ENHANCEMENT OF MICROTUBULES IN EM TOMOGRAPHY

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ABSTRACT

The interpretation of the EM tomography of microtubules is challenging due to the low SNR and low contrast of the volume data. Therefore, image enhancement is crucial for the subsequent segmentation and structural analysis of microtubules. In this paper, We propose a model based 3D image enhancement approach by combining transform domain technique and spatial domain techniques in three consecutive steps. The enhancement starts with an anisotropic invariant wavelet transform to effectively enhance the elongated features, followed by a 3D shape filter via eigen analysis to capture the local geometric properties of the tubular structure. The enhancement ends with a coherence enhancing diffusion to complete the interruptions along the microtubules. The contribution of this work is that we have tailored and improved each of the above techniques to exploit the unique geometric and photometric properties of microtubules. Experimental results indicate that our proposed approach has excellent performance in noise removal and enhancement of the tomography volume.

1. INTRODUCTION

The understanding of the structure and function of the microtubule has been a major scientific goal for several decades. Though electron microscopy is used as a mature technique to obtain the 3D structure data of microtubules, it is particularly challenging to interpret the acquired data due to its low SNR and the fact that the microtubules are in close contact to their cellular context and densely surrounded by other proteins of similar appearance, as shown in Fig.1. Therefore, the enhancement of the volume image is an indispensable step for the subsequent segmentation and structural analysis of the microtubules. However, for such extremely cluttered data, general averaging techniques can not be employed for the enhancement.

In this paper, we propose a model based enhancement approach by combining transform domain technique and spatial domain techniques in three consecutive steps. Specifically, the enhancement starts with an anisotropic invariant wavelet transform, which is shown to be advantageous for enhancing elongated features and removing clutters. A 3D shape filter exploring second order local image structure is then applied to accentuate tubular structures and attenuate isotropic features. We make the shape filter robust against noise by incorporating a cubic fitting model. The enhancement ends with a coherence enhancing diffusion to complete the interruptions along the microtubules. In addition, we propose a new coherence measurement to make this diffusion process contrast adaptive. The contribution of this work is that we have tailored each technique to fully utilize the unique structural geometry of microtubules and their photometric properties.

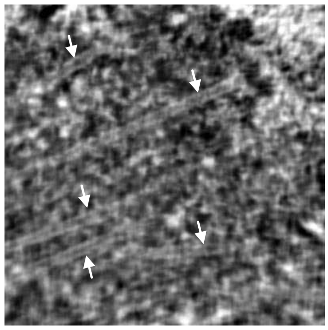


Fig. 1. Microtubules, marked with arrows, are embedded in noise and surrounding cellular materials

The remainder of this paper is organized as follows. In section 2, we describe our proposed method. In section 3, we present the experimental results on image enhancement. We then conclude with summary and conclusions.

2. PROPOSED METHOD

The proposed approach consists of techniques in both transform domain and spatial domain. The anisotropic invariant wavelet filtering is applied first to enhance the elongated features and diminish irrelevant structures. The shape filter and coherence enhancing diffusion are to enhance the anisotropic features locally and should be applied only after the wavelet enhancement, when the noise level is low enough.

2.1. Anisotropic Invariant Wavelet Filtering

Though the microtubules are the largest scale elongated objects in the image, their local features are so weak that any general averaging de-nosing technique will easily smooth out the microtubules completely. In contrast, wavelet is well established in preserving image features while reducing the noise to sufficiently low level. Considering the tubular shape of the microtubules, we propose to apply wavelet transform with anisotropic basis to enhance microtubules.

Given a 1-D orthogonal wavelet basis for L^2 as

$$\psi_{j,k}(t) = 2^{-(j/2)}\psi(2^{-j}t - k), \;\; j,k,t \in Z \tag{1}$$

the basis functions for higher dimension can be constructed by the tensor product of the 1-D wavelet basis functions. For example, the basis functions for 2-D wavelet transform can be obtained as

$$\psi_{j_1,k_1;j_2,k_2}(t_x, t_y) = \psi_{j_1,k_1}(t_x) \cdot \psi_{j_2,k_2}(t_y) \tag{2}$$

where $j_i, k_i \in Z, (t_x, t_y) \in Z^2$

With separate scale parameter for every dimension, such anisotropic basis functions have been proven superior to its one-scale multiresolution analog, the isotropic basis, for the enhancement of elongated features [1]. Moreover, we increase the anisotropy of the wavelet transform by attenuating transform coefficients on the iso-scales, which makes the wavelet transform more sensitive to elongated features.

However, for image with extremely low SNR, the denoising scheme with orthogonal wavelet will result in loss of image features, due to the lack of translation invariance [2]. In our method, to overcome this problem, the wavelet transform is made translation invariant by averaging over shift with Beylkin's algorithm [3]. The translation invariant de-noising process can be expressed as

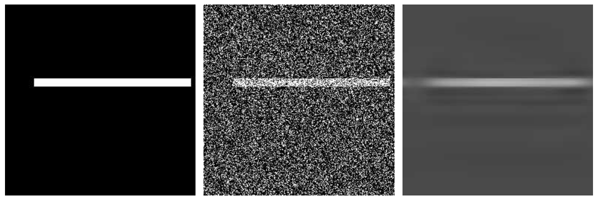
$$\bar{T}(x) = Ave_h S_{-h}(T(S_h x))) \tag{3}$$

where x is the noisy signal, T is the translation sensitive de-noising operator and S_h is the shift operation.

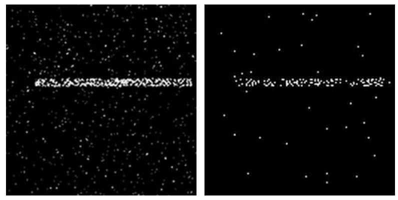
The wavelet transform is also made rotation invariant by averaging over rotation [4]. The rotation invariant denoising process can be expressed in a similar way

$$\bar{T}(x; (R_\theta)_{\theta\in\Theta}) = Ave_{\theta\in\Theta} R_{-\theta}(T(R_\theta x))) \tag{4}$$

where R_θ is a rotation operator and Θ is a set of angles. The image is rotated by a set of angles and enhanced with translation invariant wavelet transform at each angle. The enhanced images are rotated back to the original orientation and the resultant image is the average of all the enhanced images. Fig.2 shows the image enhancement by thresholding with various wavelet transforms. The results indicate that both invariance and anisotropy of the wavelet are equally important for the enhancement of elongated features.



(a) (b) (c)



(d) (e)

Fig. 2. Enhancement with various wavelet transforms: (a) the original image, (b) gaussian noise added, (c) result with anisotropic invariant wavelet, (d) result with isotropic invariant wavelet, (e) result with non-invariant anisotropic wavelet

2.2. Local Shape Filtering

To further improve the contrast between microtubules and their surroundings, we propose a 3D shape filter by exploiting the local geometric properties of the tubular structures, based on eigenvalue analysis of Hessian matrix [5].

To obtain reliable estimation of image derivatives from noisy image, we propose to compute the image derivatives analytically using a cubic fitting model, which also enables us to keep track of the estimation error and identify outliers. Assume the gray level pattern of a small neighborhood in an image can be modelled by a canonical 3D cubic polynomial:

$$\begin{aligned} I(x,y,z) &= a_1 + a_2x + a_3y + a_4z + a_5x^2 + a_6xy \\ &+ a_7y^2 + a_8yz + a_9z^2 + a_{10}xz + a_{11}x^3 + a_{12}x^2y \\ &+ a_{13}xy^2 + a_{14}y^3 + a_{15}y^2z + a_{16}yz^2 + a_{17}z^3 \\ &+ a_{18}x^2z + a_{19}xz^2 + a_{20}xyz + \epsilon \end{aligned} \tag{5}$$

ϵ is assumed to be small addictive noise of zero mean and variance σ^2. Thus each neighborhood is completely characterized by the coefficients a_is and σ^2, which can be easily solved in least square sense.

Then at voxel (x, y, z) we have the Hessian matrix as

$$H = \begin{pmatrix} 2a_5 & a_6 & a_{10} \\ a_6 & 2a_7 & a_8 \\ a_{10} & a_8 & 2a_9 \end{pmatrix} \quad (6)$$

Let λ_1,λ_2, λ_3 be the eigenvalues of the Hessian matrix and $|\lambda_1| \leq |\lambda_2| \leq |\lambda_3|$. For bright tubular structures against dark background in 3D image, it can be assumed that λ_1 is small, while both λ_2 and λ_3 have large absolute value and negative signs. Based on this idea, the following filter enhancing tubular structure while reducing other morphologies was developed [5]:

$$L = \begin{cases} 0 \quad if\, \lambda_2 > 0\, or\, \lambda_3 > 0 \\ (1 - exp(-\frac{R_A^2}{2\alpha^2}))exp(-\frac{R_B^2}{2\beta^2})(1 - exp(-\frac{S^2}{2c^2}))\; else \end{cases} \quad (7)$$

where $R_A = \frac{|\lambda_2|}{|\lambda_3|}$, $R_B = \frac{|\lambda_1|}{\sqrt{|\lambda_2 \lambda_3|}}$ and $S = ||H||_F = \sqrt{\sum_i \lambda_i^2}$. α, β and c are thresholds which control the sensitivity of the filter to the measures R_A, R_B and S.

By calculating the output value of this filter at each image point, we can obtain the confidence measure of local tubular shape, which then allows us to selectively enhance tubular structures and attenuate other structures.

2.3. Coherence Enhancing Diffusion

The last step in microtubule enhancement is to obtain continuous microtubules by connecting interrupted tubular structures through a diffusion process. The key point is to find the prominent local orientation and smooth along that direction but not across them. This leads us to coherence-enhancing diffusion[6], which is governed by the following equation:

$$I_t = div(D\nabla I) \quad (8)$$

where div is the divergence operator, ∇ is the gradient operator and the diffusion tensor D is defined to have the same eigenvector as the gradient structure tensor (GST) [7] but with adaptive eigenvalues that represent the diffusion strength in each principal directions.

The eigenvalues of the GST, μ_1,μ_2 and $\mu_3$are used to define the coherence of a structure in 3-D [8]:

$$k = \sum_{i=1}^{2} \sum_{j=i+1}^{3} (\mu_i - \mu_j)^2 \quad (9)$$

To enhance flow like structures, the diffusion tensor D is adapted to the GST

$$D = \begin{pmatrix} v_1 & v_2 & v_3 \end{pmatrix} \begin{pmatrix} d_1 & 0 & 0 \\ 0 & d_2 & 0 \\ 0 & 0 & d_3 \end{pmatrix} \begin{pmatrix} v_1^T \\ v_2^T \\ v_3^T \end{pmatrix} \quad (10)$$

To steer the diffusion along the principal orientation, Weickert [8] gave the eigenvalues for three dimension as

$$d_i := \alpha \quad (11)$$

for $i = 1, 2$ and

$$d_3 = \begin{cases} \alpha & if\, k = 0 \\ \alpha + (1 - \alpha)exp(-\frac{C}{k}) & else \end{cases} \quad (12)$$

where $C > 0$ is a threshold parameter.

The dependence of the coherence measurement k on local contrast makes it difficult to enhance weak but highly anisotropic features without inappropriate diffusion of features with strong contrast but not necessarily highly anisotropic. We therefore propose a new measurement of coherence and construct the diffusion tensor based on this new measurement.

We adopt the concept of the local shape measurement introduced by Bakker [9] and define

$$k_p = \frac{\mu_1 - \mu_2}{\mu_1 + \mu_2} \quad (13)$$

and

$$k_l = \frac{\mu_2 - \mu_3}{\mu_2 + \mu_3} \quad (14)$$

For tubular structures, it can be assumed $\mu_1 \approx \mu_2 \gg \mu_3$ which is equivalent to $k_p \approx 0$ and $k_l \approx 1$. We then choose the eigenvalues of the diffusion tensor as follows

$$d_1 = d_2 = \alpha \quad (15)$$

and

$$d_3 = \begin{cases} \alpha \quad if\, \mu_1 = \mu_2 = \mu_3 = 0 \\ \alpha + (1 - \alpha)exp(-\frac{k_p^2}{2C_p^2})(1 - exp(-\frac{k_l^2}{2C_l^2}))\; else \end{cases} \quad (16)$$

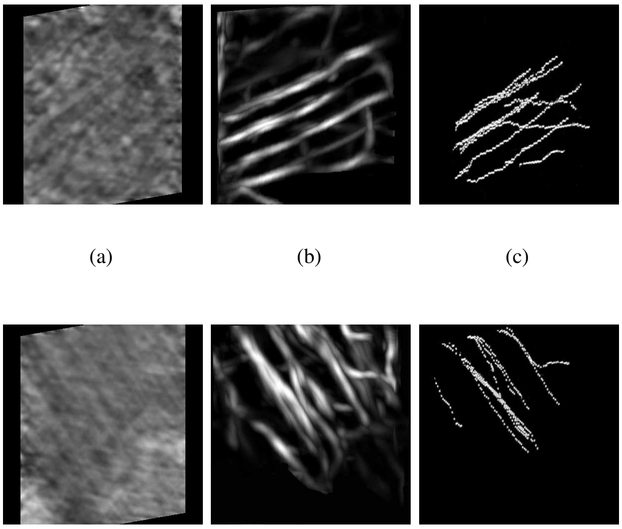
where α is a small value, C_p and C_l are threshold values.

The diffusion based on our new measurement is more suitable for enhancing anisotropic features in image with large contrast variations.

3. EXPERIMENTAL RESULTS

In this section, we present sample enhancement results. The hybrid enhancement approach was applied to tomography volumes with multiple microtubules. As shown in Fig.3(a) and (d), the microtubules are invisible in the raw volumes since they are buried in noise and surrounded by numerous cellular materials. Given the specific tomography imaging process, we can assume that the axes of the microtubules are parallel to the $x - y$ plane. Therefore, the average over rotation was performed over eight angles only in the $x - y$

plane. After the anisotropic invariant wavelet filtering, the image is further processed with local shape filtering and coherence enhancing diffusion. Fig.3(b) and (e) show in 3D that the microtubules stand out obviously after the enhancement. Fig.3(c) and (f) illustrates the microtubule central axes obtained by 3D thinning of the enhanced microtubules. Fig.4 shows the selected 2D slices of the enhanced 3D volumes.



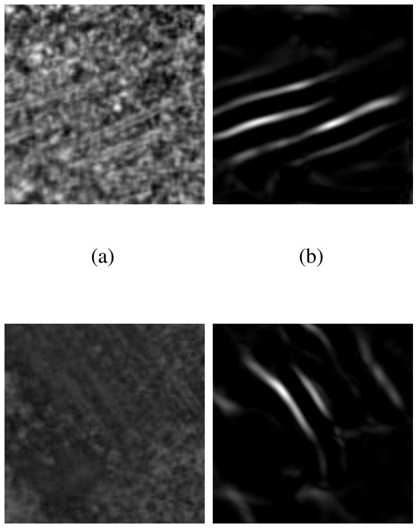
(a) (b) (c)

(d) (e) (f)

Fig. 3. Enhancement of volumes with multiple microtubules: (a)(d) the original volumes, (b)(e) the enhanced volumes, (c)(f) the central axes of microtubules

4. SUMMARY AND CONCLUSIONS

We have presented a model based approach for microtubules enhancement by combining transform domain method and spatial domain methods. This image enhancement serves as a preprocessing step for the subsequent segmentation and structural analysis of microtubules. The contribution of this work is that we have tailored each of the above methods to fully take advantage the geometric property of the microtubules and their photometric properties. We have shown that to enhance the microtubules effectively, the wavelet transform must be both anisotropic and invariant. We have also improved the discriminant ability of the 3D shape filter by incorporating cubic fitting in the eigenvalue analysis of the second order local structure. In addition, we have proposed a new coherence measurement that more effectively reflects the anisotropy of the local structure, which makes the coherence enhancing adaptive to varying image contrast. Experimental results indicate that our proposed method has excellent performance in noise removal and enhancement of the tomography volume.



(a) (b)

(c) (d)

Fig. 4. Selected enhanced slices: (a) (c) the original slices, (b)(d) the enhanced slices

5. REFERENCES

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