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Random Forests for Dura Mater Microvasculature Segmentation Using Epifluorescence Images

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Abstract

Automatic segmentation of microvascular structures is a critical step in quantitatively characterizing vessel remodeling and other physiological changes in the dura mater or other tissues. We developed a supervised random forest (RF) classifier for segmenting thin vessel structures using multiscale features based on Hessian, oriented second derivatives, Laplacian of Gaussian and line features. The latter multiscale line detector feature helps in detecting and connecting faint vessel structures that would otherwise be missed. Experimental results on epifluorescence imagery show that the RF approach produces foreground vessel regions that are almost 20 and 25 percent better than Niblack and Otsu threshold-based segmentations respectively.

I. Introduction

Angioadaptation is the process by which vascular networks in organs and tissues remodel their morphological structures and functional properties due to physiological and pathological processes. In this paper we use image analysis to quantify the role of estrogen receptors (ER) in effecting microvasculature remodeling in ER-beta wild-type and knock-out mice. We use epifluorescence imagery of the brain dura mater stained

using Alexa Fluor 488-conjugated soybean agglutinin (SBA lectin), to reliably quantify the microvessel remodeling under different conditions. Quantifying the microvascular network structure requires accurate image segmentation of the venules and arterioles comprising the microvessels. Challenges include inhomogeneous staining of the microvasculature (i.e. excess stain in the background or lack of stain within the lumen of the vasculature), different binding properties between arterioles or capillaries (distinctive boundaries), venules or veins (non-distinct boundaries), uneven contrast, low texture content, and nonlinear binding of the fluorescence dye. The motivation behind our work is to determine the effect of estrogen receptors on angiogenesis, pruning and vascular remodeling using a mouse model.

Numerous thin structure segmentation techniques have been proposed in the literature for blood vessel tree structure segmentation in retinal funduscopy, angiogram imaging or malarial retinopathy [1]–[4]. In contrast to such methods for vessel tree segmentation there has not been as much work done on extracting the microvasculature graph in epifluorescence imagery [5] which have high variance in both arteriole and venule vessel regions as seen in Figure 1 (top row). In order to accurately segment vessel boundaries, we propose a system that builds upon our previous work on multi-focus fusion and edge preserving smoothing [5]–[7]. In this work, we consider a random forest (RF) classifier with multiscale image features for mice dura mater microvascular image segmentation and automatic network vessel graph generation. We characterize vessel specific features for learning a classifier using random forest bagging for reliable binary segmentations (see Figure 1 (bottom row)).

Rest of the paper is organized as follows. Section II describes our proposed improved random forest method for segmenting microvascular structures. Section III provides detailed experimental results on epifluorescence imagery of brain dura mater from mice, and conclusions in Section IV.

II. Microvasculature Image Segmentation Using Random Forest (RF) Classifier

Traditional global segmentation methods [5] often fail to obtain reliable segmentations due to the complex nature of imaging the microvasculature in the dura mater and inhomogeneous fluorescence staining of tissues. In this paper, we use random forest [8] classifiers with vessel specific features for epifluorescence imagery. Random forests belong to a class of techniques that use an ensemble learning method for classification and regression. For classification they operate by constructing a collection of decision trees at training time and pixels are labeled using the mode of the votes from the set of decision trees. The random forest binary classifier for vessel segmentation is built using 30 trees of variable depth (MinLeafSize=1). It usually takes 4 hours in Matlab on a server with dual Intel Xeon CPUs(E5-2630) each running at a clock speed of 2.40GHz with 128GB DDR4 memory.

A. Features for segmenting microvasculature networks

In order to construct accurate microvasculature network graphs, spatially varying complex vessel shapes of the vasculature need to be extracted. We first extract a set of image features that are sensitive to linear structures across multiple scales since the vessel diameters can

vary significantly across arterioles and venules even within the same field of view as well as across images.

Frobenius norm of the Hessian matrix (6-D)—A second order vessel *structureness* term was initially defined by Frangi et al [9], [10]. The second derivative Hessian operator $\mathcal{H}$ is useful for detecting ridges, valleys and peaks. The Frobenius norm of the multiscale Hessian matrix usually has small values in the background where there are no strong linear or blob-like features; the maximum eigenvalue will be large in the foreground regions containing vessel-like structures. Such a feature is quite informative for distinguishing between heavy diffused stain in the background versus vascular structure regions. The RF feature vector uses transformed eigenvalues from the Frobenius norm at each of five scales, σ ,

$$\left\| \mathcal{H}_{\sigma}(\tilde{\lambda}_{+}, \tilde{\lambda}_{-}) \right\|_F = \sqrt{\tilde{\lambda}_{+}^2 + \tilde{\lambda}_{-}^2} \quad (1)$$

The eigenvalues are transformed using the hyperbolic tangent function:

$$\tilde{\lambda}_{+, -}(\sigma) = \frac{1 - \exp(-|\lambda_{+, -}|)}{1 + \exp(-|\lambda_{+, -}|)}. \quad (2)$$

Bright epifluorescence stained vessels have values above the mean level and the *tanh* normalization helps in enhancing the interior of vessel structures. We calculate the Frobenius norm (1) using Gaussian filters for five scales (scale normalized), $\sigma = \{1, 2, 3, 4, 5\}$ ¹, see Figure 2 first row. Additionally, the maximum response of the regular Frobenius norm ($\|\mathcal{H}_{\sigma}(\lambda_{+}, \lambda_{-})\|_F$) of the Hessian across five scales followed by z-score sigmoid normalization.

Oriented second derivatives (6-D)& Laplacian of Gaussian (LoG) filters (2-D)

—We used a subset of 26 multiscale filters from the Leung-Malik 48-filter bank [11] consisting of 18 oriented second derivative (6 orientation, 3 scale, mean normalized) and 8 isotropic LoG filters. First derivative filters were too sensitive to background noise and were not used. We used the maximum response of the 6 oriented second derivative filters over 3 scales $\sigma = \{\sqrt{2}, 2, 2\sqrt{2}\}$ ². The intuition behind this local directional second derivative filter feature was to retain the dominant filter response, regardless of orientation, corresponding to thin linear or ridge-like vascular structures. The scale specific orientation feature is included for each pixel and assigns a discrete orientation angle index/label corresponding to the maximum filter response. Two LoG-based features are the maximum response over 8 scales ($\sigma = \{\sqrt{2}, 2, 2\sqrt{2}, 4\}$ and 3σ) and the corresponding scale index.

Multiscale line detector (1-D)—The multiscale line detector feature is based on the approach of Nguyen et al [1] which improves upon the basic line detector in [2]. In this method a variable length set of oriented lines in spatial scale are used, with 12 oriented

¹Each scale uses a filtering window size of $[-3\sigma, 3\sigma]$

²<http://www.robots.ox.ac.uk/vgg/research/texclass/filters.html>

lines sampled regularly between 0 to 360 degrees. By varying this length parameter we can generate lines of different scales which can detect different diameter vessels as in [1]. We have selected the window size (W) to be 15 and the range of L is $\{3, 5, 7, 9, 11, 13, 15\}$ as in [1].

$$R_{combined} = \frac{1}{n_L + 1} \left(\sum_L R_W^L + I_{igc} \right) \quad (3)$$

where $R_W^L = I_{max}^L - I_{avg}^W$ is the response of the basic line detector within the window W , L denotes the pixel length of the required lines, I_{max}^L is the winning line, I_{avg}^W is the local intensity average at W , n_L is the number of scales, and I_{igc} is the inverted green channel pixel value.

B. Training and testing the RF

Two sets of experiments using estrogen receptor (ER- β) ovary intact mice with wild-type (WT) and knockout (KO) strains were done resulting in 20 dura mater epifluorescence high resolution images with pixel dimensions 1036×1360 . Nine images were used for training using a total of 12,680,640 labeled samples. A total of 15 features were computed over the training data to produce the classifier training vector. Details about the 15-D feature vector is given in Table I, and Figure 2. We used manually labeled ground truth segmentations of the microvasculature images supervised by an expert for pixel-based training using the green fluorescence channel. The performance evaluation in this paper is restricted to the ground-truth labels for *arterioles* only that have more well-defined boundaries compared to the venules with more diffuse boundaries that will be considered elsewhere. After obtaining the pixel-wise segmentation using RF, we utilize a fixed set of post-processing steps to improve the binary vessel segmentations including: (i) dilate to connect the small disconnections, (ii) fill the holes, (iii) remove the false positive spurious pixels, (iv) erode to return to the original vessel size (see Figure 3).

III. Experimental results

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. We utilized a set of 20 epifluorescence images consisting of 10 ER- β wild-type (012606-ERbWT), and 10 ER- β knock-out (012706-ERbKO) intact mice. Figure 4 shows six sample images (3 KO, 3 WT) with manual ground truth, two common automatic segmentation methods (Otsu and Niblack) and our RF results. Parameter sweeping was used to search for the optimal Niblack segmentation parameters like local window size and threshold. The same post-processing was used in all three segmentation methods. We evaluated the segmentation methods using the Dice similarity coefficient,

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

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. As seen in Figure 4(c) the Niblack method misses salient vessels (high false negatives shown in red) which is reflected in the low average sensitivity in Table II. Otsu thresholding results, Figure 4(d), shows a lot more false background pixels as part of the foreground (high false positives shown in blue) which is reflected in the lowest specificity compared to other methods. The Dice values in Table II show that the proposed RF performs significantly better than Niblack and Otsu global thresholding methods. Note that the Dice coefficient better characterizes the overall performance compared to accuracy which is biased towards the background class.

IV. Conclusions

We have developed a supervised forest classifier for vascular segmentation of mice dura mater in complex epifluorescence imagery that achieves an overall accuracy of 88 percent using 12.7M labeled samples across 9 images for training. The RF produces significantly better segmentation results than traditional intensity-based global segmentation methods by using vessel morphology specific features such as multiscale Hessian eigenvalues, oriented second derivatives, LoG and line detection. Experimental results for both WT and KO animal models demonstrate that the proposed RF segmentation method produces consistently high quality results compared to the other methods. We are currently using the vessel segmentations to extract morphological features to quantitatively test for any differences in vessel remodeling between wild-type and knock-out mice.

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References

1. Nguyen UTV, Bhuiyan A, Park LAF, Ramamohanarao K. An effective retinal blood vessel segmentation method using multi-scale line detection. *Pattern Recognition*. 46 (3) 703–715. 2013.
2. Ricci E, Perfetti R. Retinal blood vessel segmentation using line operators and support vector classification. *IEEE Transactions on Medical Imaging*. 26 (10) 1357–1365. 2007. [PubMed: 17948726]
3. Sironi A, Turetken E, Lepetit V, Fua P. Multiscale centerline detection. *IEEE Transactions on Pattern Analysis and Machine Intelligence*. 2015.
4. Joshi VS, Tang L, Garvin MK, Reinhardt JM, Maude RJ, Abramoff MD. Automated detection of retinal hemorrhages in malarial retinopathy. *Investigative Ophthalmology & Visual Science*. 53 (14) 4089–4089. 2012.
5. Prasath VBS, Haddad O, Bunyak F, Glinskii O, Glinsky V, Huxley V, Palaniappan K. Robust filtering based segmentation and analysis of dura mater vessel laminae using epifluorescence microscopy. 35th IEEE EMBS. 2013. 6055–6058.

6. Pelapur R, Prasath VBS, Bunyak F, Glinskii OV, Glinsky VV, Huxley VH, Palaniappan K. Multi-focus image fusion on epifluorescence microscopy for robust vascular segmentation. 36th IEEE EMBS. 2014. 4735–4738.
7. Prasath, VBS; Pelapur, R; Glinskii, OV; Glinsky, VV; Huxley, VH; Palaniappan, K. Multi-scale tensor anisotropic filtering of fluorescence microscopy for denoising microvasculature. IEEE Int. Symp. on Biomedical Imaging; 2015; 540–543.
8. Breiman L. Random forests. Machine Learning. 45 (1) 5–32. 2001.
9. Frangi AF, Niessen WJ, Vincken KL, Viergever MA. Multiscale vessel enhancement filtering. Lecture Notes in Computer Science (MICCAI). 1496: 130–137. 1998.
10. Bunyak F, Palaniappan K, Glinskii O, Glinskii V, Glinsky V, Huxley V. Epifluorescence-based quantitative microvasculature remodeling using geodesic level-sets and shape-based evolution. 30th IEEE EMBS. 2008. 3134–3137.
11. Leung T, Malik J. Representing and recognizing the visual appearance of materials using three-dimensional textons. International Journal of Computer Vision. 43 (1) 29–44. 2001.

Fig. 1.

The proposed random forest (RF) classifier obtains reliable (arteriole) segmentations for a range of cases including: (a) vessels with nonlinear contrast along boundaries, (b) diffuse blurred venules structures, (c) inhomogeneous background with excess stain. RF produces consistent results for both wild-type (WT), and knock-out (KO) as shown by the Dice values in parenthesis using manual ground-truth. Images are contrast enhanced for visualization purposes.

Fig. 2.

An example using 012706 ERbKO-19 (No. 10 in Table II, third column in Figure 4) showing the 15 vessel specific features used in the random forest classifier are shown. Frobenius norm of Hessian eigenvalues (1) at 5 scales and its sigmoid scale map, maximum response of second derivatives at 3 scales over 6 orientations and orientation maps (30 deg steps), maximum response of Laplacian of Gaussian (LoG) at 8 scales and its sigma scale map, and response of the multi-scale line detector (3).

Fig. 3.

Post-processing improves RF segmentation Dice accuracy: (a) RF segmentation, (b) after post-processing, (c) ground-truth for comparison. Dice values in parenthesis and second row zoomed views.

Fig. 4.

Compared with Niblack [5], and Otsu thresholding methods our proposed approach obtained better vessel segmentations. (a) Input images (contrast enhanced for visualization purposes), (b) expert verified ground-truth (arteriole) segmentations, (c) Niblack, (d) Otsu, and (e) our RF method. White regions represent correctly segmented pixels, red are missing (false negative) and blue are extra regions (false positive) compared to ground truth. First three columns correspond to 012706-ERbKO - 01, 03, 19, and the last three to 012606-ERbWT - 03, 05, 12.

TABLE I

Description and dimensionality of the input feature vector for the random forest (RF) classifier (see Figure 2).

Feature	Dim.
Modified Frobenius norm over 5 scales & max scale regular Frobenius	6
Maximum response of oriented 2nd derivatives over 3 scales & 3 orientation maps	6
Maximum response over eight scales of LoG filter & sigma map	2
Multiscale Line Detector	1

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TABLE II

Quantitative comparison of segmentation methods on knock-out (012706-ERbKO) and wild-type (012606-ERbWT) mice dura mater epifluorescence images for *ARTERIOLES* only.

No.	Type	Niblack	Otsu	Ours
1	KO-01	0.6103	0.2557	0.8321
2	KO-03	0.5144	0.6748	0.8677
3	KO-04	0.7105	0.7340	0.8768
4	KO-05	0.8252	0.6468	0.9167
5	KO-06	0.7905	0.7520	0.9014
6	KO-08	0.8044	0.6720	0.9094
7	KO-09	0.3588	0.5336	0.8785
8	KO-10	0.5148	0.6318	0.8413
9	KO-15	0.6998	0.5094	0.8722
10	KO-19	0.7397	0.6143	0.8811
11	WT-01	0.7839	0.5511	0.8782
12	WT-03	0.3750	0.3941	0.8749
13	WT-04	0.6022	0.8855	0.8756
14	WT-05	0.7301	0.5745	0.8936
15	WT-06	0.8504	0.7482	0.9045
16	WT-07	0.7726	0.6621	0.9245
17	WT-08	0.7640	0.6942	0.9007
18	WT-09	0.7185	0.9100	0.9098
19	WT-12	0.6762	0.5957	0.8725
20	WT-14	0.8058	0.5770	0.8748
Avg.	Dice	0.6824	0.6308	0.8843
Avg.	Sensitivity	56.05	61.60	88.75
Avg.	Specificity	99.71	95.67	99.15
Avg.	Accuracy	96.45	93.19	98.36