Non Parametric Denoising Methods Based on Wavelets: Application to Electron Microscopy Images in Low Exposure Time

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Abstract. The 3D reconstruction of the Cryo-Transmission Electron Microscopy (Cryo-TEM) and Energy Filtering TEM images (EFTEM) hampered by the noisy nature of these images, so that their alignment becomes so difficult. This noise refers to the collision between the frozen hydrated biological samples and the electrons beam, where the specimen is exposed to the radiation with a high exposure time. This sensitivity to the electrons beam led specialists to obtain the specimen projection images at very low exposure time, which resulting the emergence of a new problem, an extremely low signal-to-noise ratio (SNR). This paper investigates the problem of TEM images denoising when they are acquired at very low exposure time. So, our main objective is to enhance the quality of TEM images to improve the alignment process which will in turn improve the three dimensional tomography reconstructions. We have done multiple tests on special TEM images acquired at different exposure time 0.5s, 0.2s, 0.1s and 1s (i.e. with different values of SNR)) and equipped by Golding beads for helping us in the assessment step. We herein, propose a structure to combine multiple noisy copies of the TEM images. The structure is based on four different denoising methods, to combine the multiple noisy TEM images copies. Namely, the four different methods are Soft, the Hard as Wavelet-Thresholding methods, Bilateral Filter as a non-linear technique able to maintain the edges neatly, and the Bayesian approach in the wavelet domain, in which context modeling is used to estimate the parameter for each coefficient. To ensure getting a high signal-to -noise ratio, we have guaranteed that we are using the appropriate wavelet family at the appropriate level. So we have chosen âĂIJsym8âĂİ wavelet at level 3 as the most appropriate parameter. Whereas, for the bilateral filtering many tests are done in order to determine the proper filter parameters represented by the size of the filter, the range parameter and the spatial parameter respectively. The experiments reported in this paper demonstrate the performance of the Bilateral Filtering and the Bayesian approaches in terms of improving the SNRout and the image quality. Taken together, these results suggest that the Bayesian process has a potential to outperform all the used methods, where in the multiple noisy copies structure it gave us the best SNRout without change of the golden beads diameter. The Bayesian approach yielded enhanced average image without needing a huge amount of copies.

Keywords: Cryo-Transmission Electron Microscopy, EFTEM
PACS:

INTRODUCTION

Transmission electron microscopy (TEM) is a microscopy technique able of imaging biological samples at high resolutions in biological sciences.TEMâĂŹs technique is based on scattering an electron beam at ultra-thin specimen, which interacts with the electrons beam. This interaction between the atoms of the specimen and the electron beam causes a deviation of the beam from its initial trajectory. This technique permits TEM's to be used to visualize molecular structure of proteins and large molecules. Cryo-electron microscopy involves viewing unaltered macromolecular assemblies by vitrifying them, placing them on a grid and obtaining images by the electrons transmitted through the specimen [1]. However, there is a drawback in TEM technique if the specimen is biological, where the electron beam, may cause damage in the specimen because of the sensitivity of the biological samples to this radiation. This leads the biologist to image the specimen at very low electrons doses, which creates a new problem, the noise in the obtained images. This hinders the alignment process during the 3D reconstruction of the TEM images, which requires find a way to reduce as much as possible this noise in order to get a good 3D image quality. For this, there are many scientists interesting in creating new methods to minimize the noise in its different form, so several methods now exist to reduce the noise in images. Some of the denoising methods succeeded in eliminating the noise but damaged the image by blurring the edges as the Gaussian filtering techniques does. In contrast some methods are capable to preserve the image edges like the Bilateral Filtering which is based on both spatial and intensity distances. The effectiveness of the method refers to the capacity of distinguishing between the information and the noise, which is an important point in giving a good quality images after the denoising. In wavelet domain, Donoho and Johnston proposed the famous wavelet thresholding methods, which is widely applied in signal denoising [2], [3], specifically, the soft and hard thresholding methods. These methods are based on choosing a thresholding value, usually calculated from the details coefficients to maintain the approximations values, and then applied it to separate the significant coefficients from the noise coefficients. There are also a nonparametric methods used in image denoising, such as nonparametric Bayesian estimators in wavelet domain[4], [5], where a prior statistical model based on the -stable densities used to exploit the sparseness of the wavelet detail coefficients. In this paper, we studied the problem of noise in the TEM images using four methods combining each one with an averaging operator in multiple noisy copies. The main objective is to enhance the 3D quality after the TET (Transmission electron tomography) by using the denoised projections. We have tested the proposed structures on a set of experimental TEM test images acquired with different exposure time and organized in zones, each zone differ from the other in the acquisition order of the images. We show the effectiveness of the Bayesian method besides the wavelet thresholding and the bilateral filtering algorithmsin removing noise and enhancing the SNR of these images, thus improving the quality of the TEM images, before the alignment step during the tomographic reconstruction. The remainder of the paper is organized as follows: in Section II, we discussed the noise proprieties in the Transmission Electron Microscopy. Section III is devoted to the exposition the development of the considered methods. Section IV compares the performance of the designed structure using multiple noisy copies with previously published denoisers on an experimental database of TEM images. Finally, concluding remarks illustrate the capabilities of our proposal.

NOISE SOURCES IN TEM TECHNIQUE

Getting inside the structural molecular biology was a challenge for the scientists till the apparition of the electron tomography which has a big role in avoiding the surgical intervention. The emergence of the electron microscope allowed them to achieve their ambitions. They were able to reveal the structure of smaller objects because of the electron microscope which has a higher resolving power than a light microscope. However, the TEM technique suffers from a number of drawbacks, it necessitates ultra-thin specimen which require an extensive preparation plus the sensitivity of samples during the interaction with the electron beam. For the sake of maintaining the specimen from the electrons radiation and avoid the damaged happened in case if it highlighted with high dose electrons, the biologist uses low electron doses (measured in electrons per square Angstrom). This makes the TEM images noisy and poor in their contrast. There are two types of noise (background) in electron microscopy. The first one which is very low comes from the sensor such as the CCD camera while the second comes from the inelastic interactions of the electrons beam with the specimen. The noise from the camera is very low, so we can neglect it. In these experiments, the test TEM images are first acquired at different exposure time to get multiple noisy copies of each test images. We denoised the TEM images before their alignment to enhance the 3D reconstruction. We have considered the noise in our test images as a white Gaussian noise and applied the proposed methods combined with averaging operator for the case of multiple noisy copies. Note that the acquired test images are regrouped in different zones.

THE PROPOSED METHODS FOR THE TEM IMAGES DENOISING

Denoising Using Thresholding Methods

Thresholding technique is one of the simplest methods in the denoising domain, either for 1D or 2D signals, it is based on separating the low coefficients which are contaminated by the noise from the high coefficients, depending on the calculated thresholding value and the used methods. We have used in our studies two methods the Hard and Soft thresholding. These two methods differ in the way how the coefficients selected, where in the first methods (keep or kill) the coefficients that their values less than the thresholding value considered as zero, whereas the higher ones kept.This technique is usually used in medical image processing. While the second method the shrinkage process is calculated according to Eq.(6) [3]. In our study, we consider the following observation model :

$$y = x + \xi \tag{1}$$

where$y$ is the measured image, x is the desired image, and ξ is gaussian. Then we applied the wavelet transform to our observations:

$$D(y) = D(x) + \xi \tag{2}$$

The operator D indicates that the model is in the wavelet domain, where the good choices of the mother wavelet, the threshold type, the decomposition level and the way how the thresholding values are calculated is critical. it determines the Signal-to-Noise Ratio (SNR) after denoising. Knowing that we have tested all these parameters before doing this comparison, where in our experiment after analyzing previous results we have chosen âĂŸsym8âĂŹ as the mother wavelet beside âĂŸlevel 3âĂŹ as the decomposition level which gave us good results without affecting the images. For getting the optimal thresholding value we applied the universal ruleproposed by Donoho and Johnstone [2] given by:

$$T = \sigma\sqrt{2\log(n)} \tag{3}$$

σ is the standard deviation of the data values, whereas n is thelength of the analyzed image.This popular estimate of the noise level σ is based on the last level of the detail coefficients, and used the median absolute deviation during the calculation,according to the following equation:

$$\sigma = \frac{median(D(y))}{0.6745} \tag{4}$$

The factor in the denominator is equal to 0.6745 for normally distributed data. The thresholded wavelet coefficients are obtained using either hard or soft thresholding rule given respectively by:

$$\widehat{D}_j(y) = \begin{cases} D_j(y)\ \left|D_j(y)\right| \geq T \\ 0\ otherwise \end{cases} \tag{5}$$

$$\widehat{D}_j(y) = \begin{cases} sign\left(D_j(y)\right).\left(\left|D_j(y)\right| - T\right)\ \left|D_j(y)\right| \geq T \\ 0\ otherwise \end{cases} \tag{6}$$

Where $D_j(y)$ are the noisy wavelet coefficients at level j , the denoised wavelet coefficients and T is the threshold value.

Denoising Using Nonparametric Bayesian Estimator

Over the last decade,various estimation approaches are proposed under the wavelet domain in the context of Bayesian paradigms, where a prior distribution is provided to the wavelet coefficients [4,5]. These works proved the efficiency of the Bayesian wavelet estimators which outperform the classical wavelet term- by-term thresholding estimators. In our work, the applied Bayesian Denoiser in the wavelet domain is based on adapting a prior statistical model for $D(x)$ in Eq. (2), and then imposes it on the wavelet coefficients to describe their distribution [4,5]. It follows from Eq. (1) that:

$$\begin{cases} c_{mn} = a_{mn} + \xi \\ d_{mn}^{0j} = s_{mn}^{0j} + \xi_{mn}\ \ j = J_c, ..., J-1\ \ m,n = 0, ..., 2^j - 1 \end{cases} \tag{7}$$

Where $a_{mn}$is the approximation coefficient of the true image x (resp.y) at location (m,n), s_{mn}^{0j} the details coefficients of the original image in the wavelet domain, j and o are the scale and the orientation respectively. The complete process of the Bayesian Denoiser in wavelet domain shows in the following diagram in Fig.1.

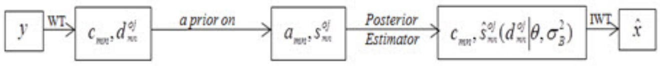

FIGURE 1. Flowchart of the used Bayesian Denoiser

We can describe the denoising process by using the BayesianDenoiser, in the following steps

step1

Calculation of the wavelet coefficients of the noisy data as in Eq.(2);

step2

Application of the Bayesian denoising algorithm to estimate the denoised wavelet coefficients. For the sake of clarity, we report here more details of this step. Different choices of loss function lead to different Bayesian rules and hence to different nonlinear wavelet shrinkage and wavelet thresholding rules. For example, it is well known that the L1-loss function corresponds to the maximum a posteriori (MAP) estimator [4]. However, except for some special cases of alpha stable distributions (e.g. $\alpha = 2$), it is not easy to derive a general analytical form of the corresponding Bayesian shrinkage rule even within the scale mixture approximation. Alternatively, the L2-based Bayes rules are used which correspond to posterior conditional means (PCM) estimates. The general expression, using the approximate prior PDF, of the PCM estimates of the wavelet coefficients $\widehat{s}$ is

$$\widehat{s}(d/\theta) = \frac{\sum_j P(j) \dfrac{d\sigma^2}{\sigma_j^2 + \sigma_B^2} \Phi\left(d; \sigma_j^2 + \sigma_B^2\right)}{\sum_j P(j) \Phi\left(d; \sigma_j^2 + \sigma_B^2\right)} \tag{8}$$

Where θ is the hyper-parameters set, $\theta_1 = \{P(j), \theta j\}$ and $\Phi(d;\theta_2)$ is the gaussian noise PDF with variance $\theta_2 = \sigma_B^2$;

step3

Reconstruction of the denoised image $\widehat{x}$ by computing the inverse wavelet transforms IWT from the estimated wavelet coefficients.

Denoising Using Bilateral Filtering

The bilateral filter is a nonlinear filter that does spatial averaging which can blur the image without smoothing its edges [6]. The key idea of the bilateral filter is that for takes the weighted sum of the nearby pixels; they also should have similar values. It means that the weights depend on both the spatial distance and the intensity distance, which preserves the edges and averages the noise. The bilateral filter at a pixel location x ,is defined by:

$$I(x) = \frac{1}{c} \sum_{y \in N(x)} e^{\frac{-\|y-x\|^2}{2\sigma_d^2}} e^{\frac{-\|I(y)-I(x)\|^2}{2\sigma_r^2}} I(y) \tag{9}$$

where σ_d and σ_r are parameters controlling the fall-off of the weights in spatial and intensity domains, respectively, is a spatial neighbourhood of and is the normalization constant [6].

$$C = \sum_{y \in N(x)} e^{\frac{-\|y-x\|^2}{2\sigma_d^2}} e^{\frac{-\|I(y)-I(x)\|^2}{2\sigma_r^2}} \tag{10}$$

In our study, we applied the bilateral filter to denoise our TEM noisy data. In order to compare the performance of this filter to the other proposed methods, we have done previous analyses where derived the appropriate parameters for the bilateral filter we want to use (window size,σ_d and σ_r). The proposed structure of multiple noisy copies, based on wavelet transform, consists of three major modules: (i) a subband representation function that utilizes the wavelet transform, (ii) applying one of the considered denoising algorithms and (iii) the traditional averaging operation. As shown in Fig. 4, the denoised copies (the outputs of the denoiser blocs) are combined by averaging operation in order to obtain the final recovered image.

EXPERIMENTAL RESULTS

In this section, the assessments of the denoising results are reported. Due to space limitation, we present some of the obtained results.

Test Images Dataset

In order to verify the proposed methods, we worked with Cryo Microscopy images acquired by using a JEOL 2200FS transmission electron microscope operating at 40000X (Name microscope Mag 10000x) at different exposure times, and different zones.The order of acquisitions of each zone is as follows: zone1: 2s(1x), 1s(2x), 0.5s(4x), 0.2s(10x), 0.1s(20x), zone2: 1s(2x), 0.5s(4x), 0.2s(10x), 0.1s(20x), 2s(1x), zone3: 0.5s(4x), 0.2s(10x), 0.1s(20x), 2s(1x), 1s(2x), zone4: 0.2s(10x), 0.1s(20x), 2s(1x), 1s(2x), 0.5s(4x), zone5: 0.1s(20x), 2s(1x), 1s(2x), 0.5s(4x), 0.2s(10x).We should note that the order of acquisitions is taken in account. This order is put intentionally by the biologists because it is affecting the images which area golden beads whose size is a quality control of the methods, and frozen water in the glassy state.

Denoising Quality, and Computational Performance

-Evaluation using the circularity coefficient: we calculated the gold beads circularity for the sake of getting a good assessment of the used methods. We calculate this parameter by using ImageJ 1.47 [7] according to the following equation:

$$circularity = 4\pi \frac{Air}{(perimeter)^2} \tag{11}$$

If the circularity is equal to 1 this means that the beads are circular and we should keep it as one or improve it if is not equal to one. We can also check the circularity of the Gold beads through the aspect ratio parameter AR. It is defined as the ratio of the Axis Major to the Axis minor of the Gold beads by using Eq. (12). This parameter is also measured by using ImageJ 1.47. image analysis software. If the resulting aspect ratio equals one, the Gold beads are round. These two parameters, the Circularity or the AR indicate the accuracy of the proposed denoising approach.

$$Ar = \frac{(AxisMajor)}{(AxisMinor)} \tag{12}$$

If the resulting aspect ratio equals one, the Gold beads are round. These two parameters, the Circularity or the AR indicate the accuracy of the proposed denoising approach.

Experimental Denoising Results

-For one copy: We first, show the results where we denoised each image separately using the Soft, Hard Thresholding, Bilateral filtering and the Bayesian Approach. The results are shown in Tab.1. The first row contains the name of the original images from zone1, where we chose to take one image as an example from each series due to limitation space, 0.1s-001 from 0.1s series, 0.2s-001 from 0.2s series, 0.5s-001 from 0.5s and 2s. We can see that the proposed method successfully enhances the SNR_{out} compared to the SNR_{in} of these images. It is also seen that the Bayesian Approaches gave higher SNR_{out} for 0.5s, 1s and 2s images than the obtained by applying Bilateral Filtering and the Thresholding methods. The original images are shown in Fig.2, Fig.3 shows the denoised ones.

TABLE 1. The SNRin and SNRout Results of the analysed denoising methods for one noisy copy

Zone1/ Images	SNR_in	Methods	SNR_out
0.1s-001	17.1395	Bayesian	19.1895
		Bilateral Filtering	20.7715
		Soft	18.4397
		Hard	18.4324
0.2s-001	20.2354	Bayesian	22.1332
		Bilateral Filtering	22.4993
		Soft	21.4752
		Hard	21.4687
0.5s-001	24.0256	Bayesian	25.8649
		Bilateral Filtering	25.0961
		Soft	25.2006
		Hard	25.1946
1s-001	26.7572	Bayesian	28.3607
		Bilateral Filtering	27.2268
		Soft	27.8743
		Hard	27.8684
2s	29.2794	Bayesian	30.7616
		Bilateral Filtering	29.4420
		Soft	30.3088
		Hard	30.3024

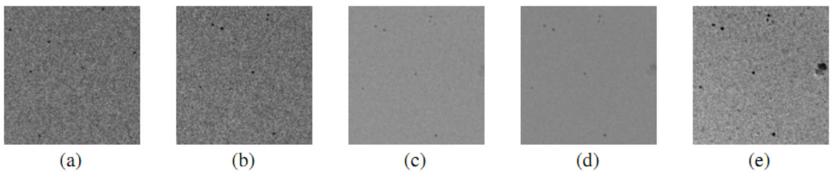

FIGURE 2. The Denoised image 0.1s-001from zone1 after applying :(a) The bilateral Filtering, (b) The Bayesian Approach,(c) Soft Thresholding,(d) Hard Thresholding

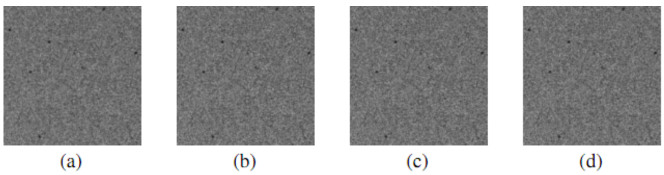

FIGURE 3. Original images from zone1,(a) 0.1s-001,(b) 0.2s-001,(c) 0.5s-001,(d) 1s-001 and (e) 2s

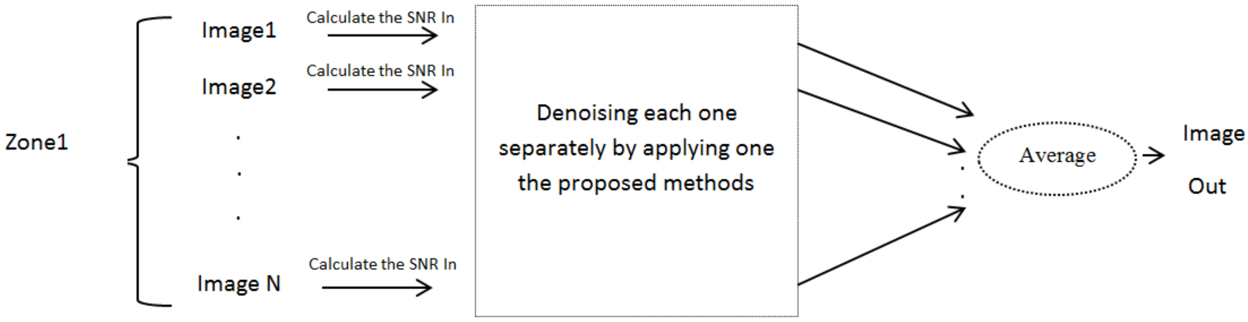

FIGURE 4. (Multiple noisy copies structure)

-For multiple noisy copies: in the multiple noisy copies structure shown in Fig.4, one of the considered denoising methods is first applied to each noisy TEM image independently. So, we get a partial denoised TEM image. The final recovered image is then obtained by computing the average of all denoised copies. This is done for each zone separately. We can see from Tab.2 that the bilateral filtering gave the higher SNRout and each time we increase the number of copies, the SNRout is increased (the higher number of copies, the higher value of SNRout). Visual results are shown in Fig.5.

TABLE 2. The SNRin and SNRout Results of the proposed structure using multiple noisy copies

		SNRout			
Images	Number of noisy copies	BayesianApproach	BilateralFiltering	Soft	Hard
	3	23.7583	25.2676	23.2447	23.2348
2-6	6	26.6453	28.0739	26.1398	26.1297
2-6	9	28.2426	29.6044	27.7485	27.7379
0.1s 2-6	12	29.3453	30.6455	28.8588	28.8485
2-6	15	30.1496	31.4018	29.6777	29.6683
2-6	18	30.8125	32.0129	30.3494	30.3391
2-6	20	31.2103	32.3731	30.7411	30.7325
	3	26.6256	26.9765	26.1161	26.1062
0.2s 2-6	6	29.3366	29.6684	28.8574	28.8481
2-6	9	30.8405	31.1623	30.3947	30.3845
	2	28.5501	27.8415	28.0556	28.0453
0.5s 2-6	3	30.0221	29.3583	29.56	29.5499
2-6	4	30.9816	30.3649	30.5579	30.5491
1s	2	30.8249	29.7785	30.3673	30.3585

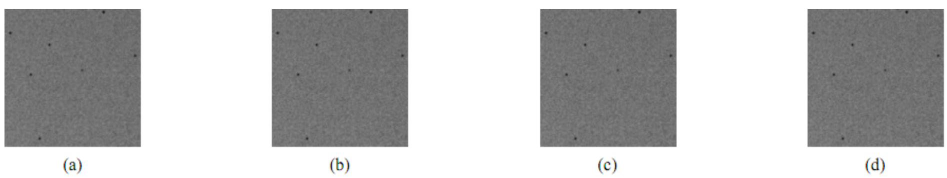

FIGURE 5. The Denoised image 0.1s-001from zone1 after applying :(a) Bilateral Filtering, (b) Bayesian Approach, (c) Soft Thresholding,(d) Hard Thresholding, by using three copies

TABLE 3. The Circularity before and after the Denoising

			Circularity			
Images	Circularity	Number of copies	BayesianApproaches	BilateralFiltering	Soft	Hard
		3	0.752	0.786	0.774	0.782
3-7		6	0.785	0.784	0.783	0.776
3-7		9	0.763	0.772	0.785	0.75
0.1s 3-7	0.702	12	0.784	0.754	0.78	0.729
3-7		15	0.757	0.776	0.784	0.775
3-7		18	0.757	0.771	0.782	0.769
3-7		20	0.784	0.769	0.777	0.77
		3	0.76	0.779	0.783	0.77
0.2s 3-7	0.742	6	0.761	0.783	0.78	0.773
3-7		9	0.764	0.769	0.772	0.747
		2	0.873	0.873	0.782	0.781
0.5s 3-7	0.781	3	0.809	0.869	0.771	0.783
3-7		4	0.785	0.782	0.773	0.722
1s	0.761	2	0.786	0.774	0.695	0.687

CONCLUDING REMARKS

In this paper, we proposed to use the Bayesian approach combined with an averaging operator besides the bilateral filtering and the Thresholding methods to denoiseTEM noisy images. We show that enhancements of image quality and accuracy are achieved by using multiple noisy copies. The goal of using multiple copies is to reduce the radiation damage of the biological samples by reducing the exposure time. We first analysed the first structure (one copy) where we showed that the bilateral filter gave higher SNRout compared to the other methods for the data acquired with exposure time equal to 0.1s and 0.2s, in contrast to the acquired data with 0.5s, 1s and 2s , where the Bayesian gave better SNRout values. Then, we extended our experiments to the multiple noisy copies instead of treating each image separately under each series. From Tab.2, we observed that by applying the Bilateral filter we get a higher SNRout compared to the other methods for the required data at exposure time equal to 0.1s, in contrast to the obtained results obtained for exposure time equal to 0.5s. Tab.2 showed us that the Bayesian Approach gives increasing values for the SNRout, the higher the number of multiple noisy copies, the higher SNRout. There are several interesting points we considered here: first we studied which wavelet family we want to use and the best decomposition level we could apply, then for the bilateral filter we choose the size of the filter we want to employ. These permit us to achieve the best SNRout and a good quality for the TEM images. To sum up, our work we have presented an efficient method in denoising the TEM images based on the Bayesian approach, this method didnâĂŹt give a higher SNRout than the bilateral filter but it results a better visual quality images (no changes in the circularity) than the other proposed methods especially for the multicopy structure. By using multiple noisy copies, we can reduce exposure time considerably. This is an important result for the acquisition of data from biological samples.

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