Published in final edited form as:

Proc IEEE Int Symp Biomed Imaging. 2015 April ; 2015: 540–543. doi:10.1109/ISBI.2015.7163930.

MULTISCALE TENSOR ANISOTROPIC FILTERING OF FLUORESCENCE MICROSCOPY FOR DENOISING MICROVASCULATURE

V. B. S. Prasath¹, R. Pelapur¹, O. V. Glinskii^{2,3,5}, V. V. Glinsky^{3,4,5}, V. H. Huxley^{2,3}, and K. Palaniappan¹

¹Department of Computer Science, University of Missouri-Columbia, Columbia, MO 65211 USA

²Department of Medical Pharmacology and Physiology, University of Missouri-Columbia, Columbia, MO 65211 USA

³National Center for Gender Physiology, University of Missouri-Columbia, Columbia, MO 65211 USA

⁴Department of Pathology and Anatomical Sciences, University of Missouri-Columbia, Columbia, MO 65211 USA

⁵Research Service, Harry S. Truman Memorial Veterans Hospital, Columbia, MO 65201 USA

Abstract

Fluorescence microscopy images are contaminated by noise and improving image quality without blurring vascular structures by filtering is an important step in automatic image analysis. The application of interest here is to automatically extract the structural components of the microvascular system with accuracy from images acquired by fluorescence microscopy. A robust denoising process is necessary in order to extract accurate vascular morphology information. For this purpose, we propose a multiscale tensor with anisotropic diffusion model which progressively and adaptively updates the amount of smoothing while preserving vessel boundaries accurately. Based on a coherency enhancing flow with planar confidence measure and fused 3D structure information, our method integrates multiple scales for microvasculature preservation and noise removal membrane structures. Experimental results on simulated synthetic images and epifluorescence images show the advantage of our improvement over other related diffusion filters. We further show that the proposed multiscale integration approach improves denoising accuracy of different tensor diffusion methods to obtain better microvasculature segmentation.

Keywords

Fluorescence; microscopy; anisotropic filtering; multiscale; vasculature; denoising

1. INTRODUCTION

Florescent imaging technique is becoming vitally important for visualizing and analyzing the dynamic biological processes, which benefits from the advent of fluorescent labeling techniques plus the sophisticated light microscopes. Vessel extraction or detection from microscopy imagery is an important application in biomedical imaging. Due to uneven contrast and illumination due to high intensity vessel wall regions (foreground) versus low intensity non-vascular regions (background) there is a need for effective filtering methods for microscopy images [1-4].

Anisotropic diffusion [5-7] based partial differential equations (PDEs) have been widely utilized for image filtering and recently various improvements have been proposed to adapt it to microscopy images. Kriva et al [8] utilized an advection term along with non-linear anisotropic diffusion. Mosaliganti et al [9] used a Hessian matrix driven diffusion model and similarly Manniesing et al [10] used a Hessian eigensystem combined with nonlinear anisotropic diffusion scheme for vessel enhancement. Drblikova [11] modified the Weickert [12] coherence enhancing diffusion for cell membranes. Recently, Pop et al [13] proposed a plane confidence measure along with coherence. Despite their successes in microscopy denoising each of these schemes use only a single scale for smoothing (linear/nonlinear) and thus tend to smooth out vessel structures smaller than the predefined scale parameter. Moreover, the methods by [8,11] apply the same diffusion to the background membrane thus producing spurious artifacts, while Hessian based models [9,10] are sensitive to noise.

In order to improve upon these limitations, we propose here a multiscale integration of smoothing scales thereby avoiding the loss of microstructures which are in the planar structure of dura mater laminae. Following [13], we utilize a multiscale anisotropic tensor diffusion which depends on anisotropic diffusion smoothed structure tensor. By utilizing a range of scales and combining corresponding edge maps into a diffusion tensor formulation, we obtain better restoration of microscopy images compared to previous approaches [3, 13]; see Figure 1 where we compare the segmentation method (adaptive Niblack's thresholding studied in [3]), and it shows that our multiscale anisotropic diffusion improves segmentation accuracy. We study the effects of integration of multiscale in a tensors within an anisotropic diffusion filtering framework for fluorescence microscopy images. Comparison with related anisotropic diffusion based smoothing methods [8-13] indicate we obtain better results in terms of signal to noise ratio and structural similarity measure on simulated synthetic images. Further, experiments on epifluorescence and microscopy images indicate the preservation of vascular structures without oscillations. The multiscale estimation of the smoothing parameter utilized here can be incorporated into any of the tensor anisotropic diffusion PDEs and as experiments indicate perform better across different images.

The rest of the paper is organized as follows. Section 2 after briefly reviewing tensor anisotropic diffusion, we detail the proposed multiscale filtering approach. Section 3 comparison with related approaches on synthetic and real epifluorescence imagery are given to illustrate the advantages of our proposed method. Finally, Section 4 concludes the paper.

2. DIFFUSION FILTERS

2.1. Tensor anisotropic diffusion

The unified tensor anisotropic diffusion model [12] is given by the following PDE,

$$\begin{cases} \frac{\partial u(x,t)}{\partial t} = \nabla \cdot (\mathcal{D}(u(x,t)) \nabla u(x,t)), & x \in \Omega, \\ u(x,0) = u_0(x), & x \in \Omega, \\ \frac{\partial u}{\partial n} = 0, & x \in \partial\Omega. \end{cases} \quad (1)$$

where ∇ is the gradient operator, $x = (x_1, x_2)$ the pixel coordinates, and t denotes the time variable, $\nabla \cdot \xi$ represents the divergence (*div*) operation, and u/n denotes the normal derivative on the boundary. Here $\mathcal{D}$ is the 3D diffusivity matrix,

$$\mathcal{D} = (\vec{v}_1, \vec{v}_2, \vec{v}_3) \text{diag}(\lambda_1, \lambda_2, \lambda_3) (\vec{v}_1, \vec{v}_2, \vec{v}_3)^T \quad (2)$$

where $(\lambda_1, \lambda_2, \lambda_3)$ represent the amount of diffusion, and $(\vec{v}_1, \vec{v}_2, \vec{v}_3)$ are eigenvectors of the isotropic structure tensor defined by,

$$J_\rho(\nabla u_\sigma) := G_\rho \star (\nabla u_\sigma \otimes \nabla u_\sigma). \quad (3)$$

Here $G_\rho(\mathbf{x}) = (\rho\sqrt{2\pi})^{-1} \exp(-|\mathbf{x}|^2/2\rho^2)$ is the isotropic Gaussian kernel, $\star$ denotes the convolution operator, $\otimes$ tensor product, and superscript T is the transpose. The function $u_\sigma := G_\sigma \star u$ encodes the *noise scale* σ and is directly related to the amount of noise present in a given image. Moreover, structures smaller than scale σ are smoothed out and choosing this parameter is important in obtaining better restorations. Let the eigenvalues of the tensor (3) be $\mu_1 \ \mu_2 \ \mu_3 \ 0$ and they capture average contrast in the eigenvector directions.

2.2. Cell membrane enhancement

Recently, Pop et al [13] proposed to use the following eigenvalues system for better contrast enhancement and continuity of membrane structures.

$$\begin{cases} \lambda_1 = \psi(\mu_1) \\ \lambda_2 = \max(\lambda_1, 0) - (\max(\lambda_1, 0) - \lambda_3) \times h_\tau(C_{\text{plane}}) \\ \lambda_3 = 1, \end{cases} \quad (4)$$

where ψ is modeled after diffusion flux and chosen as ($K > 0$ contrast parameter):

$$\psi(s) = \frac{1}{1+(s/K)^2} - \frac{2(s/K)^2}{1+(s/K)^2},$$

and $C_{\text{plane}} = \mu_1 - \mu_2 / \mu_1 + \mu_2$ a planar confidence measure and the function h_τ is a thresholding function $[0, 1]$ and helps in identifying membrane structures ($h_\tau \rightarrow 1$) and isotropic/1D homogenous ($h_\tau \rightarrow 0$ regions, see [14] for more details. The above system has the following characteristics:

- Membrane regions: $\mu_1 \gg \mu_2 \approx \mu_3 \approx 0$, $h_\tau \rightarrow 1$ giving $\lambda_2 = \lambda_3 = 1$ thus enabling diffusion along the tangential plane ($\vec{v}_1^\perp$)
- Isotropic/1D homogeneous structures: $\mu_1 \approx \mu_2 \approx \mu_3 \approx 0$, $\lambda_1 \rightarrow 1$, $h_\tau \rightarrow 0$, thus all λ values are closer to 1 enabling strong diffusion.
- 1D structures: $\mu_1 \approx \mu_2 \gg \mu_3 \approx 0$, $\lambda_2 \rightarrow \lambda_1 \approx 0$, thus diffusion takes place along smallest variation of contrast ($\vec{v}_3$).

2.3. Multiscale tensor diffusion

Pop et al [13] advocate using an anisotropic version of the tensor (3) where the integration scale $\rho > 0$ in (3) is avoided using tensor anisotropic diffusion (1) using the diffusivity matrix consist of eigen-values in (4) applied to each component of the tensor $J(\nabla u_\sigma) = (\nabla u_\sigma \otimes \nabla u_\sigma)$. However, this method still inherits blurring of edges associated with the tensor diffusion model as well as they perform poor on highly noisy images. To avoid these problems we consider a multiscale approach which is robust to noise and preserves small scale microvasculature structures better. We define the following multiscale tensor by aggregating products across scale,

$$\mathcal{J}(u) = \bigoplus_{\sigma=L_1}^{L_2} (\nabla u_\sigma \otimes \nabla u_\sigma) \quad (5)$$

where L_1 is the finest scale and L_2 is the coarsest scale. The calculation across different scales allows us to detect cell membranes of different sizes and further apply anisotropic diffusion (1) using the system (4) while avoiding blurring thin cell membrane boundaries at smaller scales as shown in Figure 2. These characteristics will be further useful in denoising and segmentation processes as we will see next.

3. EXPERIMENTAL RESULTS

The multiscale PDE system (1)-(4)-(5) is implemented using an explicit time discretization and finite difference approximations for the derivatives. All the schemes utilized here were implemented on a MacBook Pro laptop with 2.3GHz Intel Core i7 processor, 8GB RAM memory and MATLAB R2012a is used for the calculations and visualizations. To speed up the calculations further we used a box filter for approximating the Gaussian convolution in (5). Overall our scheme takes 0.17 seconds for an image of size 256×256 . Optimizing and utilizing C/C++ will decrease overall computational times further. The range of scales $[L_1, L_2] = [1, 4]$ is chosen to reflect microvascular imagery. We use the root mean squared error (RMSE), mean ellipse intensity (MEI), and structural similarity (SSIM) [15] for estimating denoising results quantitatively. We compare our scheme with the approaches of Kriva et al [8], Weickert [12], Drblikova et al [11], Mosaliganti et al [9], Pop et al [13], Manniesing et al [10].

3.1. Synthetic images

Figure 3 shows synthetic images and their corresponding denoised results using various schemes are shown in Figure 4. The first synthetic image is generated following a similar

set-up as the one in [13] by simulating boundary thickness close to real cells and 50% of the membrane pixels are randomly removed. Note that this simulates the acquisition of 2D membrane structures under a 3D fluorescence microscope and we show only 2D cross sections for clarity. In Figure 4 we show in each row, denoised results, residual ($|u_0 - u|$), and corresponding SSIM maps. Note red pixels in SSIM maps indicate that the denoised results are structurally similar to ground-truth noise free image. We see that our method performs well in terms of junction preservation, noise removal and background artifact reduction when compared with other methods. Table 1 shows that our proposed method outperforms other schemes in different error metrics.

3.2. Fluorescence microscopy images

Figure 5 shows a comparison of different schemes for denoising and enhancement on a microscopy image. As can be seen, the proposed approach yields a better result without membrane boundary blurring as observed in [8, 9, 11, 13] while removing background noise effectively and junctions are preserved in contrast to [3, 10, 12] which excessively smooths them.

3.3. Segmentation improvements

Figure 6 shows the effect of denoising on segmentation of mice dura mater microvasculature laminae images using an adaptive Niblack thresholding utilized in [3]. The endothelial cells were stained using AlexaFluor 488 fluorescently labeled WGA lectin, to yield a high contrast image of the vascular walls (foreground) and low intensity avascular background. The epifluorescence-based high resolution images were acquired using a video microscopy system (Laborlux 8 microscope from Leitz Wetzlar, Germany). To compare quantitatively we used ground-truth data provided by an experienced physiologist in computing Dice coefficient. Figure 6(a) shows applying thresholding without denoising generates false positives. We compare the effect denoising methods on the final segmentation map by sweeping the parameters to obtain optimal segmentation in terms of the maximum Dice coefficient. Figure 6(b)-(c) show the segmentation after applying the schemes of Pop et al [13] and our scheme respectively. Comparison of different Dice coefficients show that our multiscale tensor anisotropic diffusion obtains the best accuracy, see also Figure 1 for another example¹.

4. CONCLUSIONS

In this paper we study a multiscale anisotropic tensor diffusion method for vasculature network denoising and enhancement. Using a tensor anisotropic diffusion driven by a planar confidence measure based eigen-system from the structure tensor we obtain better restorations without blurring across vessel boundaries. Due to the multiscale nature of the method we can preserve small scale edges while background noise is more robustly smoothed as shown in the simulated synthetic images. Experiments on microvasculature images shows that our scheme obtains good results. We also show that better denoising improves microvasculature morphology segmentation. Finally, we note that the proposed

¹Further results and corresponding error metric values are available at <http://cell.missouri.edu/pages/VesselDiffusion>.

filtering scheme is not restricted to microscopy images but can be applied to other imagery which contain thin membrane- or vessel-like linear structures.

Acknowledgment

This research was supported in part by the Award #1I01BX000609 from the Biomedical Laboratory Research & Development Service of the VA Office of Research and Development (VVG), the National Cancer Institute of the National Institutes of Health Award #R01CA160461 (VVG).

REFERENCES

- [1]. Frangi, AF.; Niessen, WJ.; Vincken, KL.; Viergever, MA. MICCAI. 1998. Multiscale vessel enhancement filtering; p. 130-137.
- [2]. Bunyak, F., et al. IEEE EMBS. 2008. Epifluorescence-based quantitative microvasculature remodeling using geodesic level-sets and shape-based evolution; p. 3134-3137.
- [3]. Prasath, VBS., et al. IEEE EMBC. 2013. Robust filtering based segmentation and analysis of dura mater vessels using epifluorescence microscopy; p. 6055-6058.
- [4]. Pelapur, R., et al. IEEE EMBC. 2014. Multi-focus image fusion on epifluorescence microscopy for robust vascular segmentation; p. 4735-4738.
- [5]. Perona P, Malik J. Scale-space and edge detection using anisotropic diffusion. IEEE PAMI. 1990; 12(7):629–639.
- [6]. Aubert, G.; Kornprobst, P. Mathematical problems in image processing: Partial differential equation and calculus of variations. Springer; New York, USA: 2006.
- [7]. Prasath VBS, Vorotnikov D. On a system of adaptive coupled PDEs for image restoration. J Math. Imag. Vis. 2014; 48(1):35–52.
- [8]. Kriva Z, et al. 3D early embryogenesis image filtering by nonlinear partial differential equations. Med. Image Anal. 2010; 14(4):510–526. [PubMed: 20457535]
- [9]. Mosaliganti, K., et al. IEEE ISBI. 2010. Anisotropic plate diffusion filtering for detection of cell membranes in 3D microscopy images; p. 588-591.
- [10]. Manniesing R, et al. Vessel enhancing diffusion: A scale space representation of vessel structures. Med. Image Anal. 2006; 10(6):815–825. [PubMed: 16876462]
- [11]. Drblikova, O., et al. SSVM. Springer; 2009. The nonlinear tensor diffusion in segmentation of meaningful biological structures from image sequences of zebrafish embryogenesis; p. 63-74. LNCS 5567
- [12]. Weickert J. Coherence enhancing diffusion. IJCV. 1999; 31:111–127.
- [13]. Pop, S., et al. IEEE ICIP. 2011. Image filtering using anisotropic structure tensor for cell membrane enhancement in 3D microscopy; p. 2041-2044.
- [14]. Romulus, T., et al. ACIVS. Springer; 2005. Flow coherence diffusion. linear and nonlinear case; p. 316-322. LNCS 3708
- [15]. Wang Z, et al. Image quality assessment: from error visibility to structural similarity. IEEE TIP. 2004; 13(4):600–612.

Fig. 1.

High background noise levels can affect the denoising performance and thereby affect further vessel segmentations. Using our multiscale version of the anisotropic diffusion method we improve segmentation performance. (a) Input microscopy image along with ground-truth segmentation. Segmentations using denoising schemes from (b) Prasath et al [3] (c) Pop et al [13] and (d) our multiscale version. In each sub-figure we show (left) denoised (right) segmentation.

Fig. 2.

Multiscale version improves capturing better cell membrane boundaries. (a) Input microscopy image. (b) First eigenvalue computed in (4) following Pop et al [13] (c) Our multiscale version.

Fig. 3.

Noisy synthetic images used in our experiments. (a) Original (b) Noisy (top: *Circles* image obtained by sampling 50% and with noise level $\sigma = 80$, bottom: *Phantom* image obtained with noise level $\sigma = 30$) (c) Residue ($|GT - u_0|$) (d) SSIM maps.

Fig. 4.

Synthetic images denoising and enhancement. (a) Kriva et al [8] (b) Weickert [12] (c) Drblikova et al [11] (d) Mosaliganti et al [9] (e) Pop et al [13] (f) Manniesing et al [10] (g) Prasath et al [3] (h) Proposed method. We show denoising results, residual ($|u_0 - u|$) and SSIM maps in each case. Better viewed online and zoomed in.

Fig. 5.

Cell membrane image denoising and enhancement on the input image Figure 2(a). (a) Kriva et al [8] (b) Weickert [12] (c) Drblikova et al [11] (d) Mosaliganti et al [9] (e) Pop et al [13] (f) Manniesing et al [10] (g) Prasath et al [3] (h) Proposed method. Better viewed online and zoomed in.

(a) Noisy, Dice 0.6523

(b) Pop [13], Dice 0.6170

(c) Our, Dice 0.7049

Fig. 6.

Structure preserving denoising especially in the background regions improves segmentation accuracy. (a) Input image is segmented without denoising and with denoising using the methods of (b) Pop et al [13], and (c) our multiscale method. Here white regions are correctly segmented pixels (TP), red are missing (FN) and blue are extra regions (FP) compared to the ground truth segmentation. The epifluorescence images are contrast stretched for visualization. Better viewed in color and online.

Table 1

RMSE/MSSIM values for automatic denoising methods when compared with manual ground truth for two synthetic images in Figure 4. Lower RMSE and MSSIM closer to one indicate good denoising performance.

Figure 4		(a)	(b)	(c)	(d)	(e)	(f)	(g)	(h)
Ref.	Noisy	[8]	[12]	[11]	[9]	[13]	[10]	[3]	Our
<i>Circles</i> 256 × 256	<u>80.245</u>	<u>24.1248</u>	<u>19.5873</u>	<u>18.801</u>	<u>30.6419</u>	<u>22.1995</u>	<u>14.8963</u>	<u>25.5306</u>	<u>10.6394</u>
	0.0755	0.3799	0.2246	0.2842	0.4059	0.3563	0.4486	0.3255	0.7670
<i>Phantom</i> 512 × 512	<u>29.9902</u>	<u>21.6928</u>	<u>14.2349</u>	<u>18.1891</u>	<u>31.9928</u>	<u>22.8848</u>	<u>12.1397</u>	<u>9.3521</u>	<u>9.5863</u>
	0.6031	0.9241	0.8393	0.8712	0.8370	0.9133	0.9797	0.9534	0.9921