Brain vascular image Segmentation Based on Fuzzy Local Information C-Means Clustering

Chaoen Hu[a,b,c], Xia Liu[a], Xiao Liang[b,c], Hui Hui[b,c], Xin Yang[b,c], Jie Tian[b,c]
[a]School of Automation, Harbin University of Science and Technology, Harbin 150080, China
[b]Key Laboratory of Molecular Imaging, Institute of Chinese Academy of Sciences, Beijing 100190, China
[c]Beijing Key Laboratory of Molecular Imaging, Institute of Automation, Beijing, 100190, China

ABSTRACT

Light sheet fluorescence microscopy (LSFM) is a powerful optical resolution fluorescence microscopy technique which enables to observe the mouse brain vascular network in cellular resolution. However, micro-vessel structures are intensity inhomogeneity in LSFM images, which make an inconvenience for extracting line structures. In this work, we developed a vascular image segmentation method by enhancing vessel details which should be useful for estimating statistics like micro-vessel density. Since the eigenvalues of hessian matrix and its sign describes different geometric structure in images, which enable to construct vascular similarity function and enhance line signals, the main idea of our method is to cluster the pixel values of the enhanced image. Our method contained three steps: 1) calculate the multi-scale gradients and the differences between eigenvalues of Hessian matrix. 2) In order to generate the enhanced micro-vessels structures, a feedforward neural network was trained by 2.26 million pixels for dealing with the correlations between multi-scale gradients and the differences between eigenvalues. 3) The fuzzy local information c-means clustering (FLICM) was used to cluster the pixel values in enhance line signals. To verify the feasibility and effectiveness of this method, mouse brain vascular images have been acquired by a commercial light-sheet microscope in our lab. The experiment of the segmentation method showed that dice similarity coefficient can reach up to 85%. The results illustrated that our approach extracting line structures of blood vessels dramatically improves the vascular image and enable to accurately extract blood vessels in LSFM images.

Keywords: Brain vascular image enhancement, Light Sheet Microscopy, Feedforward Neural Network, Blood vascular segmentation, Fuzzy local information c-means clustering

1. INTRODUCTION

Blood vessel segmentation is one of the most important tasks in image analysis for biological applications, especially in tumor study. Semi-automatic [1] or automatic blood vessel region extraction approaches can provide useful information for image registration and three-dimensional reconstruction in image post-processing procedure. In the literature, various methods have been proposed for blood vessel segmentation and feature extraction, for instance intensity-based methods [2], matched filtering [3], model based approaches [4-5] and so on.

Generally, the vessel segmentation can be divided into two steps. Firstly, a vessel enhancement procedure is performed to enhance vessel structures using vessel enhancement filtering. Then, various segmentation algorithms are applied for delineating the vessels in acquired images. To enhance the line structures in blood vascular network, the classical way is to apply vessel enhancement filtering based on Hessian matrix [6-7]. With the purpose of extracting line structures of blood vessels, the correlative linear objectives of the eigenvalues of Hessian matrix was used to construct vascular similarity function in these filters. However, the design of accurate and efficient vessel segmentation algorithms is still challenging, due to the variety and complexity of images, especially for brain blood vessel segmentation. As the emergence of new theories and techniques, machine learning techniques [8-10] and statistical theory [2] are widely used for blood vessel segmentation.

Imaging, Manipulation, and Analysis of Biomolecules, Cells, and Tissues XV, edited by Daniel L. Farkas, Dan V. Nicolau, Robert C. Leif, Proc. of SPIE Vol. 10068, 100680Q
 · doi: 10.1117/12.2251725

In this paper, a feedforward neural network trained by 2.26 million pixels for dealing with the correlations between multi-scale gradients and the differences between eigenvalues was used to enhance the vascular image, and then FLICM was used to cluster the pixel values in enhance line signals.

2. METHOD

1.1 Multi-scale features calculation

In this work, the vascular similarity function is trained by using the multi-scale gradients and the differences between eigenvalues of Hessian matrix.

$$\begin{cases} L(w,h,\sigma) = G(w,h,\sigma) * I(w,h) \\ G(w,h,\sigma) = \dfrac{1}{2\pi\sigma^2}\exp\left(-\dfrac{w^2+h^2}{2\sigma^2}\right) \end{cases} \tag{1}$$

where $I(w,h)$ is the gray level in position (w,h) of image to segment. $G(w,h,\sigma)$ is the Gaussian kernel function. σ is defined as scale. Notation $*$ denotes the 2-D convolution operation. The gradient of image I over w and h direction, namely L_w^σ and L_h^σ, are defined as,

$$L_w^\sigma(w,h) = \left(\frac{-w}{2\pi\sigma^2}\exp\left(-\frac{w^2+h^2}{2\sigma^2}\right)\right) * I(w,h), L_h^\sigma(w,h) = \left(\frac{-h}{2\pi\sigma^2}\exp\left(-\frac{w^2+h^2}{2\sigma^2}\right)\right) * I(w,h) \tag{2}$$

The Hessian matrix of image I in position (w,h) is defined as

$$H^\sigma(w,h) = \begin{bmatrix} L_{xx}^\sigma & L_{xy}^\sigma \\ L_{xy}^\sigma & L_{yy}^\sigma \end{bmatrix} \Rightarrow d\lambda^\sigma(w,h) = \lambda_1^\sigma - \lambda_2^\sigma = \sqrt{\left(L_{xx}^\sigma - L_{yy}^\sigma\right)^2 + 4L_{xy}^{\sigma\,2}} \tag{3}$$

where $d\lambda^\sigma$ is difference between λ_1^σ and λ_2^σ, which are the eigenvalues of Hessian matrix and $\left|\lambda_1^\sigma\right| \geq \left|\lambda_2^\sigma\right|$.

1.2 Vascular structure enhancement

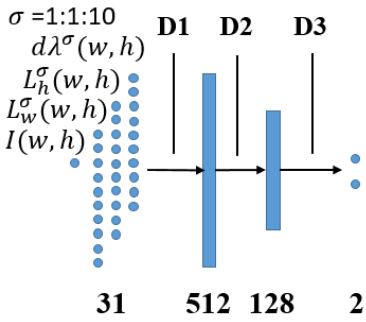
In this subsection, a feedforward neural network (FNN) trained by 2.26 million pixels is used to enhance the vascular structure, as shown in Figure 1. The input layer of FNN contains 31 units, which are represented by $I(w,h)$ $L_w^\sigma(w,h)$, $L_h^\sigma(w,h)$ and $d\lambda^\sigma(w,h)$ (σ from 1 to 10 with 1 step in pixel w and h). Next, three fully connected layers (D1-D3) are used in FNN, with each output unit connected to all inputs. These layers are able to capture correlations between features computed by equation (2) to (3). Parametric Rectified Linear Unit (PReLU) [11] is also used as an activation function after all fully connected layers because of nonlinearity purposes, so the output unit with all input's units is defined by

$$P\,\mathrm{ReLu}(x) = \begin{cases} x & x > 0 \\ ax & x \leq 0 \end{cases}, u_j = P\,\mathrm{ReLu}\left(\sum_{i=0}^{n} w_{ij} \times u_i + \theta_j\right) \tag{4}$$

where a is a learned array with the same shape as x, u_j and u_i are the j-th output unit and the i-th input unit respectively, n is the number of input units, w_{ij} is the weight between u_j and u_i. θ_j is the bias of the j-th output unit. For the output layer that includes two units, the activation value is converted into probability distribution over the class labels by using a binary softmax function, which is defined by

$$p_k = \frac{e^{u_k}}{e^{u_0} + e^{u_1}}, k \in \{0,1\} \tag{5}$$

where μ_k is the k-th input of output layer, p_k the probability assigned to the k-th class.



	Input layer	Dense1	Dense2	Dense3	Output layer
Input shape	31	31	512	128	2
Output shape	31	512	128	2	2

Figure 1. FNN architecture.

For each training sample, the goal of FNN training is to minimize the cross-entropy loss function. Suppose that y_n is the true label for a given input pixel, the loss function is defined as:

$$loss = -\frac{1}{N}\sum_{n=1}^{N}\left[y_n \log \hat{y}_n + (1-y_n)\log(1-\hat{y}_n)\right] \tag{6}$$

where the $\hat{y}_n$ is the output of FNN. The weights of the FNN are initialized with the Xavier algorithm [12] and updated by the stochastic gradient descent (SGD) [13] algorithm.

1.3 Fuzzy Local Information C-Means Clustering

FLICM is an effective tool to segment the intensity homogeneity image. Firstly, FLICM has a fuzzy partition matrix,

$$P_{ik} = \left\|x_j - v_k\right\|^2, G_{ki} = \sum_{j\in N_i, i\neq j}\frac{1}{d_{ij}+1}(1-\mu_{kj})^m P_{jk}, \mu_{ki} = 1/\left(\sum_{j=1}^{c}\left(\frac{P_{ik}+G_{ki}}{P_{ij}+G_{ji}}\right)^{\frac{1}{m-1}}\right) \tag{7}$$

where the i-th pixel is the center of the local window, k is the reference cluster and the j-th pixel belongs in the set of the neighbors falling into a window around the i-th pixel (Ni). d_{ij} is the spatial Euclidean distance between pixels i and j, μ_{ki} is the probability of the i-th pixel belong to the k-th cluster, m is a real parameter, c is the number of the cluster prototypes , the prototype of the center of cluster k is

$$v_k = \left(\sum_{i=1}^{N}\mu_{ki}^m x_i\right)/\left(\sum_{i=1}^{N}\mu_{ki}^m\right) \tag{8}$$

where x_i is the value of the i-th pixel, N is the number of pixels in the image to segment. Therefore, the detailed steps of FLICM algorithm are described as follows.

Initialize : $c = 2, m = 2, \varepsilon = 10^{-5}$, *Maxsteps* $= 100$.

the loop counter : $l = 0$.

Initialize randomly the fuzzy partition matrix.

Do

{ $l = l + 1$;

update v_k^l *by equation* (8).

update μ_{ki}^l *by equation* (7).}

while $\left(l < Maxsteps \text{ or } \max\left\{\left|\mu_{ki}^l - \mu_{ki}^{l-1}\right|\right\} < \varepsilon\right)$

The pixel i is assigned to the class C by

$$C_i = \arg_k \left\{ \max \left\{ u_{ki} \right\} \right\}, \mathrm{k} \in \{1,2\}. \tag{9}$$

3. APPLICATION OF MOUSE BRAIN VASCULAR IMAGES SEGMENTATION

In this work, the new-born (5 days) C57BL/6J mice were sacrificed to prepare intact brain samples. Alexa Fluor-488 fluorescence antibody to CD31 were used to stain mice brain vessels, and then brain samples were cleared by using the iDISCO method [14]. In order to acquire a series of mouse brain vascular images, the intact mouse brain was imaged by light-sheet microscope (Lavision Biotech Ultrascope II) equipped with a sCMOS camera and 2x/NA 0.5, 6mm working distance dipping cap. The FFN was trained by 2.26 million pixels that manual labeled from a part of mouse brain vascular images. The information (I , L_w^σ, L_h^σ and $d\lambda^\sigma$, σ from 1 to 10 with 1 step) of all pixels in image to be segmented were fed into the FNN with enhanced vascular images. Then FLCM was used to segment the enhanced vascular images, as shown in Figure 2. Figure 3(a) shows the image to be segmented. The enhanced image in Figure 3(b) shows that the trained FNN is adept at fitting vascular similarity function. Figure 3(b) shows that proposed method is suitable for extracting vessel even for intensity inhomogeneity, and the dice similarity coefficient (DSC) [15] is 85%.

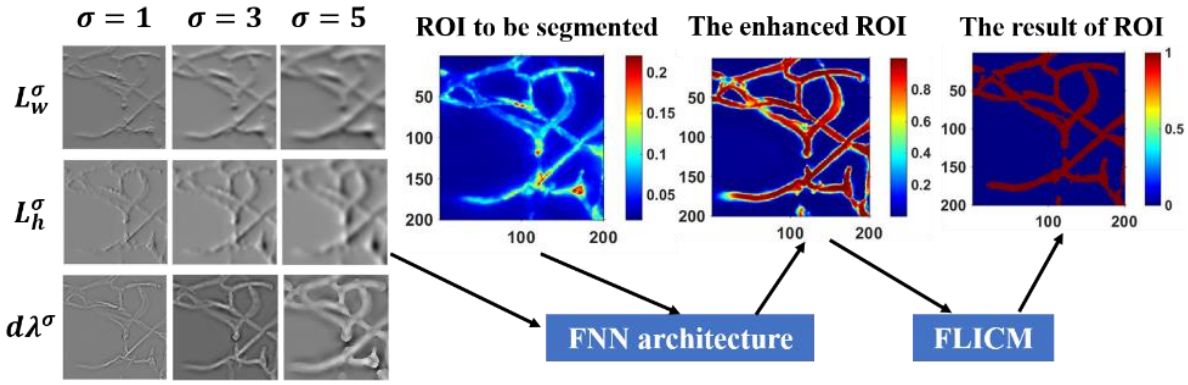
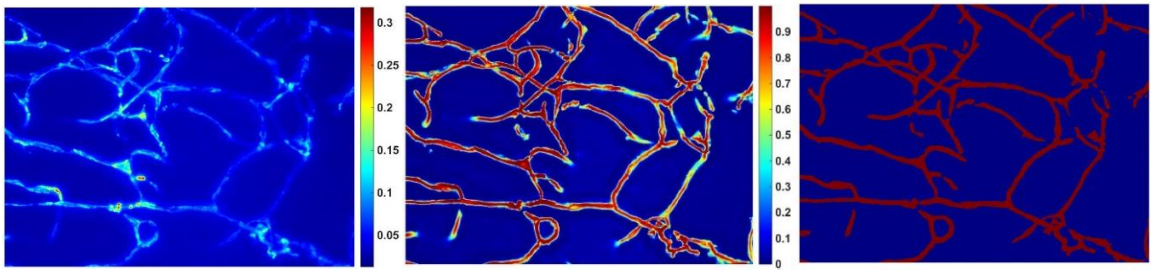


Figure 2. The schematic of the method for brain vessel segmentation



(a) (b) (c)

Figure 3. (a) The acquired brain vessel image, (b) the enhanced image of (a) by using FNN and (c) is the segmentation results.

4. CONCLUSION

In this paper, we have proposed a vascular image segmentation method by clustering the enhanced image with FLCM. The main contributions of this work are to suppress intensity inhomogeneity and enhance vascular contrast, which directly improve the segmentation result. In our method, a FNN architecture designed to fitting vascular similarity function has been used to enhance vascular image, and then cluster the pixels' value of enhanced image. We have

applied our method to mouse brain vascular images acquired by LSFM, and the experimental results demonstrated that the proposed method effectively extracted blood vessels for both large vessels and micro-vessels. It is noted that FNN fits vascular similarity function by some priori features. In future work, a modified Convolutional Neural Network by more training pixels will be involve in the method.

ACKNOWLEDGEMENT

This paper is supported by the National Natural Science Foundation of China under Grants 81527805, 8130134, 81227901, 61231004 Scientific Research and Equipment Development Project YZ201457, Strategic Priority Research Program XDB02060010 of Chinese Academy of Sciences, Beijing Municipal Science & Technology Commission No. Z161100002616022.

REFERENCES

[1] Wang Z, Kyono H, Bezerra H G, et al. "Semiautomatic segmentation and quantification of calcified plaques in intracoronary optical coherence tomography images." Journal of Biomedical Optics 15(6), 061711-061711(2010).
[2] Krinidis, S., & Chatzis, V. "A robust fuzzy local information c-means clustering algorithm." IEEE Transactions on Image Processing, 19(5), 1328-1337(2010).
[3] Yousefi, S., Qin, J., Zhi, Z., & Wang, R. K. "Label-free optical lymphangiography: development of an automatic segmentation method applied to optical coherence tomography to visualize lymphatic vessels using hessian filters." Journal of Biomedical Optics, 18(8), 86004(2013).
[4] Liu, W., Liu, T., Song, W., Yi, J., & Zhang, H. F. "Automatic retinal vessel segmentation based on active contours method in Doppler spectral-domain optical coherence tomography." Journal of Biomedical Optics, 18(1), 61-66 (2013).
[5] Lu, S., Huang, H., Liang, P., Chen, G., & Xiao, L. "Hepatic vessel segmentation using variational level set combined with non-local robust statistics." Magnetic Resonance Imaging, 36, 180-186 (2016).
[6] Yang, J., Ma, S., Sun, Q., Tan, W., Xu, M., & Chen, N., et al. "Improved hessian multiscale enhancement filter." Bio-medical materials and engineering, 24(6), 3267-75(2014).
[7] Chen, K., Yin, Q., Jia, X., & Lu, M. "Image enhancement based improved multi-scale hessian matrix for coronary angiography." International Journal of Computer Applications, 126(10) (2015).
[8] Dan, C. C., Giusti, A., Gambardella, L. M., & Schmidhuber. "Deep neural networks segment neuronal membranes in electron microscopy images." Advances in Neural Information Processing Systems, 25, 2852--2860(2012).
[9] Havaei, M., Davy, A., Warde-Farley, D., Biard, A., Courville, A., & Bengio, Y., et al. "Brain tumor segmentation with deep neural networks." Medical Image Analysis, 35, 18-31(2016).
[10] Prentašić, Heisler, P., Mammo, M., Lee, Z., Merkur, S., & Navajas, A., et al. "Segmentation of the foveal microvasculature using deep learning networks." Journal of Biomedical Optics, 21(7), 075008-075008 (2016).
[11] He, K., Zhang, X., Ren, S., & Sun, J. "Delving deep into rectifiers: surpassing human-level performance on imagenet classification." 2015 IEEE International Conference on Computer Vision (ICCV), 00(0), 1026-1034 (2015).
[12] Glorot, X., & Bengio, Y. "Understanding the difficulty of training deep feedforward neural networks." Journal of Machine Learning Research, 9, 249-256(2010).
[13] Lecun, B. Y. et al, "Gradient-based learning applied to document recognition." Proceedings of the IEEE, 86(11), 2278-2324 (1998).
[14] Renier, N., et al., "iDISCO: a simple, rapid method to immunolabel large tissue samples for volume imaging. " Cell 159(4), 896–910 (2014).
[15] Zhang, Y. J. "A survey on evaluation methods for image segmentation." Pattern Recognition, 29(8), 1335-1346 (1996).