Noise Analysis and Removal in 3D Electron Microscopy

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Abstract. Recent research in several fields such as Biotechnology and Healthcare has uncovered a vast number of applications where 3D Electron Microscopy (EM) is useful. However, images produced by 3D EM are in most cases severely degraded. These degradations arise due to a multitude of reasons, e.g. the complex electronics in the system, magnetic lens aberration, heating and motion stability, charging, etc. Although the raw, degraded images are currently used for analysis, their usefulness is limited because the degradations make visual distinction and automated analysis of biological features very difficult. In this work, we give an analysis of noise, as one of the most important degradations in 3D EM imaging. Next, we propose a Non-Local Means image restoration algorithm that exploits the derived noise characteristics. The proposed algorithm yields significant improvements compared to other state-of-the-art image restoration algorithms.

1 Introduction

Microscopy has been a crucial subject within fields such as biological and medical research. Where Light Microscopy (LM) is able to magnify up to a factor of 2.000, recent Electron Microscopes (EM) allow us to increase this factor up to 2.000.000. Therefore, it is possible to image biological samples up to nanometer resolution.

However, acquired EM images are severely degraded with various artifacts such as noise (Fig. 1), making it sometimes difficult for field experts to visually distinct biological structures. Current methods used in EM for dealing with these obstacles involve low-pass filtering techniques resulting into low-quality image restorations. Further automated processing such as e.g. segmentation will, as a consequence, become less accurate.

Image restoration requires a detailed description of the image artifacts. For example, taking into account for noise correlation yields significant improvements in several existing techniques [1], [2], [3], [4]. We will therefore perform an analysis of EM noise, which is one of the most notable artifacts in EM images.

The derived noise characteristics will be used in a Non-Local Means (NLMS) framework. NLMS [5] is a state-of-the-art denoising algorithm exploiting non-local repetitive structures in images. In order to do this, it assumes white, Gaussian noise in the corrupted image, an assumption which is not always satisfied.

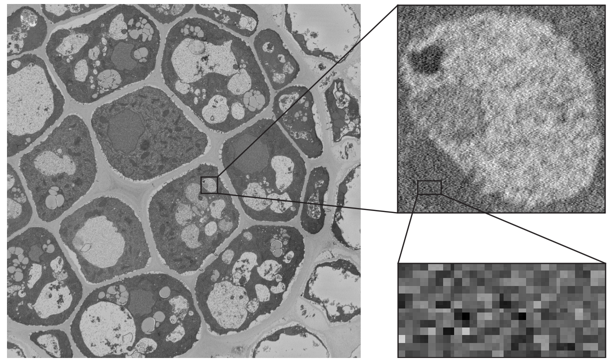


Fig. 1. Noisy EM image

We will therefore extend this algorithm in order to remove correlated, signal-dependent noise.

The structure of this paper is as follows: in Sect. 2, we will discuss the image acquisition process and noise sources in 3D EM. Furthermore, a noise analysis within degraded EM images is derived. Next, the proposed NLMS-based algorithm will be discussed in Sect. 3. We evaluate the proposed algorithm in Sect. 4. Finally, Sect. 5 recapitulates this paper.

2 EM image acquisition and degradation

Image artifacts such as noise are caused by a variety of sources: complex electronics, heating, signal amplification, etc. A study of how these artifacts are introduced in the resulting EM images will allow us to suppress them more effectively.

2.1 Image acquisition & noise sources

In general, two types of EM can be distinguished: Transmission and Scanning EM (respectively TEM and SEM). Both types focus a beam of electrons (using a magnetic lens system) on the specimen's surface. The difference between TEM and SEM lies in the type of detected electrons. Where TEM detects electrons passing through the specimen, SEM detects backscattered electrons. In 3D SEM, a diamond knife makes a next slice after every acquired image in order to capture the next image. This way, a 3D stack of 2D images is obtained. This is called serial block face (SBF) SEM. As the name specifies, SEM scans the image in a grid, cell by cell[1]. Fig. 2 shows the workflow of this type of EM and the most important noise sources it contains.

In order to suppress noise, we have to analyse its characteristics in EM images [6], [7]. A detailed image degradation knowledge allows us to model them more

[1] Not in the biological sense, but cells within the grid.

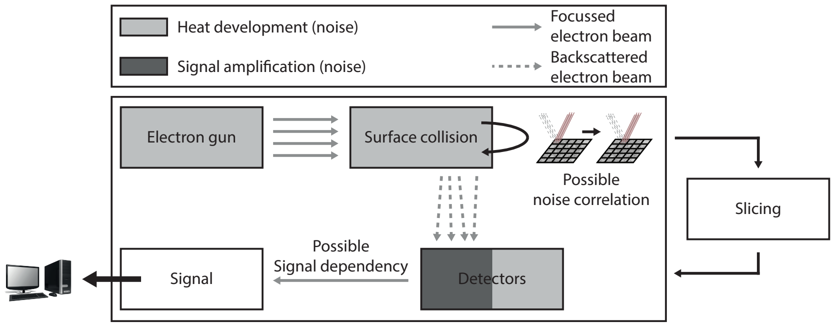


Fig. 2. 3D SEM workflow and its image artifact sources: an electron gun repetitively fires an electron beam at the surface of the specimen according to the scanning principle. Backscattered electrons are detected and transformed into a digital signal. When a slice is completely imaged, the next slice is prepared to start imaging

accurate and therefore remove the noise more effectively, without destroying the image content.

2.2 Noise model

An accurate noise model allows a more accurate estimate of the ideal image and therefore a better restoration [1], [4], [8], [9]. We propose to model the noise by means of four characteristics:

- Distribution: the noise distribution gives information about which noise values are expected to be least and most common. The variance of the distribution tells us how much the noise is varying within the image and is therefore a good measure of how much the noise degrades the image.
- Stationarity: noise may be dependent of the spatial position within the image. This type of noise is called *non-stationary*, whereas spatially independent noise is called *stationary*.
- Spatial correlation: noise intensities may be mutually dependent. In this case, certain patterns may be noticed in the noise signal. Spatially correlated noise is referred to as *colored* noise, whereas spatially uncorrelated noise is called *white*.
- Signal dependency: another possibility is that of a dependency between the noise and the observed image intensity. In special cases, high intensity (bright) regions are inflicted with more noise (i.e. noise with higher variance) than low intensity (dark) regions. This type of noise is called *Poisson* noise.

The above mentioned characteristics will be used to form our proposed degradation model:

$$\boldsymbol{y} = \boldsymbol{x} + \boldsymbol{H}\boldsymbol{\Sigma}(\boldsymbol{x})\boldsymbol{n} \ , \tag{1}$$

where $\boldsymbol{x}$ and $\boldsymbol{y}$ are MN-length vectors representing the ideal and observed $M \times N$ image (in e.g. raster scanning order), respectively, $\boldsymbol{n}$ is a stochastic MN-length vector (assumed having mean $\mathbf{0}$ and covariance matrix $\boldsymbol{I}$, where $\boldsymbol{I}$ is the identity matrix) representing the noise, $\boldsymbol{H}$ is an $MN \times MN$ circulant matrix representing the low-pass filter which correlates the noise and $\boldsymbol{\Sigma}(\boldsymbol{x})$ is an $MN \times MN$ diagonal $\boldsymbol{x}$-dependent matrix incorporating the noise signal dependency. The kth diagonal element of $\boldsymbol{\Sigma}(\boldsymbol{x})$ expresses the standard deviation of the kth noise sample, given a certain measured intensity $(\boldsymbol{x})_k$.

There are several remarks to this model. First, we assume an additive noise model with a signal-dependent adaptation whereas most models assume multiplicative, Poisson noise. This way, all the noise characteristics are clearly split in the noise model given by Eq. (1): $\boldsymbol{n}$, $\boldsymbol{H}$ and $\boldsymbol{\Sigma}(\boldsymbol{x})$ express the noise distribution, correlation and signal dependency, respectively. Second, the signal dependency matrix $\boldsymbol{\Sigma}(\boldsymbol{x})$ is assumed to be diagonal and therefore only assuming the noise variance (not the covariance between noise intensities) to be signal-dependent. In an additive model, the signal-dependent noise variance $\sigma^2(i)$ may be written as [10]:

$$\sigma^2(i) = \alpha i + \sigma_0^2 \ , \tag{2}$$

where i is the measured intensity and α and σ_0 are parameters to be estimated. Note that σ_0^2 corresponds to the noise variance in absence of signal, α expresses the significance of the signal dependency.

2.3 Noise analysis

An ideal denoising algorithm, following Eq. (1), should give $\boldsymbol{x}$ as output for an observed image $\boldsymbol{y}$. However, in practice an approximation $\hat{\boldsymbol{x}}$ is derived. An analysis of the noise characteristics allows us to determine the parameters in Eq. (1) more accurately and therefore allows a more qualitative estimation of $\boldsymbol{x}$.

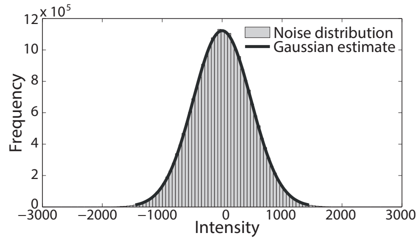
In order to analyse the noise, we need to separate the noise from the signal. We made this possible by switching off the electron beam of the EM ($\boldsymbol{x} = \mathbf{0}$):

$$\boldsymbol{y_0} = \boldsymbol{H\Sigma}(\mathbf{0})\boldsymbol{n} \ . \tag{3}$$

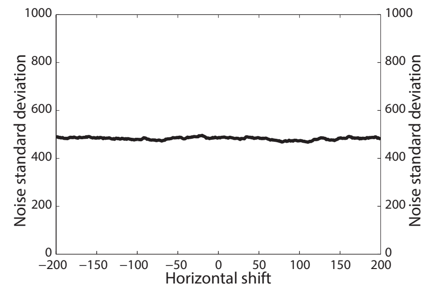
Given this kind of noise signal $\boldsymbol{y_0}$, we can observe the corresponding histogram (Fig. 3(a)). It is clearly a reasonable approximation to assume EM noise to be Gaussian distributed: $\boldsymbol{n} \sim \boldsymbol{\mathcal{N}}(\mathbf{0}, \boldsymbol{I})$.

In order to analyse spatial, statistical noise dependency, we computed the noise standard deviation for a reference block and a number of shifted blocks using the noise images given by Eq. (3). Non-stationary noise would reveal a non-constant structure of these standard deviations for variable shifts. Fig. 3(b) illustrates this experiment for horizontal shifts and a constant vertical position[2]. It can be seen that the noise is stationary, since the different block standard deviations differ at most 20 intensity units. This is negligible, compared to the dynamic range of a typical EM image, which is several thousands of intensity units wide.

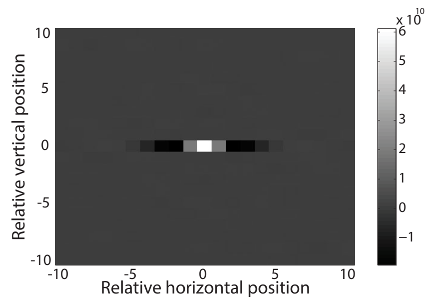
[2] 2D shifts would complicate the visuality of the results, but yields similar results.



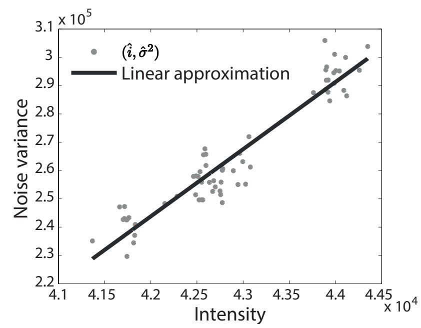
(a) Noise histogram



(b) Stationarity of the noise. The horizontal shift expresses the relative horizontal position of the observed block to the reference block



(c) Autocorrelation function of the noise



(d) Signal dependency of the noise. The values for σ_0^2 and α from Eq. (2) can be estimated by a linear least squares fit

Fig. 3. Noise characteristics

Another noise property is spatial correlation. To analyse this, we computed the autocorrelation function (ACF) of the noise. The ACF of a signal is found by applying a convolution between the signal and a mirrored version of itself. For this reason, the noise ACF is ideal to find mutual dependencies between noise samples. We obtain a horizontal correlation between neighbouring noise samples (Fig. 3(c)). This is the reason why we find a horizontal striping effect in the noisy EM images (Fig. 1). Because the ACF describes relative noise sample correlation, we will normalise it (i.e. divide by the sum of its elements) to obtain normalised a low-pass filter representing the noise correlation.

The analysed images given by Eq. (3), i.e. the images without any signal, do not allow to study signal dependency. To solve this, we extracted several patches $\boldsymbol{y_i}$ with different expected intensity i (but approximately constant within every patch). Next, the expected intensity is subtracted from the patch to obtain a noise signal:

$$\begin{aligned} \boldsymbol{y}_{\boldsymbol{i}}' &= \boldsymbol{y}_{\boldsymbol{i}} - \boldsymbol{i} \\ &= \boldsymbol{i} - \boldsymbol{i} + \boldsymbol{H}\boldsymbol{\Sigma}(\boldsymbol{i})\boldsymbol{n} \\ &= \boldsymbol{H}\boldsymbol{\Sigma}(\boldsymbol{i})\boldsymbol{n} \ , \end{aligned} \tag{4}$$

where $\boldsymbol{i}$ is a constant NM-length vector with $(\boldsymbol{i})_k = i$ (for all k). The expected intensity i is approximated as the mean patch intensity, since the additional noise is assumed to be zero-mean.

In order to estimate signal dependency, several patches with an expected constant intensity $\hat{i}$ are acquired. For each patch, the noise variance estimate $\hat{\sigma_i}^2$ is computed. Plotting the points $(\hat{i}, \hat{\sigma_i}^2)$ would reveal a signal-(in)dependent structure. This plot is shown in Fig. 3(d). Clearly, we can conclude that the noise is signal-dependent, since an increase of 1000 intensity units leads to an increase of 20.000 in noise variance. Given that the noise variance is of order 10^5, this is a significant increase and may not be ignored.

3 Image restoration

Our proposed algorithm, based on the degradation model given by Eq. (1) and parameter settings through the procedures in 2.3, will exploit the derived characteristics of the noise in EM images. Furthermore, it is based on the Non-Local Means (NLMS) algorithm, which was first proposed in [5]. It estimates a noise free pixel by computing weighted averages of all the pixels. The effectiveness of the algorithm lies in the choice of weights. Our framework will allow to use the NLMS algorithm in the presence of signal-dependent, correlated noise.

3.1 Non-Local Means

The NLMS algorithm assumes white, Gaussian noise in the degradation model (a simplified version of Eq. (1) where $\boldsymbol{H} = \boldsymbol{I}$ and $\boldsymbol{\Sigma}(\boldsymbol{x}) = \sigma\boldsymbol{I}$):

$$\boldsymbol{y} = \boldsymbol{x} + \sigma\boldsymbol{n}, \tag{5}$$

where σ is a positive constant representing the noise standard deviation. In order to obtain the restored pixelvalue $(\hat{\boldsymbol{x}})_i$, the NLMS algorithm computes a weighted average of all pixels:

$$(\hat{\boldsymbol{x}})_i = \frac{\sum_{j=0}^{MN-1} w_{ij}(\boldsymbol{y})_j}{\sum_{j=0}^{MN-1} w_{ij}} \ . \tag{6}$$

The essential idea behind NLMS is the proposed definition for the weights w_{ij}. Similar regions corresponding to the pixels at position i and j will receive a relative large weight w_{ij}, non-similar regions will lead to smaller weights. In [8], the so-called modified bisquare weights have proven to be very effective:

$$w_{ij} = \begin{cases} \left(1 - \frac{\|\boldsymbol{y}_{N_i} - \boldsymbol{y}_{N_j}\|^2}{h^2}\right)^8 & \left(\|\boldsymbol{y}_{N_i} - \boldsymbol{y}_{N_j}\| \leq h,\, i \neq j\right) \\ 0 & \left(\|\boldsymbol{y}_{N_i} - \boldsymbol{y}_{N_j}\| > h,\, i \neq j\right) \\ 1 & (i = j)\ . \end{cases} \tag{7}$$

We will use these weights in the following sections.

3.2 Proposed denoising algorithm: NLMS-SC

In order to improve the original NLMS algorithm, our proposed method will apply a pre-whitening step, which will decorrelate the noise term. To do so, we observe the assumed noise model, given by Eq. (1). The correlation whithin the noise is due to the matrix $\boldsymbol{H}$, which filters the signal-dependent noise term $\boldsymbol{\Sigma}(\boldsymbol{x})\boldsymbol{n}$. In the Fourier domain, this will correspond to a point-wise multiplication of their respective Fourier transforms $\tilde{\boldsymbol{H}}$ and $\widetilde{\boldsymbol{\Sigma}(\boldsymbol{x})}\boldsymbol{n}$. In order to decorrelate the noise term, we therefore apply the inverse operation to the observed image in the Fourier domain:

$$(\tilde{\boldsymbol{y}}')_i = \frac{(\tilde{\boldsymbol{y}})_i}{\max\left(\varepsilon, \left(\tilde{\boldsymbol{H}}\right)_i\right)}\ , \tag{8}$$

where ε is a small, positive constant which takes care of the numerical instability of the point-wise division. In a similar way, we apply a second pre-processing step in order to make the noise signal-independent. The signal dependency is due to the matrix $\boldsymbol{\Sigma}(\boldsymbol{x})$. Because this matrix is assumed diagonal, its influence is nothing but a point-wise multiplication of its diagonal elements with the noise term $\boldsymbol{n}$. Therefore, we can transform the image[3] given by Eq. (8) into a signal-independent version by point-wise dividing with the diagonal elements of $\boldsymbol{\Sigma}(\boldsymbol{x})$:

$$(\boldsymbol{y}'')_i = \frac{(\boldsymbol{y}')_i}{\alpha(\boldsymbol{y}')_i + \sigma_0}\ , \tag{9}$$

where α and σ_0 are the parameters corresponding to the signal-dependency estimation. Eqs. (8) and (9) will make sure the spectrum of the noise becomes flat, i.e. the noise becomes decorrelated and signal-independent. Next, the weights w'_{ij}, given by Eq. (7), are determined, based on the transformed version $\boldsymbol{y}''$ instead of $\boldsymbol{y}$. Because $\boldsymbol{y}''$ is now an image, inflicted with ordinary, white noise, these weights will be more effective for noise reduction. Similar as in NLMS, we obtain the denoised image by Eq. (6) using the weights w'_{ij} instead of w_{ij} and the observed image $\boldsymbol{y}$. We will refer to this NLMS variant as the NLMS-SC algorithm.

[3] This image is defined in the Fourier domain. The actual 'signal independency transformation' is obviously applied on its inverse Fourier transform $\boldsymbol{y}'$.

4 Results and discussion

As an example, we have added signal-dependent, correlated noise, i.e. the same type of noise that degrades SBF-SEM images, to the *Barbara* image (Fig. 4(a)) and various images of the Kodak dataset. The result in Fig. 4(e) shows a significant increase in PSNR and visual quality compared to the NLMS algorithm [5] and state-of-the-art BM3D denoising method [11]. Both the NLMS and BM3D results (respectively Fig. 4(c) and 4(d)) tend to reveal artifacts due to noise correlation in flat areas. Because of the signal-independency assumption, bright regions still contain noise, where as darker regions become more blurry. The proposed NLMS-SC result shows none of these artifacts. This can especially be seen in the cropped restoration results (Fig. 4(f), 4(g) and 4(h)).

The proposed algorithm is compared to several, frequently used, denoising techniques. Anisotropic diffusion [12] filters a noisy image locally in horizontal and vertical direction, depending on local edge information. Another type of image restoration algorithm is a MAP estimator. We used a basic MAP estimator assuming a Gaussian distributed image with mean $\bar{\boldsymbol{x}}$ and variance σ_x^2 (MAP-S). Fig. 5 illustrates the effectiveness of the NLMS-SC algorithm in terms of PSNR compared to the previously mentioned denoising algorithms.

We have also applied the NLMS variant to EM images. Fig. 4(j) illustrates the significant improvement, compared to the original noisy version (Fig. 4(i)). The EM images are both visualised within their intensity domain. Because of the noisy pixel intensities, the degraded image therefore has less contrast. The NLMS-SC algorithm manages to remove most of the noise in the degraded EM image without losing valuable image information. Smaller structures whitin the cell become clearer in the denoised image. Field experts have confirmed that the restored EM images are more suitable for analysis than the acquired images.

5 Conclusion

Analysis of 3D EM images is a difficult task for researchers, because of the significant amount of noise in the acquired images. Therefore, we propose an effective image restoration algorithm. This algorithm should use all the characteristics of EM noise in order to model accurately. For this reason, we performed an analysis of the noise properties, that were exploited in an NLMS framework (NLMS-SC). The NLMS-SC algorithm shows a significant improvement compared to other state-of-the-art denoising algorithms, in terms of PSNR as well as in visual quality.

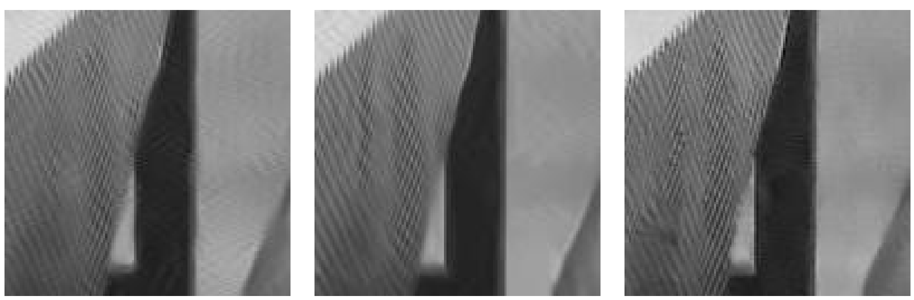
For future work, we noticed EM images are not solely corrupted by noise. Another relevant artifact is blur, caused by magnetic lens abberation. Further deconvolution research will possibly yield even better restoration results.

References

1. Matrecano, M., Poggi, G., Verdoliva, L.: Improved BM3D for Correlated Noise Removal. VISAPP (1) (2012)

(a) Original image (b) Noisy image $(21,03\ \mathrm{dB})$

(c) NLMS restoration $(28,86\ \mathrm{dB})$ (d) BM3D restoration $(29,57\ \mathrm{dB})$ (e) Proposed NLMS-SC restoration $(29,79\ \mathrm{dB})$



(f) NLMS restoration (crop) (g) BM3D restoration (crop) (h) Proposed NLMS-SC restoration (crop)

(i) Original EM image (j) Proposed NLMS-SC restoration

Fig. 4. Noisy image restoration using NLMS [5], BM3D [11] and NLMS-SC

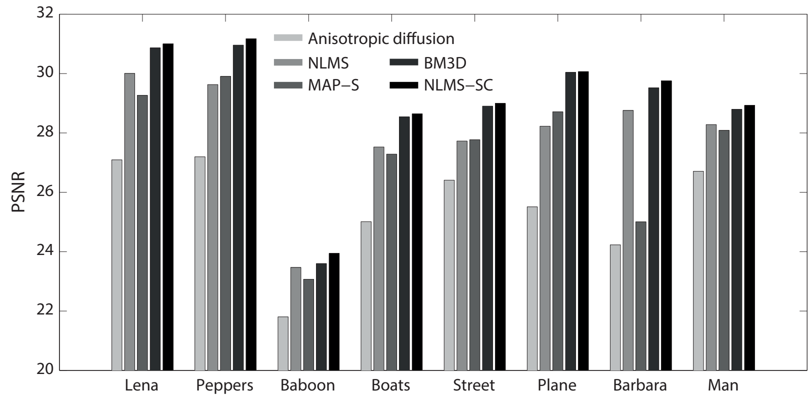


Fig. 5. NLMS-SC compared to the other denoising techniques.

2. Goossens, B., Pizurica, A., Philips, W.: Removal of Correlated Noise by Modeling the Signal of Interest in the Wavelet Domain. IEEE Transactions on Image Processing (2009)
3. Bosco, A., Bruna, R.A., Giacalone, D., Battiato, S., Rizzo, R.: Signal-Dependent Raw Image Denoising using Sensor Noise Characterization via Multiple Acquisitions. Digital Photography VI (2010)
4. Oktem, R., Egiazarian, K., Lukin, V.V., Ponomarenko, N.N., Tsymbal, O.V.: Locally Adaptive DCT Filtering for Signal-Dependent Noise Removal. IEEE Transactions on Image Processing (2011)
5. Buades, A., Coll, B., Morel, J.M.: A Non-local Algorithm for Image Denoising. IEEE Computer Society Conference on Computer Vision and Pattern Recognition (2005)
6. Pyatykh, S., Hesser, J., Zheng, L.: Image Noise Level Estimation by Principal Component Analysis. IEEE Transactions on Image Processing (2013)
7. Liu, C., Szeliski, R., Kang, S.B., Zitnick, C.L., Freeman, W.T.: Automatic Estimation and Removal of Noise from a Single Image. IEEE Transactions on Pattern Analysis and Machine Intelligence (2006)
8. Goossens, B., Luong, H., Pizurica, A., Philips, W.: An Improved Non-local Means Algorithm for Image Denoising. International Workshop on Local and Non-Local Approximation in Image Processing (2008)
9. Fehrenbach, J., Weiss, P., Lorenzo, C.: Variational Algorithms to Remove Stationary Noise: Application to SPIM Imaging. IEEE Transactions on Image Processing (2011)
10. Foi, A., Trimeche, M., Katkovnik, V., Egiazarian, K.: Practical Poissonian-Gaussian Noise Modeling and Fitting for Single-Image Raw-Data. IEEE Transactions on Image Processing (2007)
11. Dabov, K., Foi, A., Katkovnik, V., Egiazarian, K.: BM3D Image Denoising with Shape-Adaptive Principal Component Analysis. Proc. Workshop on Signal Processing with Adaptive Sparse Structured Representations (2009)
12. Perona, P., Malik, J.: Scale-Space and Edge Detection Using Anisotropic Diffusion. IEEE Transactions on Pattern Analysis and Machine Intelligence (1990)