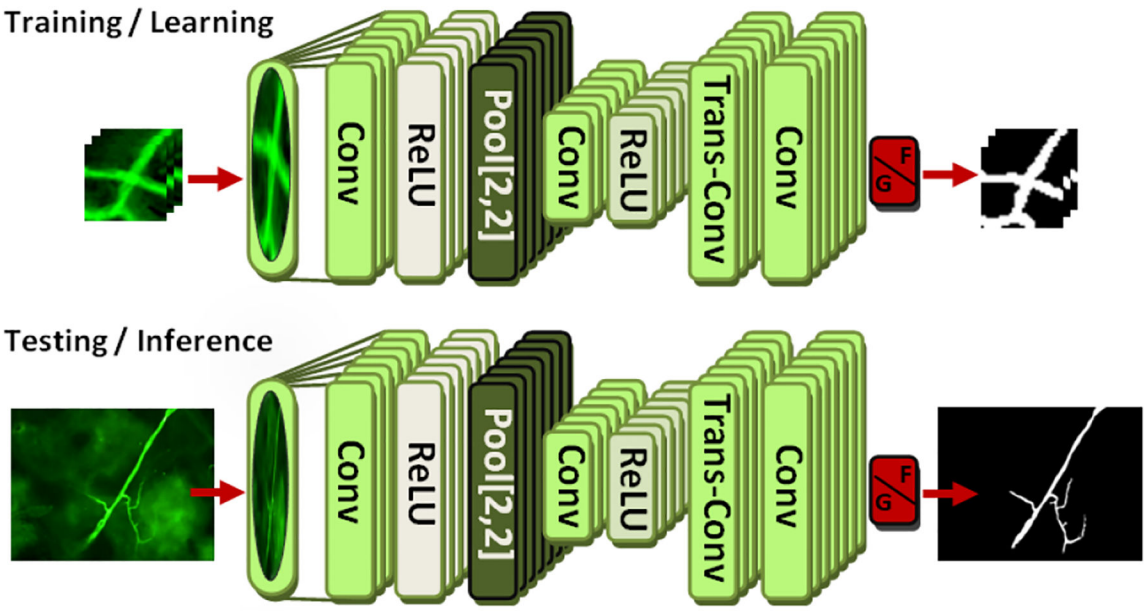

Figure 1.

The top network is used for training, it accepts input patches with (32×32×1) dimension, feature map's depth is 64×64, convolution filters are (3×3), transposed convolution filters are (4×4), the output of last convolution layer is either foreground (vessel) pixel or background (non-vessel) pixel, softmax with stochastic gradient descent to learn network parameters. At testing stage shown at the bottom, the learned model is used to test the whole image

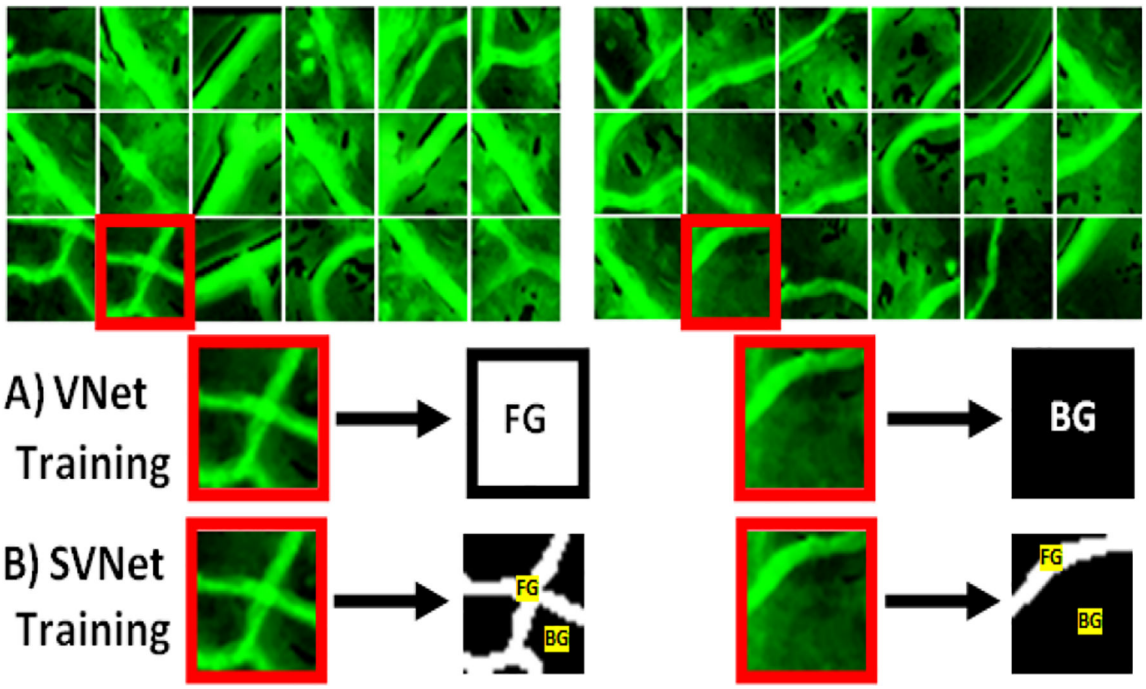

Figure 2.

Advantage of SVNet over VNet [1] in terms of training that lead to fast and better convergence (a) In VNet, the patch considers as vessel/foreground(FG) if the vessel pass through the center of the patch otherwise it is a non-vessel/background(BG), (b) In our proposed SVNet, the network will learn pixel wise the FG pixels and BG pixels corresponding to the manual ground truth as depicted by the yellow small boxes.

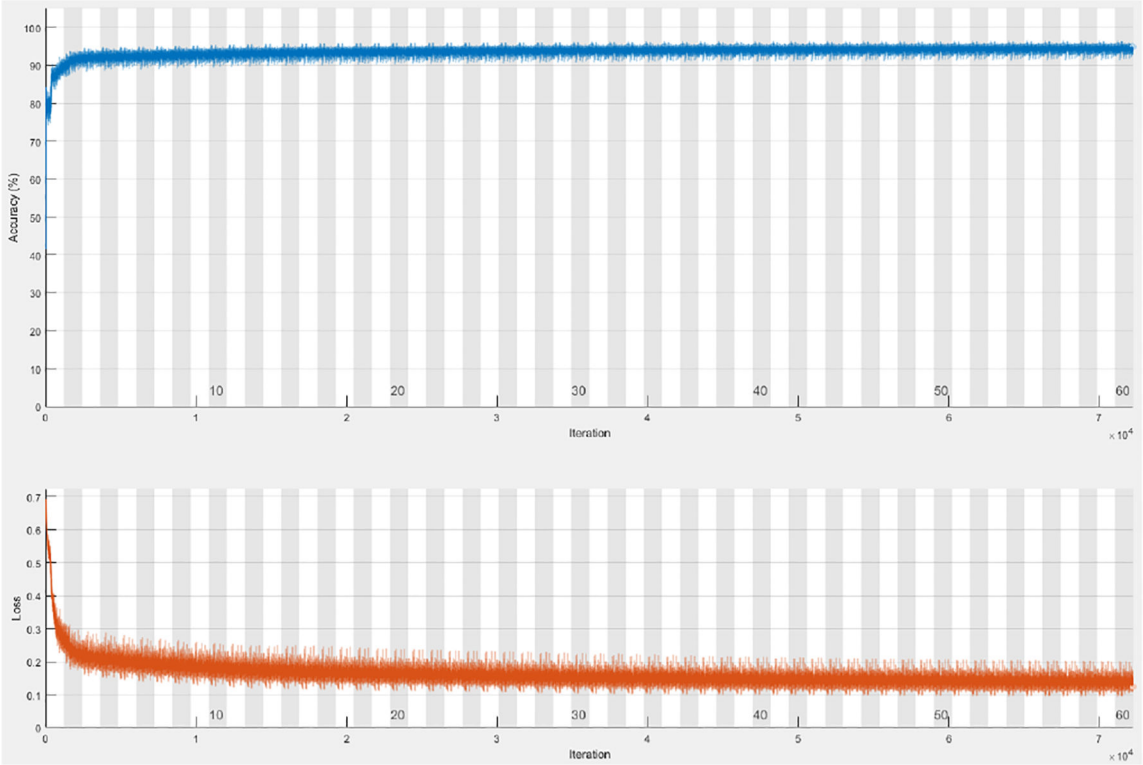

Figure 3.

Reported accuracy taken from the report produced by Matlab 2017b, it shows the accuracy and loss for 60 epochs, it can be observed that our training accuracy has fast convergence as it is above 90 within the first 10 epochs, we consider 60 epochs just to compare with [1]

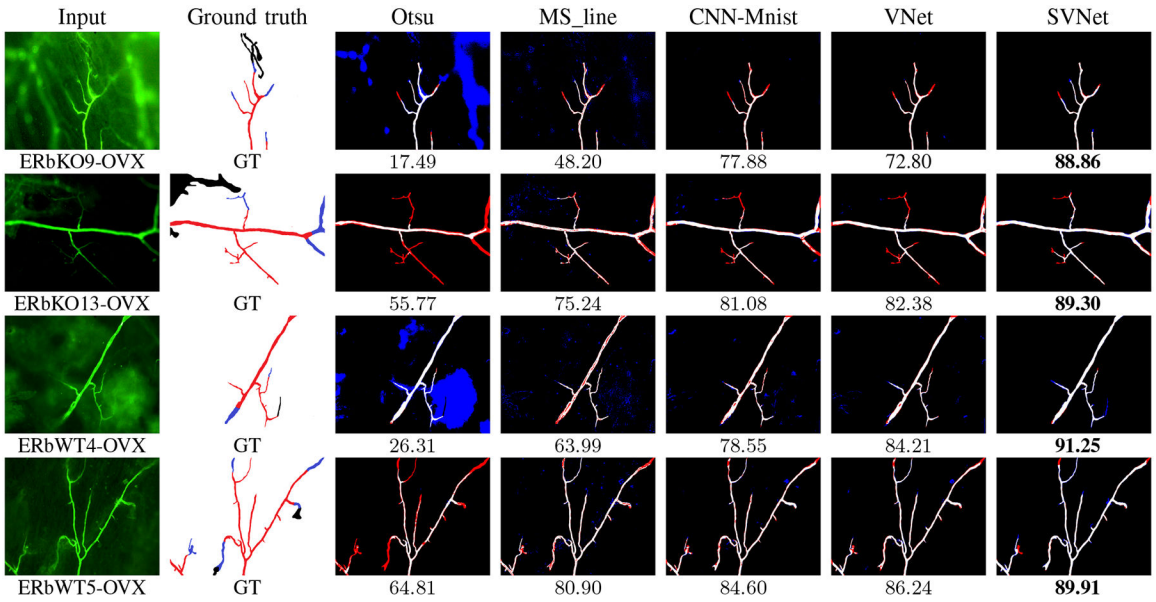

Figure 4.

Comparison of segmentation methods on ERβ- knock-out (KO) images 9, 13 and ERβ-wild type (WT) images 4, 5. We show the optimal segmentation (with respect to Dice metric) of different methods. (a) Input image, (b) Manually drawn ground truth (GT) of arteries and veins produced and validated by 3 physiologists, (c) Otsu thresholding [23], (e) Multiscale line detector (MS_line) [24], (f) Deep network with 8 layers built on top of MatConcNet to recognize digits (CNN-Mnist), (g) Deep network with 14 layers (VNet) [1]. (h) Our proposed semantic network with 9 layers (SVNet). White regions represent correctly segmented foreground pixels, red are missing (false negative). and blue are extra regions (false positive) compared to GT.

TABLE 1.

QUANTITIVE COMPARISON OF SEGMENTATION METHODS ON POST OVARY ACTOMY (OVX) MICE DURA MATER EPIFLUORESCENCE IMAGES WITH OTSU (GLOBAL THRESHOLDING) [23], MULTI-SCALE LINE DETECTOR [24], CNN-MNIST [25], VNET (VESSEL NET) [1] AND OUR PROPOSED SVNET (SEMANTIC VESSEL NET). WE SHOW THE OVERALL AVERAGE DICE, SENSITIVITY, SPECIFICITY, AND ACCURACY VALUES FOR A SET OF 20 EPIFLUORESCENCE MICROSCOPY IMAGES

Arteriole Segments						Arteriole and Venule Segments				
Method	**Otsu Thres.**	**Multiscale Line Det.**	**CNN Mnist**	**VNet**	**SVNet**	**Otsu Thres.**	**Multiscale Line Det.**	**CNN Mnist**	**VNet**	**SVNet**
Dice	55.91	65.56	80.16	85.26	**87.96**	51.79	66.22	77.02	81.55	**84.53**
Sens	67.41	79.48	76.51	79.52	**90.03**	59.26	73.40	69.75	74.06	**83.56**
Spec	95.33	97.13	99.54	**99.79**	99.51	95.70	97.77	99.54	**99.54**	99.42
Acc	94.16	97.80	98.79	99.11	**99.21**	93.72	96.62	98.23	97.77	**98.71**

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