doi:10.1017/S1431927617001386
Microsc. Microanal. 23 (Suppl 1), 2017


# Deep Learning Segmentation for Epifluorescence Microscopy Images

Yasmin M. Kassim[1], Olga V. Glinskii[2,5], Vladislav V. Glinsky[3,5], Virginia H. Huxley[2,5] and Kannappan Palaniappan[1]

[1.] Computational Imaging and VisAnalysis (CIVA) Lab, Department of Electrical Eng. and Computer Science/University of Missouri-Columbia, Columbia, MO, USA.
[2.] Department of Medical Pharmacology and Physiology/University of Missouri-Columbia, Columbia, MO, USA.
[3.] Department of Pathology and Anatomical Sciences/University of Missouri-Columbia, Columbia, MO, USA.
[4.] National Center for Gender Physiology/University of Missouri-Columbia, Columbia, MO, USA.
[5.] Research Service, Harry S. Truman Memorial Veterans Hospital, Columbia, MO, USA.

Vessel segmentation and quantification is important in many application such as early disease detection, quantify the effect of some drugs or hormone and discover some differences between certain species and gender. Automatic segmentation [2, 3] is widely required since marking the images manually is tedious that needs much time and effort. Most of the existing algorithms and papers are suitable for retinal images such as DRIVE, STARE, and CHASE, however there has not been as much work done in microscopy epifluorescence segmentation.

Our work considers vessel localization for dura mater in the upper part of the brain in the mouse. The goal is to quantify the effect of estrogen receptor between ovary intact (OV) versus post ovary ectomy in both wild type and knock-out mice. This work needs an accurate segmentation algorithm for later quantification and analysis. For that reason we utilize deep learning convolutional network for this purpose since it proves that it achieves the state of the art results in many domains and applications. Our images suffer from uneven contrast, noisy background mainly because of the leakage of epifluorescence dye, depth occlusion and the existence of blood cells that looks attached to the vessel which lead to be a false detection. Our proposed method has less error and more accurate in dealing with such challenges.

In typical classification and pattern recognition problems, convolution neural network (CNN) takes an image as input and produces a probability map as output. It basically performs multiple operations through hidden layers to produce some high level features that can potentially represent the target classes. The important operations that usually occurs are convolution, max pooling and rectified linear units (ReLU).

In our work, we utilized deep CNN for the purpose of microvasculature segmentation from epifluorescence microscopy imagery. Our adapted CNN receives 32*32 patches of the microscopy images, and outputs two classes for microvasculature network as foreground, and other regions as background. This network has been built on top of the existing MatConvNet toolbox [1]. In the training phase, the goal is to let the network learn how to identify a given patch as vessel or non-vessel. We considered each patch as vessel if the corresponding ground truth has 1's in the middle of the patch, i.e. if the four middle pixels in the ground truth are 1, then the corresponding patch will be labeled as part of a foreground vessel, otherwise, it is considered as background. Our new CNN architecture consists of 9 convolution layers, 3 ReLU and 2 pooling layers. The filter size is 5*5 for the first 4 layers, then it is 3*3 for the next 3 layers, with the last two fully connected. Some layers have padding to keep the spatial

sizes the same, and others have not. The last softmax layer is to discriminate between the two classes to produce a patch which is either a foreground or background. As with any classification and deep learning approaches, the network need to be trained first, and then used that model to test the images. The training phase takes patches from different 10 epifluorescence microscopy images with stride equal to 4 (correspond to 880600 number of patches). The training continues for a specific number of epochs which is set at 60 for our data. In the testing phase, we used overlapped patches through the network to obtain smooth binary segmentations.

In this paper, we proposed a deep learning architecture (CNN) for our challenging epifluorescence microscopy imagery. In this work, we consider the arteriole part of the vessel and compared our work with one of the well know algorithms in extracting the vessel network for fundoscopy imagery. Our network produces noticeable results with high accuracy in different evaluation methods. For the future work we will consider semantic segmentation and pixel based prediction to improve our results and to decrease the prediction time for overlapped patches.

Table 1 shows our results compared with one of the state of the art segmentation methods [2] in retinal images in terms of Dice, Sensitivity, Precision and Accuracy. Figure 1 shows some examples of our images with ground truth (GT) and segmentation results for two methods.

References:

[1] A. Vedaldi *et al*, ACM Inter. Conf. Multi. (2015) p. 689.
[2] U. T. V. Nguyen, A. Bhuiyan and L. A. F. Park, Pattern Recognition **46** (2013), p. 703.
[3] A. Sironi, E. Turetken and V. Lepetit, IEEE Trans. Patt. Anal. Mach. Intell. **38** (2015), p. 1327.

| Method | Multi scale line detector | Our method |
|---|---|---|
| Dice | 65.56 | 85.26 |
| Sensitivity | 79.48 | 79.52 |
| Precision | 59.66 | 93 |
| Accuracy | 97.8 | 99.11 |

**Table 1**. Comparison of evaluation methods

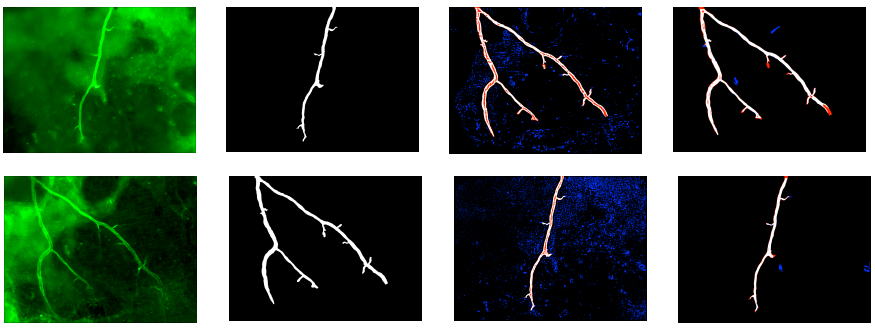


**Figure 1**. Segmentation results, blue color represent false positive, red represent false negative

https://doi.org/10.1017/S1431927617001386 Published online by Cambridge University Press