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Denoising for 3D Photon-limited Imaging Data Using Non-separable Filterbanks

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Abstract

In this paper, we present a novel frame-based denoising algorithm for photon-limited 3D images. We first construct a new 3D non-separable filterbank by adding elements to an existing frame in a structurally stable way. In contrast with the traditional 3D separable wavelet system, the new filterbank is capable of using edge information in multiple directions. We then propose a data-adaptive hysteresis thresholding algorithm based on this new 3D non-separable filterbank. In addition, we develop a new validation strategy for denoising of photon-limited images containing sparse structures, such as neurons (the structure of interest is less than 5% of total volume). The validation method, based on tubular neighborhoods around the structure, is used to determine the optimal threshold of the proposed denoising algorithm. We compare our method with other state-of-the-art methods and report very encouraging results on applications utilizing both synthetic and real data.

Index Terms

3D Image Denoising; Molecular and Cellular Bioimaging; and Non-separable Filterbanks

I. Introduction

Photon-limited imaging systems such as confocal and multi-photon microscopes are obtaining high-resolution optical sections through relatively thick specimens (e.g., live brain tissues) by excluding out-of-focus light. By precise focusing at different depth and scanning through the whole specimen, imaging of an entire volume can be achieved. However, during acquisition of the volume data, noise is embedded. Several sources of noise can be identified for photon-limited imaging data [1]: i) thermal noise induced by the photomultiplier; ii) photon shot noise which accounts for the component of the noise that varies locally with intensity and can best be described using the Poisson noise model; iii) biological background or autofluorescence noise which can be described using the additive Gaussian white noise model; and iv) non-uniform fluorophore noise. There are also other imaging artifacts due to possible mismatches of refractive indices and tissue scattering.

Denoising for photon-limited imaging data has been an active research field in the last several decades and a number of effective methods have been proposed. Most of the early methods

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employed statistical technologies in the spatial domain due to the special properties of the Poisson noise [2], [3]. The maximum likelihood estimator (MLE) was the most popular one and was routinely applied in scientific and clinical practice [4], [5], [6], [7], [8]. Lately, with the fast development of the theory of wavelets and their great successes in the field of signal estimation, wavelet-based denoising methods for photon-limited imaging data have been developed [9], [10], [11], [12], [13]. These methods make full use of the excellent ability of the wavelet transform to sparsely represent the underlying intensity function. For example, Dima *et al.* [14], [15] proposed an effective wavelet denoising method for 3D confocal microscopy imaging data that includes a multiresolution validation scheme to detect edges and to suppress the responses from noise and variations of contrast. Recently, it was realized that the commonly used separable wavelet system fails to efficiently deal with edges involved in high dimensions and several new systems have been developed [16], [17], [18]. In contrast with the separable wavelet systems, these systems allow one to analyze a function in a multidirectional fashion. Hence, one can deal with straight and curve singularities more effectively. Some of these new systems were applied to denoising for photon-limited images and obtained results of very high quality [19].

Despite the advances mentioned above, the denoising problem for photon-limited imaging data of neurons is considered one of the most challenging problems due to the high complexity of the components of the different noise sources. Furthermore, a typical 3D image of a neuron has a low ratio of structure occupied voxels to total voxels (Fig. 1). Thus, special attention to morphological reconstruction of neuron cells is needed (where the structure of interest is less than 5% of the total volume [20], [21], [22], [23]) since small but important structures (dendrites) must be denoised without corrupting their structural information.

As far as validation methodology, there is an unmet need for appropriate computational phantoms in validating various denoising methods for photon-limited imaging data. Validation requires testing denoising methods on synthetic data which resemble the real data under consideration. To the best of our knowledge, there is no reliable computational phantom for fluorescence microscopy data. For example, Willett *et al.* [11] only considered the Shepp-Logan phantom and Poisson noise. Bernad *et al.* [24] only considered a simple apple-shaped textured structure and additive white noise. Furthermore, for neuron imaging data containing sparse structures, the metrics employed (mean squared error, peak-signal-to-noise ratio, and confusion matrix) in [11] and [24] to evaluate denoising methods do not account for the fact that the relevant structure is very sparse.

In this paper, we propose a new 3D frame-based denoising method for photon-limited imaging data that facilitates the morphological reconstruction of neurons by denoising small tubular structures while maintaining their original topology. To that end, we present a general framework to construct 3D non-separable frames from separable ones. In particular, we develop a non-separable 3D Parseval frame based on a 1D, piecewise linear, spline Parseval frame. The filterbank that generates this 3D frame is multidirectional, capable of detecting edges along the main axes and diagonals in 3D. In addition, we propose a simple but effective adaptive hysteresis threshold strategy based on the new 3D frame. Finally, we develop a novel validation strategy for denoising sparse 3D structures. This strategy allows us to accurately estimate classical metrics related to noise elimination.

The remainder of the paper is organized as follows¹. Section II discusses the construction of the 3D non-separable Parseval frame. Section III describes our frame-based hysteresis threshold denoising algorithm and how the synthetic model and validation strategy are

¹Parts of this work have appeared in [20], [21].

constructed. Section IV presents results on synthetic and real data. Concluding remarks are presented in Section V.

II. Construction of 3D Non-separable Parseval Frame

In this section, we describe a method to construct 3D non-separable frames in Hilbert spaces using existing frames-based digital filters. More specifically, we define in Section II-A the notion of a Parseval frame and we briefly describe in Section II-B the mathematical framework for constructing and lifting frames using filterbanks. In Section II-C, we construct the new 3D non-separable filterbanks used in this paper.

A. Parseval Frame

The theory of frames in Hilbert spaces plays a fundamental role in signal and image processing. A frame is a redundant collection of vectors in a given Hilbert space, generalizing the notion of orthogonal basis. A frame satisfies the property of perfect reconstruction: any vector of the Hilbert space can be recovered from its inner products with the frame vectors. The linear frame transform, from the initial space of coefficients (obtained by taking the inner product of a vector with the frame vectors), is injective and hence admits a left inverse [25]. Perfect reconstruction together with redundancy make the use of frames successful in a broad spectrum of applications, including signal/image processing and quantum information theory [26], [27], [28], [29], [30].

Let us recall that a *digital filter* is a vector $K \in \ell^2(\mathbb{Z}^d)$ for which the Fourier transform $\hat{K} = k$ is a bounded function. This filter acts on every digital signal by the convolution operator C_K , defined as $C_K(s) = s * K$, for all $s \in \ell^2(\mathbb{Z}^d)$. On $\ell^2(\mathbb{Z}^d)$ we will also consider the translation operator $T_{\mathbf{n}}$, defined by $T_{\mathbf{n}}s(\mathbf{m}) = s(\mathbf{m} - \mathbf{n})$, for every $\mathbf{n}, \mathbf{m} \in \mathbb{Z}^d$ and $s \in \ell^2(\mathbb{Z}^d)$.

A *frame* in a Hilbert space H , with inner product $\langle \cdot, \cdot \rangle$, is a collection of vectors $\{v_i\}_{i \in I} \subset H$, which satisfies the frame inequalities:

$$A\|x\|^2 \leq \sum_{i \in I} |\langle x, v_i \rangle|^2 \leq B\|x\|^2, \text{ for all } x \in H,$$

where $A \leq B$ are positive constants called frame bounds. For our purposes, I is a countable index set. A *Parseval frame* is a frame for which $A = B = 1$; for this frame the inequality above becomes the well-known Parseval identity. Parseval frames generalize orthogonal bases: the same vectors used in analysis (decomposition) can be used in synthesis (reconstruction). In summary, using the notation above, for a Parseval frame $\{v_i\}_{i \in I} \subset H$ we have the following perfect reconstruction formula:

$$x = \sum_{i \in I} \langle x, v_i \rangle v_i, \text{ for all } x \in H. \quad (1)$$

B. Augmenting a Frame

The power and efficiency of frames comes from their redundancy, a key ingredient in accurate reconstruction. To enhance redundancy, we will add elements to a given frame in a structurally stable way, thus obtaining new improved frames. We use our previous framework for augmenting frames [26], [27].

A finite set $\{K_0, \dots, K_l\}$ of $\ell^2(\mathbb{Z}^d)$ generates a frame in $\ell^2(\mathbb{Z}^d)$ if the family $\{T_{\mathbf{n}}K_j : \mathbf{n} \in \mathbb{Z}^d, j = 0, \dots, l\}$ of all possible translates is a frame in $\ell^2(\mathbb{Z}^d)$. The following result provides a characterization for the sets of digital filters that generate frames.

Proposition 1—Let $K_j, j = 0, \dots, l$, be a finite set of digital filters. Then $\{T_{\mathbf{n}}K_j : \mathbf{n} \in \mathbb{Z}^d, j = 0, \dots, l\}$ is a frame of $\ell^2(\mathbb{Z}^d)$ if and only if there exists constants $A, B > 0$ such that for almost every $\omega \in [-\pi, \pi]^d$ the following inequality holds:

$$A \leq \sum_{j=0}^l |\widehat{K}_j(\omega)|^2 \leq B.$$

Moreover, this frame is a Parseval frame if and only if $A = B = 1$.

As a consequence of this characterization, we have the following useful Corollary that allow us to augment frames. For a given positive integer Q , let U be a $2\pi\mathbb{Z}^d$ -periodic $(Q+1) \times (R+1)$ matrix-valued function whose entries $(U(\omega))_{q,r}$ are continuous. The matrix multiplication:

$$U(\omega)(\widehat{K}_0(\omega), \dots, \widehat{K}_R(\omega))^t = (\widehat{F}_0(\omega), \dots, \widehat{F}_Q(\omega))^t,$$

defines a new family of digital filters F_q , with $q = 0, 1, \dots, Q$. As a consequence of Proposition 1, under certain assumptions on the matrix U , the new family of filters will generate a frame.

Corollary 1—If there exists $A > 0$ such that for almost every $\omega \in [-\pi, \pi]^d$ we have $A\|\mathbf{x}\| \leq \|U(\omega)\mathbf{x}\|$ for all $\mathbf{x} \in C^{R+1}$, then the integer translates of the new family of digital filters $F_q, q = 0, 1, \dots, Q$ also form a frame for $\ell^2(\mathbb{Z}^d)$. If, in particular, $U(\omega)$ is an isometry for almost every $\omega \in [-\pi, \pi]^d$, then the resulting and the original frames have the same frame bounds.

Note that Corollary 1 is a general tool that can be used in constructing frames in any dimension. In Section II-C we construct a separable Parseval frame for the Hilbert space $\ell^2(\mathbb{Z}^3)$, and then augment it with the lifting scheme from Corollary 1 using a constant matrix U that implements an isometry (i.e., preserves distances). As a result, we are able to build a non-separable Parseval frame incorporating multidirectional edge detectors.

C. Non-separable 3D Frame from Multi-directional Filters

We begin our construction with the 1D frame described by Ron and Shen [31] as being the simplest example of a compactly supported tight spline frame. Consider the following Riesz scaling function ϕ and corresponding wavelets ψ_1 and ψ_2 :

$$\begin{aligned} \widehat{\phi}(\omega) &= \frac{\sin^2(\frac{\omega}{2})}{(\frac{\omega}{2})^2}, \quad \widehat{\psi}_1 = i \sqrt{2} \frac{\cos(\frac{\omega}{4}) \sin^3(\frac{\omega}{4})}{(\frac{\omega}{4})^2}, \text{ and} \\ \widehat{\psi}_2(\omega) &= -\frac{\sin^4(\frac{\omega}{4})}{(\frac{\omega}{4})^2}. \end{aligned}$$

The associated low-pass k_0 , band-pass k_1 , and high-pass k_2 filters are defined as follows:

$$k_0(\omega) = \cos^2\left(\frac{\omega}{2}\right), \quad k_1(\omega) = i\left(\frac{\sqrt{2}}{2}\right)\sin(\omega), \text{ and} \\ k_2(\omega) = \sin^2\left(\frac{\omega}{2}\right).$$

The filters are normalized so that:

$$|k_0(\omega)|^2 + |k_1(\omega)|^2 + |k_2(\omega)|^2 = 1, \quad (2)$$

for all $\omega \in [-\pi, \pi)$. By Proposition 1 the translates $T_{\mathbf{n}}$ ($\mathbf{n} \in \mathbb{Z}$) of the corresponding impulse responses $\widehat{k}_0 = K_0 = (1/4)[1, 2, 1]$, $\widehat{k}_1 = K_1 = (1/4) [\sqrt{2}, 0, -\sqrt{2}]$ and $\widehat{k}_2 = K_2 = (1/4) [-1, 2, -1]$ form a 1D Parseval frame for $\ell^2(\mathbb{Z})$. Note that K_1 is a first-order singularity detector (edge-detector) while K_2 is a second-order singularity detector.

To extend to 3D, we take the 3-folded tensor products of this frame with itself. Fourier calculus, the perfect reconstruction condition (Eq. 1), and Proposition 1 are needed to show that the Fourier transforms of:

$$k_{p,3^2+q,3+r}(\omega_1, \omega_2, \omega_3) = k_p(\omega_1)k_q(\omega_2)k_r(\omega_3), \quad (3)$$

with $p, q, r \in \{0, 1, 2\}$, are digital filters that generate a *separable* 3D Parseval frame with 27 filters. The term *separable* refers to the fact that the 3D filters are obtained by direct multiplication of filters from lower dimensions, in our case 1D filters. This set of 3D filters preserves the perfect reconstruction property, and incorporates detection of first-order singularities along the coordinates axes in the 3D space. Next, we will further augment this frame to incorporate additional first-order singularity detectors.

We focus our attention on the following set of filters: K_1, K_3, K_9 , the impulse responses of k_1, k_3, k_9 , respectively. These separable, unidirectional edge detector operators are tuned to detect edges in the three principal axes. We wish to augment our frame with non-separable filters capable of detecting edges along other desired directions (e.g., the main diagonals in 3D space). Let $\theta \in [0, 2\pi)$ be the angle in 3D measured counterclockwise from $(0, 1, 0)$ towards $(0, 0, 1)$ and on the positive x -axis let $\varphi \in \left[0, \frac{\pi}{2}\right]$. Then,

$$K(\theta, \varphi) = K_9 \cos \varphi + K_3 \cos \theta \sin \varphi + K_1 \sin \theta \sin \varphi,$$

represents (up to normalization) a unidirectional edge detector along the vector $(\cos \varphi, \cos \theta \sin \varphi, \sin \theta \sin \varphi)$, while K_9 corresponds to the choice $\varphi = 0$, K_3 corresponds to $\left\{\varphi = \frac{\pi}{2}, \theta = 0\right\}$ and K_1 corresponds to $\left\{\varphi = \frac{\pi}{2}, \theta = \frac{\pi}{2}\right\}$.

To incorporate additional directions to the frame, we would need to add more choices of pairs of angles in such a way that the resulting set of filters and their translates still form a Parseval frame. We apply Corollary 1 for the above frame, with $R = 27$ and $Q = R + N$, N being the number of new directional filters. We will choose U to be a constant $(R + N) \times (R)$ matrix implementing an isometry. Since it will preserve distances, the matrix U will satisfy

automatically the hypothesis required in Proposition 1. We may reorganize the filters into vector so that K_9 , K_3 and K_1 are the last 3 elements, in this order. We will only use the last 3 columns of U to augment the frame, since only these columns will affect the last 3 elements of the input vector. Therefore, we can write U as the block matrix:

$$U = \begin{pmatrix} I_{R-3} & 0_{R-3,3} \\ 0_{N+3,R-3} & V_{N+3,3} \end{pmatrix},$$

where I_k is the $k \times k$ identity matrix, and $0_{k,l}$ is the $k \times l$ zero matrix. U is an isometry whenever V is, and we define V as:

$$\begin{pmatrix} a & 0 & 0 \\ 0 & a & 0 \\ 0 & 0 & a \\ b \cdot \cos \varphi_1 & b \cdot \cos \theta_1 \sin \varphi_1 & b \cdot \sin \theta_1 \sin \varphi_1 \\ \vdots & \vdots & \vdots \\ b \cdot \cos \varphi_N & b \cdot \cos \theta_N \sin \varphi_N & b \cdot \sin \theta_N \sin \varphi_N \end{pmatrix}.$$

For V to be an isometry, it suffices that the columns are orthogonal and of norm one:

$$\begin{aligned} b^2 \sum_{i=1}^N \cos \varphi_i \sin \theta_i \sin \varphi_i &= 0, \\ a^2 + b^2 \sum_{i=1}^N \cos^2 \varphi_i &= 1, \\ b^2 \sum_{i=1}^N \cos \varphi_i \cos \theta_i \sin \varphi_i &= 0, \\ a^2 + b^2 \sum_{i=1}^N \cos^2 \theta_i \sin^2 \varphi_i &= 1, \end{aligned}$$

and finally:

$$\begin{aligned} b^2 \sum_{i=1}^N \cos \theta_i \sin \theta_i \sin^2 \varphi_i &= 0, \\ a^2 + b^2 \sum_{i=1}^N \sin^2 \theta_i \sin^2 \varphi_i &= 1. \end{aligned}$$

We have augmented the frame with two choices of angles and constants a , b . One of our choices

for the angles is $N=4$, $\varphi_i = \frac{\pi}{2}$, $i=1, \dots, 4$ and $\theta_i = \frac{\pi}{4} + (i-1) \cdot \frac{\pi}{2}$. This leads to $a = \frac{\sqrt{3}}{2}$ and $b = \frac{\sqrt{2}}{4}$ and the filters obtained by applying U to $(\hat{K}_0(\omega), \hat{K}_1(\omega), \dots, \hat{K}_R(\omega))^t$ are edge detectors along the main diagonals in 3D.

Table I presents our choice of U by listing the result of applying the operations associated with the augmentation process. We call the resulting filterbank the *UH Lifted Spline Filterbank (UH-LSF)*. All the other 24 frame elements that are not listed remain unchanged. The new frame incorporates F_1 , F_3 , F_9 which are scaled versions of the separable original filters. They

are edge detectors capable of detecting edges parallel to coordinate axes. It also defines a set of new directions by containing non-separable filters (F_{27} , F_{28} , F_{29} and F_{30}) that are tuned along the main diagonals, as depicted in Fig. 2. For example, F_{27} estimates the directional derivative in the direction of the vector $(1, 1, 1)^t$ while F_{30} estimates the directional derivative in the direction of the vector $(1, -1, -1)^t$.

Another choice for angles is $N = 8$, $\varphi_i = \frac{\pi}{2}$, and $\theta_i = (i - 1) \cdot \frac{\pi}{4}$ for all $i = 1, \dots, 8$. For this choice, one can easily verify that $a = \frac{\sqrt{2}}{2}$ and $b = \frac{\sqrt{2}}{4}$. This frame will contain, in addition to the edge detectors along the main diagonals, the detectors for edges along all the diagonals in the coordinate axes planes.

A similar construction can be performed starting from any set of 1D filters that generate a frame. For example, the filters corresponding to the Haar scaling and wavelet functions are, $\frac{1}{\sqrt{2}} [1 \ 1]$ and $\frac{1}{\sqrt{2}} [1 \ -1]$, respectively. The first filter is the low pass, averaging filter and retains most of the energy. The second filter is the detail filter, again an edge detector. Since they are created via a multi-resolution analysis, the integer translates of these filters will again generate a 1D frame.

III. Frame-based Denoising

Based on the constructed 3D non-separable filterbank, *UH-LSF*, we will present a simple but effective algorithm for noise removal in 3D photon-limited images in Section III-A. The algorithm thresholds the noisy frame coefficients based on two adaptive threshold bounds that depend on subsets of the frame elements, which is very different from the traditional wavelet shrinkage algorithm in the literature. To determine the optimal thresholds and evaluate the performance of the presented algorithm, we will develop a generalized method to construct computational phantoms that resemble the real fluorescence microscopy data of neurons in Section III-B.

A. Frame-based Affine Hysteresis Thresholding

Assume that $\{F_r\}_{r=0, \dots, R}$ is a filterbank whose integer translates form a Parseval frame for $\ell^2(\mathbb{Z}^d)$. Let $I = \{(r, \mathbf{n}) : 0 \leq r \leq R, \mathbf{n} \in \mathbb{Z}^d\}$ be the index set and let $v_{(r, \mathbf{n})} = T_{\mathbf{n}}(F_r)$ for all $(r, \mathbf{n}) \in I$. With this notation, our assumption is that $\{v_{(r, \mathbf{n})} | (r, \mathbf{n}) \in I\}$ is a Parseval frame. Let $\mathbf{X}$ represent a d -dimensional input noisy signal ($d \geq 1$). By padding $\mathbf{X}$ in all directions with zeros, we can embed $\mathbf{X}$ in $\ell^2(\mathbb{Z}^d)$. We will always consider input signals as elements of $\ell^2(\mathbb{Z}^d)$. A simple computation shows that the perfect reconstruction condition (Eq. 1) can be written as:

$$\mathbf{X} = \sum_r (\mathbf{X} * F_r^t) * F_r,$$

where F_r^t is a copy of F_r flipped about the origin: $F_r^t(\mathbf{n}) = F_r(-\mathbf{n})$ for all $\mathbf{n} \in \mathbb{Z}^3$. To see this, let $\mathbf{Y}_r = \mathbf{X} * F_r^t$. Then $\{\mathbf{Y}_r\}_r$ contains all the frame coefficients of the translates of F_r . Furthermore:

$$\begin{aligned}
Y_r(\mathbf{n}) &= \sum_{\mathbf{m} \in \mathbb{Z}^3} X(\mathbf{m}) F_r'(\mathbf{n} - \mathbf{m}) \\
&= \sum_{\mathbf{m} \in \mathbb{Z}^3} X(\mathbf{m}) F_r(\mathbf{m} - \mathbf{n}) \\
&= \langle \mathbf{X}, T_{\mathbf{n}}(F_r) \rangle \\
&= v(r, \mathbf{n}).
\end{aligned}$$

Let Λ_r be the maximum of the absolute values of frame coefficients corresponding to F_r . For denoising purposes, we set a low threshold bound $B_1(r) = a_1 \cdot \Lambda_r$ and an upper threshold bound $B_2(r) = a_2 \cdot \Lambda_r$, where a_1 and a_2 are constants (we present the optimal values of a_1 and a_2 in Section III-B). To take full advantage of the redundancy of the frame, we implement a hysteresis algorithm that modifies the frame coefficients as follows. If a coefficient's value $|c_{(r, \mathbf{n})}|$ exceeds $B_2(r)$, the coefficient remains unmodified. If this absolute value is less than $B_1(r)$, then the coefficient will be replaced by 0. If the absolute value is between $B_1(r)$ and $B_2(r)$, to decide whether to retain this coefficient we check all other filters for coefficients that correspond to the same spatial location (i.e., voxel in 3D) given by $\mathbf{n}$. If there is at least one more filter $F_{r\sim}$ with $r\sim \neq r$ and for which $|c_{(r\sim, \mathbf{n})}|$ is higher than the lower threshold bound $B_1(r\sim)$, the coefficient $c_{(r, \mathbf{n})}$ is retained but modified with an affine threshold function given by:

$$\rho_{B_1, B_2}(x) = \begin{cases} \frac{B_2}{B_2 - B_1}(x - \text{sign}(x)B_1). \end{cases}$$

In summary, the affine hysteresis thresholding is formulated as follows:

$$\tilde{c}_{(r, \mathbf{n})} = \begin{cases} c_{(r, \mathbf{n})} & , \text{ if } |c_{(r, \mathbf{n})}| > B_2(r); \\ \rho_{B_1, B_2}(c_{(r, \mathbf{n})}) & , \text{ if } B_1(r) < |c_{(r, \mathbf{n})}| \leq B_2(r), \\ & \text{and} \\ & |c_{(\tilde{r}, \mathbf{n})}| > B_1(\tilde{r}), \tilde{r} \neq r; \\ 0 & , \text{ otherwise.} \end{cases} \quad (4)$$

The choice of the affine function was motivated by the fact that it will enhance the smoothness of the structure of interest. With $\tilde{\mathbf{Y}}_r(\mathbf{n}) = c_{\sim (r, \mathbf{n})}$ containing the altered frame coefficients, the reconstructed volume will be $\tilde{\mathbf{X}} = \sum_r \tilde{\mathbf{Y}}_r * F_r$.

In contrast with the classical wavelet threshold approach, our method takes advantage of filter information with a voting-based correction scheme. Augmented frames provide at each voxel location more detailed information than a standard separable wavelet decomposition and the correction scheme will use this detailed information to decide if a coefficient will be modified or not. The proposed algorithm Frames-based Adaptive hySteresis Thresholding (UH-FAST) can be summarized as follows:

Algorithm 1: UH-FAST.

Input: The noisy data $\mathbf{X}$ and the number of decomposition levels L .

Output: Denoised data.

Step 1: Recursively decompose the volume $\mathbf{X}$ up to level L using the filterbank to obtain $\{\mathbf{Y}_r\}_r$.

Step 2: Compute $\{\tilde{\mathbf{Y}}_r\}_r$ by applying the approach described in Eq. (4).

Step 3: Reconstruct $\tilde{\mathbf{X}}$ from $\{\tilde{\mathbf{Y}}_r\}_r$ using the same filter-bank.

As compared with most existing wavelet-based denoising algorithms, our UH-FAST algorithm processes all high frequency subbands but keeps the lowpass subband unchanged.

B. Computational Phantom and Validation Strategy

In the following, we will outline the construction of computational phantoms for fluorescence microscopy data that can be used for the validation of the performance of a denoising algorithm. We will use these computational phantoms to determine the optimal threshold bounds for our denoising algorithm.

The construction has the following steps: i) create binary volume; ii) simulate intensity decay; iii) create tubular neighborhoods; and iv) add noise.

Create binary volume—Based on the Duke-Southampton database of cells [32], we create a volume sampled at the desired resolution where the voxels occupied by the cylinder are labeled 1 and background voxels are labeled 0 (note such a binary volume can be used, for example, as the ground truth for dendrite morphology reconstruction tasks).

Simulate intensity decay—Construct a volume in which the intensity decays linearly in the voxels that correspond to the neuron and simulates the diffusion of the dye in the dendrites. The collection of cylinders is presented in a tree-like description with the root in the soma. The linear intensity decay is based on the tree-distance of the cylinders to the soma. Such volume will be used as the original for the denoising tasks considered in this paper.

Create tubular neighborhoods—Create tubular neighborhoods around the neuron, based on a prescribed ratio of volumes and surfaces. We can create several neighborhoods in which the neuron occupies at least 5% of the total volume of the neighborhood. These neighborhoods will be used both to create several types of synthetic noise and to define accurate metrics.

Add noise—Add to the tubular neighborhoods decaying speckle noise with randomly generated statistical parameters. To obtain the local Poisson noise, we apply a Poisson noise with variance (e.g., 0.1 of the local intensity). To compensate for the intensity gap between the layers of noise, we filter the image with a zero mean gaussian noise with local intensity dependent variance. For more realistic effects, we convolve the volume with the theoretical point spread function derived using the parameters of the microscope used to acquire the real data.

Figure 3(a) depicts a binary volume with dimensions of $374 \times 158 \times 57$ and isotropic voxels, created using the descriptions of n120.swc from the Duke-Southampton database [32]. Noise is added in several layers of tubular neighborhoods to obtain the data depicted in Fig. 3(b), as described above.

Using the constructed computational phantoms, we will determine the optimal threshold bounds for our denoising algorithm. As pointed out by Dima *et al.* [14], for 3D neuron data denoising applications the Mean Square Error (MSE) computed at the level of the entire volume would not produce good results due to the small structure-to-volume ratio in images depicting neurons. In fact, Dima *et al.* do not consider this metric at all, concluding that its value is

meaningless for this class of volumes. We hence need to define several new metrics that account for sparse structures of neurons.

Our strategy, as outlined in Fig. 3(c), is to consider metrics in the regions close to the structure, according to tubular neighborhood constructed above. Our approach permits a local neighborhood evaluation. Let I be the original and I_d be the denoised image, both with dimensions $n_1 \times n_2 \times n_3$. Let χ_{LN} be the indicator function of the local neighborhood in which the metrics are evaluated. We define new metrics, namely, local neighborhood MSE (LN-MSE), local neighborhood Root MSE (LN-RMSE), local neighborhood signal-to-noise-ratio (LN-SNR) and local neighborhood peak-signal-to-noise-ratio (LN-PSNR) as follows:

$$\begin{aligned} \text{LNMSE}(I, I_d) &= \frac{\|(I - I_d) \chi_{LN}\|_2^2}{n_1 n_2 n_3}, \\ \text{LNRMSSE}(I, I_d) &= \sqrt{\text{LN-MSE}(I, I_d)}, \\ \text{LNSNR}(I, I_d) &= 10 \log_{10} \frac{\|I \chi_{LN}\|_2}{\|(I - I_d) \chi_{LN}\|_2}, \text{ and} \\ \text{LNPSNR}(I, I_d) &= 20 \log_{10} \frac{\max_I}{\text{LN-RMSE}(I, I_d)}. \end{aligned}$$

Similarly, for the evaluation of preservation of structure, we also can define the local confusion matrix [20], [21].

Obviously, the lower and upper thresholds of our algorithm play a key role for noise removal of the photon-limited data. It is natural to determine the optimal thresholds employing these computational phantoms constructed above. The simplest and most straightforward criterion is to choose the thresholds that lead to minimum of the LN-MSE. Experimentally, we found that the optimal value for the lower threshold is in the range $[0.5-0.6]\Lambda_r$, and for the upper threshold is in the range $[0.7-0.8]\Lambda_r$. In addition, we also found that these optimal thresholds do not change significantly with the data and noise considered, which is indeed desirable for practical applications. In this paper, we set the optimal lower and upper threshold to be $0.5\Lambda_r$ and $0.75\Lambda_r$, respectively. Experiments on synthetic and real data both demonstrated that such a choice leads to very encouraging results for the proposed algorithm, as will be shown in Section IV.

IV. Results and Discussion

We compare our algorithm to median filtering, nonlinear anisotropic diffusion filtering, and a threshold algorithm based on the 3D separable Haar system. The median filter is a non-separable edge-preserving smoothing filter. It is usually applied as a preprocessing step to reduce the amount of noise. The filter sorts pixels covered by a $N \times N \times N$ mask according to their intensity; the center pixel is then replaced by the median of these pixels. We used a $3 \times 3 \times 3$ mask in our experiments. Nonlinear anisotropic diffusion filtering employs an iterative, ‘tunable’ filter introduced by Perona and Malik [33]. Perona and Malik formulated it as a diffusion process that encourages intraregion smoothing while preserving the edges. Broser *et al.* [34] used the anisotropic diffusion filtering to average noise along the local axis of the neuron’s tubular-like dendrites in order to maintain morphological structure. In all the experiments, the parameters used for the anisotropic diffusion were set to 50 iterations with a time step of 0.0625, a conductance parameter equal to 3, were implemented using the Insight Segmentation and Registration Toolkit (ITK) [35]. The most widely-used wavelet threshold algorithm first estimates the noise level according to the median of absolute value of coefficients in the high frequency subband, then determines the threshold based on the estimated noise level. Surprisingly, we found such an algorithm does not work for both synthetic and real volumes for the 3D separable Haar system. Specifically, the estimated

threshold for our test data is about zero or a very small number. The reason is that the neuron data are very sparse and the majority of noise components are signal-dependant (e.g., the Poisson noise component) in the 3D photon-limited case. As a result, most wavelet coefficients are near zero and the estimated threshold too. Instead of estimating the threshold using the median value method, we set the threshold to be half of the maximum of the absolute value of coefficients in a subband for the threshold algorithm based on the 3D separable Haar system.

For the proposed algorithm, we employ the 3D filterbank constructed in Section II-C, namely, the *UH-LSF*.

A. Synthetic data

We first test our algorithm on synthetic noisy volumes – the computational phantoms constructed using the method presented in Section III-B. Two synthetic noisy volumes are used in our experiments. The first, depicted in Fig. 3(b), is of dimensions $374 \times 158 \times 57$. The second, depicted in Fig. 4(b), is of dimensions $180 \times 66 \times 40$. The first noisy volume is used here to simulate the neuron imaging data as a whole, while the second one provides us the opportunity to investigate the behavior of our denoising algorithm on more detailed structures of a neuron (e.g., the basal dendrites).

To assess the performance of our denoising algorithm, we use local metrics computed in the tubular neighborhoods, as defined in Section III-B. The performance for two synthetic data sets is presented in Tables II and III, respectively.

From Tables II and III, it is clear that all algorithms used in our experiments can significantly suppress noise components in the data. Our algorithm produces the best results for both noisy volumes (as measured by all local metrics). For example, for the noisy volume depicted in Fig. 3(b), our algorithm is 6.89 dB better than the nonlinear anisotropic diffusion filtering in terms of LN-PSNR; for the noisy volume depicted in Fig. 4(b), our algorithm is 187.53 units better than the median filtering in terms of LN-MSE. For visual comparison, we present denoising results using the four algorithms for the noisy volume depicted in Fig. 4(b). In Fig. 4(c), we observe that the median filtering destroys fragile details, which in fact include very important information needed for the morphological analysis of neurons. The nonlinear anisotropic diffusion method and the threshold algorithm based on the 3D Haar system tends to remove most of the noise (Figs. 4(d)-(f)). However, by carefully inspecting the corresponding results, we can find some sections of the structure of interest that are not preserved. By comparison, our algorithm not only removes most background noise, but also preserves the tubular structure — even in regions with very fine details (Fig. 4(e)).

Table IV depicts the running time of each denoising method². Among the four algorithms, median filtering is the most computationally effective. Our method runs slower than the median filtering and the thresholding algorithm based on the 3D separable Haar system, but faster than anisotropic diffusion filtering.

B. Multi-photon and Confocal Microscopy data

We have tested our method on both multi-photon and confocal microscopy data sets and compared quantitatively and qualitatively its performance with the other three filtering methods.

²The platform we used for our experiments is as follows: Hardware Architecture: PC; Operating System: Windows XP; Processor: 2.6 GHz; Memory Size: 4G.

With respect to multi-photon imaging, the cells of interest were mouse cortical CA1 pyramidal neuron cells from rat hippocampi. Images were acquired with a customized multi-photon Galvo microscope and the cell was injected with Alexa Fluor 594 dye. Our ORION Team [36] has acquired twelve image datasets consisting of seven or more partially overlapping stacks with size of $640 \times 480 \times 150$ each, and voxel size of $0.3 \times 0.3 \times 1.0 \mu\text{m}^3$. Excitation wavelength was set to 810 nm while the lens and index of refraction both correspond to water. Figure 5(a) depicts the maximum intensity projection of a registered volume along the $x - y$ and $x - z$ axis respectively; where the volume dimensions are $2362 \times 1126 \times 146$ voxels along the x , y and z axis.

We define a structural-quantitative measure as the length of the dendrites obtained from the largest connected component after applying a global threshold obtained from the denoising results. This allow us to assess the sensitivity of each algorithm to produce ‘gaps’ among dendrites (tubular structures) and how well the overall connectivity is preserved. Figure 5(b) depicts a detail of the original volume, while Figs. 5(c)–(e) depict the projection of the binary segmented volume with a threshold value set to 10 in the $x - y$ axis. Notice that fragile details are lost in the case of median filtering and anisotropic diffusion filtering. We note that some background noise is present after denoising with wavelets in the first level of decomposition (Fig. 5(f)), whereas in the second level the background noise is mostly removed (Fig. 5(g)). The effect of the separable filter can be observed in Fig. 5(g), where a block effect is introduced. This effect is visible in the binarized volume. This block effect in the binary volume is not desired since it does not allow capturing local structures (spines) which populate the dendrites. In addition, we observe that some line segments are broken when applying median filtering and anisotropic diffusion. By comparison, our algorithm can preserve more edges, even the weak ones, as depicted in Fig. 5(e) without producing an aliasing effect (our transform is undecimated). Figure 6 depicts the estimated length from the binarized largest connected component at different threshold values. We observe that in low threshold values our method preserves more structure than the other three filtering methods. In high threshold values we observe that the performance of the anisotropic diffusion is better than our method and median filtering, and the largest difference with respect to our method and the median filtering is at the value of 40. This effect can be explained since the anisotropic diffusion preserves well only strong edges. In addition, it should be noted that while we increase the threshold value, the relation between the energy of the signal (of the volume of interest) and the ability to capture details at very low energy levels is indirectly proportional.

The confocal data sets that we used in our experiments were acquired from neuron cells either loaded with Alexa Fluor 555 and 488 dyes or taken from a line expressing enhanced green fluorescent protein. The current cells of interest are CA1 pyramidal cells from mice or rat hippocampi. These experimental data sets consist of three or more partially overlapping stacks with an approximate resolution of $1024 \times 1024 \times 120$ each. The voxel size is $0.23 \times 0.23 \times 0.5 \mu\text{m}^3$. Note that each stack may exhibit a different noise level as they are acquired independently.

Figure 7(a) depicts a confocal imaging volume with a selected region of interest. For comparison, a manually segmented version of the structure in the selected region is depicted in Fig. 7(c). As it can be observed, both median filter and our algorithm obtain satisfactory results in this case. On the contrary, the nonlinear anisotropic diffusion tends to destroy fine details (Fig. 7(e)). Again, the block effect can be easily observed in the result due to the separability 3D of the Haar wavelet (Fig. 7(g)).

C. Discussion

In the experiments above, we have demonstrated the high efficiency of the constructed 3D non-separable system for noise removal of neuron photon-limited imaging data. Compared with other two algorithms, namely, the median filtering and the nonlinear anisotropic diffusion, our

algorithm has a significant advantage – it is preserving the edge information. The main reason, we believe, is the high efficiency of the new 3D non-separable system to capitalize on directional information. In Section II-C, we constructed the 3D non-separable system by adding new filters into existing separable systems. These new filters in fact correspond to new directions that the separable system cannot address effectively. More precisely, these new filters correspond to the main diagonals in 3D. Furthermore, we have investigated the energy distribution of different subbands of the new 3D system. As usual, for the new system, most of the energy (i.e., the l_2 -norm) is contained in the first filter: the low-pass filter F_0 . The energy contribution of remaining 30 filters is presented in Fig. 8. Most of the energy is captured by the detectors of first and second order singularities. Notice that the subbands due to newly-added filters (F_{27} , F_{28} , F_{29} , and F_{30}) have significant energy, even more than that of existing filters. This means that the data have energy in a main diagonal direction and this directional information has been captured by the new 3D non-separable system.

For our problem domain, “structural noise” are those structures in the background which do not belong to the neuron cell – mainly blob-like structures. Figures 9(a),(b) depict the maximum intensity projection along the x - y axis of a raw dataset and after denoising with our method, respectively. Structural noise corresponding to blob-like structures is enclosed in yellow boxes before and after denoising. We emphasize that our denoising method is not intended to remove structural noise. As can be observed from Fig. 9(b), those structures in the background of considerable size are not removed, but neither are they enhanced.

V. Conclusion

We have presented a novel method that allows us to separate structure from noise in volume data from photon-limited imaging systems. Based on the design of a Parseval frame, this method produces results of significant visual fidelity. Although the results presented in this application are from a particular domain, our algorithms apply to any biomedical imaging dataset with similar assumptions about the sources of noise. By choosing a well designed set of filters, such as the multidirectional edge detectors presented in this paper, we can exploit the redundancy that comes from expressing the data in terms of this new type of basis - the resulting frame. Redundancy is the key element in preserving structure while thresholding the frame coefficients. This makes the frame transform a versatile and effective denoising tool.

We have compared our method to commonly used denoising methods and we have shown that it outperforms them. We have provided evidence of the visual fidelity offered by our method in comparison with the other methods. We have applied our method to neuron data acquired with confocal and multi-photon microscopes, where dendrites are extremely fragile elements and their correct identification and estimation are crucial to this type of imaging experiments.

We are considering further automation of lower threshold bound estimators, as well as the design of other frames (e.g., based on curvelets). These topics are currently under investigation.

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Fig. 1.
Depiction of neuron morphology.

Fig. 2.
Depiction of the directional derivatives.

Fig. 3. Maximum intensity projection of volume data from synthetic neuron n120; (a) binary volume; and (b) with added noise. (c) Depiction of the local neighborhood associated with noise removal.

Fig. 4.

Denoising results due to different algorithms on a synthetic noisy volumes. (a) Original volume; (b) noisy volume; (c) result due to median filtering; (d) result due to anisotropic diffusion; (e) result due to the FAST algorithm; and (f) result due to thresholding algorithm based on the 3D Haar wavelet.

Fig. 5.

Results of applying our denoising, anisotropic diffusion, median filtering and thresholding using the 3D Haar wavelet. (a) Maximum intensity projection in the x - y axis of the volume of interest. (b) Details of the selected region of interest (yellow square in (a)). Results of applying a global threshold with value $T = 10$; (c) median filtering; (d) anisotropic diffusion filtering; (e) UH-FAST denoising algorithm; and (f),(g) threshold algorithms based on the 3D Haar wavelet with one and two levels of decomposition, respectively.

Fig. 6.

Performance evaluation of the length estimation in function of the detected largest component volume at a given threshold (the number of levels of decomposition of the wavelet transform was two).

Fig. 7.

Results of denoising in a selected region. (a) Original volume; (b) selected region; (c) manually segmented result; (d) result due to median filtering; (e) result due to anisotropic diffusion filtering; (f) result due to the UH-FAST algorithm; and (g) result due to thresholding using 3D Haar wavelets.

Fig. 8. Energy distribution other than the low pass filter in each subband of the *UH-LSF* for the 3D neuron data of Fig. 5(a).

Fig. 9. Structural noise. (a) Maximum intensity projections in the x - y axis of the volume of multiphoton data (the yellow boxes indicate structural noise areas), (b) denoised with the UH-FAST algorithm.

TABLE I
Uh Lifted Spline Filterbank: Selected Frame Elements

$F_1 =$	$\frac{\sqrt{3}}{2}K_1$
$F_3 =$	$\frac{\sqrt{3}}{2}K_3$
$F_9 =$	$\frac{\sqrt{3}}{2}K_9$
$F_{27} =$	$\frac{1}{4}(K_9 + K_3 + K_1)$
$F_{28} =$	$\frac{1}{4}(K_9 - K_3 + K_1)$
$F_{29} =$	$\frac{1}{4}(K_9 - K_3 - K_1)$
$F_{30} =$	$\frac{1}{4}(K_9 + K_3 - K_1)$

TABLE II
Performance Evaluation on the Noisy Volume Depicted in Fig. 3

Metric	Noisy	Anisotropic Diffusion Filtering	Median Filtering	3D Haar Threshold	UH-FAST
LN-MSE	67.13	63.49	16.17	20.28	12.97
LN-RMSE	8.19	7.96	4.02	4.50	3.60
LN-SNR (dB)	-4.19	-3.95	1.98	1.00	2.94
LN-PSNR (dB)	45.25	45.49	51.43	50.45	52.39

TABLE III
Performance Evaluation on the Noisy Volume Depicted in Fig. 4

Metric	Noisy	Anisotropic Diffusion Filtering	Median Filtering	3D Haar Threshold	UH-FAST
LN-MSE	749.06	720.16	448.82	349.23	261.28
LN-RMSE	27.36	26.83	21.18	18.68	16.16
LN-SNR (dB)	1.36	1.53	3.58	4.67	5.93
LN-PSNR (dB)	34.77	34.94	37.00	38.09	39.35

TABLE IV
Performance Evaluation - Time (Unit: Second)

	Anisotropic Diffusion Filtering	Median Filtering	3D Haar Threshold	UH-FAST
Noisy Volume Fig. 3(b)	687.95	58.59	71.97	374.76
Noisy Volume Fig. 4(b)	28.85	2.09	3.80	14.20