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AN INVESTIGATION ON VARIOUS STANDARD STRATEGIES FOR FLUORESCENCE IMAGE DENOISING OF CARDIO FIBROBLAST CELLS

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1 Introduction

Fluorescence microscopy imaging became a common tool in biomedical research since it allows the study of the dynamics of living cells in an almost non-invasive manner. The phenomenon of fluorescence consists on the emission of light with a longer wavelength than the one of the incident radiation, by excited molecules within nanoseconds after the absorption of photons. The fluorophore is the component of the molecule responsible for its capability to fluoresce (Isabel Rodrigues et al 2010).

The photoblinking/photobleaching (PBPB) effects lead to an intensity fading of a fluorescent probe along the experiment time (Schuster et al 2007). This effect is caused by quantum phenomena associated with the electronic excitation and photochemical reactions among the fluorescent and the surrounding molecules induced by the incident radiation that temporarily or irreversibly destroy their ability to fluoresce. Since illumination is needed to excite and observe the tagging fluorescent proteins in the specimen and all the fluorophores will eventually photobleach upon extended excitation, the acquisition of this type of images becomes a hard task for long exposures. The reduction of the intensity of the incident radiation can attenuate this effect but leads to a decreasing in the signal to noise ratio (SNR) of the acquired images. Successful denoising algorithms are consequently indispensable before visualization and analysis of these images. This research would present various noise removal approaches used for fluorescence images to remove the noises in efficient way.

2 Literature Review

Lefkimmiatis et al (2009) often say that the removal of Poisson noise is performed through the following three-step procedure. First, the noise variance is stabilized by applying the transformation to the data. This produces a signal in which the noise can be treated as additive Gaussian with unitary variance. Second, the noise is removed using a conventional denoising algorithm for additive white Gaussian noise. Third, an inverse transformation is applied to the denoised signal, obtaining the estimate of the signal of interest. Recently, efficient denoising methods were also developed based on sparsity and redundant representations over learned dictionaries.

There is a variety of Poisson intensity estimation techniques proposed in the literature by Zhang et al (2008) with the Bayesian methods allowing incorporation of prior knowledge about the underlying intensity to be estimated. In particular, multiscale Bayesian methods are becoming increasingly popular since they can provide significant simplifications to the problem

3 Problem Specification

One of the fundamental challenges in the field of image processing and computer vision is image denoising, where the underlying goal is to estimate the original image by suppressing noise from a noise-contaminated version of the image. Image noise may be caused by different intrinsic (i.e., sensor) and extrinsic (i.e., environment) conditions which are often not possible to avoid in practical situations. Therefore, image denoising plays an important role in a wide range of applications such as image restoration, visual tracking, image registration, image segmentation, and image classification, where obtaining the original image content is crucial for strong performance. While many algorithms have been proposed for the purpose of image denoising, the problem of image noise suppression remains an open challenge, especially in situations where the images are acquired under poor conditions where the noise level is very high. (Florian Luisier, Thierry Blu, and Michael Unser,2011)

4 Motivation

The search for efficient image denoising methods is still a valid challenge at the crossing of functional analysis and statistics. In spite of the sophistication of the recently proposed methods, most algorithms have not yet attained a desirable level of applicability Image processing is a widely growing field as many of the nowadays applications are making use of it. Therefore, there is also a need of image denoising techniques due to introduction of noisy elements during image acquisition. White noise is one of the most common problems in image processing. (Luisier, F, Vonesch, C, Blu, T and Unser, M, 2010) Even a high-resolution photo is bound to have some noise in it. For a high-resolution photo a simple box blur may be sufficient, because even a tiny features like eyelashes or cloth texture will be represented by a large group of pixels. Unfortunately, this is not the case with video where real-time noise reduction is still a subject of many researches. So, the main motivation is to come up with qualitatively and computationally efficient algorithm for denoising time-lapse (2D+time) fluorescence microscopy images.

5 Research Contribution

In the first part of work, present an extensive experimental analysis using the OWT-SURELET denoising algorithm for Fluorescence imaging. In this the Fluorescence image is gaussianized first then the resulting image is given as an input to the OWT SURELET to remove the Gaussian white noise. In the second part of research, the consequential image is denoised using conservative denoising algorithms for additive white Gaussian noise such as BLS-GSM and OWT-SURELET and finally the inverse transformation is done on the denoised image.

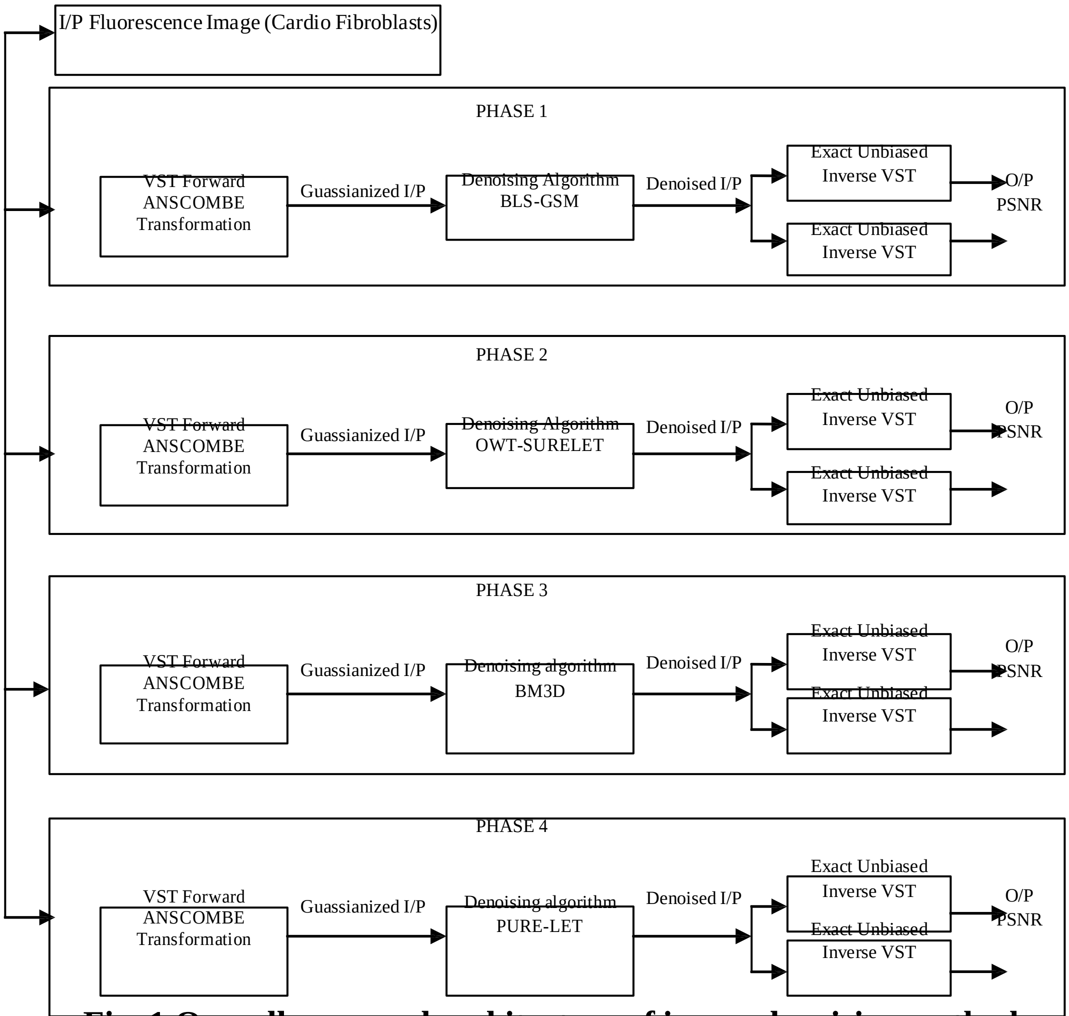


Fig. 1 Overall proposed architecture of image denoising methods

In the third part of work, considering the importance of cardio fibroblasts, reflect on various denoising strategies for diminution of noise in the images which could lead to some life saving observations. The entire work representation is illustrated in Figure 1.

5.1 Poisson Noise Removal from Fluorescence Images using Optimized Variance-Stabilizing Transformations and Standard Gaussian Denoising

The GSM is capable of approximating the non-Gaussian marginal response with a complete mathematical framework. It has seen many successful applications in image processing. The BLS-GSM modeling based image denoising is introduced in the image Curvelet domain to get the uncontaminated image signals with Bay inference. Given an image I, the observed value of the Curvelet coefficients with multiscales and multi directionalities after the Curvelet transformation is denoted as y, which is contaminated by additive Gaussian white noise. It is known that any block of coefficient x with the center of x_c can be subjected to the GSM model. In this research, simply consider the additive Gaussian noise, the observation coefficients y can be expressed as the following equation:

$$-$$

where represent the identical distribution, is the noise coefficients which obey normal distribution , is the noise covariance matrix, is the noise-free image signals, is a process viable with normal distribution, and $-$ is positive scale variable independent with . Given , it is explicit to know the covariance of the observe coefficients .

5.2 Improved Image Denoising using Optimized Variance Stabilizing Transformations

While the asymptotically unbiased inverse provides good results for high-count data, applying it to low-count data leads to a biased estimate.

Exact unbiased inverse

Provided a successful denoising, i.e. D is treated as the exact unbiased inverse of the Anscombe transformation I_c is an inverse transformation that maps the values E to the desired values :

Let us remark that if the exact unbiased forward inverse is applied to the denoised data D with some errors in the sense that then the estimation error can include variance as well as bias components. In general, the unbiasedness of Ic holds only provided that exactly, as it is assumed.

5.3 Fluorescence Image Denoising using Diverse Strategies and their Performance Evaluation

Low illumination environment in Fluorescence microscopy, create arbitrary variations in the photon emission and detection process that manifest as Poisson noise in the captured images. Therefore study the effect of Standard denoising algorithms wherein the noise is either transformed to Gaussian or the denoising is done on the Poisson noise itself. (Anscombe, FJ, Biometrika,1948) In the first strategy the noise is Gaussianized by applying the Anscombe root transformation to the data, to produce a signal in which the noise can be treated as additive Gaussian and then the consequential image is denoised using conservative denoising algorithms for additive white Gaussian noise such as BLS_GSM and OWT_SURELET and finally the inverse transformation is done on the denoised image (Thierry Blu and Florian Luisier, 2007). The choice of the proper inverse transformation is vital for fluorescence images in order to reduce the bias error which arises when the nonlinear forward transformation is applied. The Latter strategy considers PURELET technique where the denoising process is a linear expansion of thresholds (LET) that optimize results by depending on a purely data-adaptive unbiased estimate of the mean-squared error (MSE), derived in a non-Bayesian framework (PURE: Poisson–Gaussian unbiased risk estimate) (Qunli Li, 2012). Experimental results are compared with existing work on how the ISNR changes with the change in algorithms for fluorescence images.

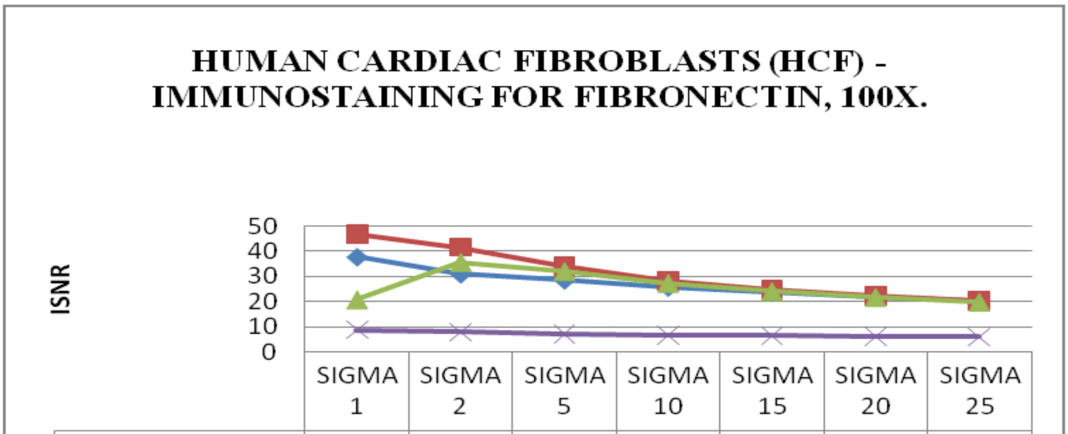
5.4 Validation of Various Standard Strategies for Fluorescence Image Denoising of Cariofibroblast Cells

Fluorescence microscopy imaging is a common biomedical tool for researchers make use in the study of active processes occurring inside live cells. Although fluorescent confocal microscopes are consistent instruments, the acquired images are normally corrupted by a severe type of Poisson noise owing to the small amount of acquired radiation (low photon-count images) and also the huge opto-electronics amplification. These effects are still more destructive when very low intensity incident radiation is employed to avoid photo toxicity. To validate various standards of denoising algorithms to denoising the cardio fibroblast cells, in which all the fluorescence images are affected by Poisson Gaussian Noise.(Portilla, J, Strela, V, Wainwright, MJ and Simoncelli, EP,2003) These images are considered especially as a cardio fibroblasts contribute to structural, biochemical, mechanical and electrical properties of the myocardium. The denoising approaches employed here can directly act on Poisson noise like PURELET or use approaches wherein gaussianize the noise by means of standard VST algorithms and then Gaussian denoising algorithms like BLS_GSM, BM3D and OWT SURELET are proposed.

Results from the various denoising algorithms show that the BLS_GSM denoising and PURE-LET denoising provides stable performance when compared to BM3D and OWT-SURELET for almost all fibroblast images. OWT-SURELET approaches provide higher ISNR when the low sigma value. As the sigma value increases there is a sharp decrease in the signal to noise ratio. BM3D has shown variations which kept back fluctuating based on intensity of input images. They did not provide significant improvement in the SNR and showed poor performance as sigma increased. All algorithms show deterioration in SNR as sigma increased. The BLS_GSM and OWT-SURELET showed improvement when using the exact unbiased transform when compared to asymptotic inverse transform. The performance improvement gets slow when there is increase in sigma values. The total comparison of results shows that the PURELET BLS_GSM or OWT SURELET strategies can be used for low sigma values. As standard deviation increases it is better to stick on to BLS_GSM or PURELET strategy.

6 Results and Discussion

The experiment is conducted in MATLAB and evaluates the performance in terms of PSNR. The approaches used here are the OWT-SURELET, BLS_GSM, BM3D and PURELET for the denoising and the inversion is done with either the exact unbiased inverse or the asymptotically unbiased inverse. The graphical representation Human cardiac fibroblast (HCF) immunostaining for different fibernectin is shown in the figure 2. The BLS-GSM ISNR,BM3D, PURELET ISNR and OWT ISNR are compared with each other.



	SIGMA 1	SIGMA 2	SIGMA 5	SIGMA 10	SIGMA 15	SIGMA 20	SIGMA 25
BLS-GSM ISNR	37.8428	31.0561	28.5991	25.7441	23.5622	21.7441	20.2357
OWT ISNR	46.7551	41.4257	33.8798	27.9955	24.5189	22.0427	20.118
PURELET ISNR	20.8908	35.776	32.1599	27.4417	24.2312	21.8843	19.9718
BM3D	8.7798	7.9604	7.1867	6.665	6.3493	6.1268	5.9379

Fig. 2 ISNR comparison graph

The graphs show that the performance of removing the denoising using OWT-ISNR in Human Cardiac fibroblasts is better than the other algorithms. The graph shows that OWT-ISNR and BLS-GSM ISNR are varied gradually for different sigma values where as rapid variation is seen in PURE LET ISNR.

Table 1 Test Results Using PURE-LET

Sigma Σ	ISNR (using PURE-LET)					
	Sample 1	**Sample 2**	**Sample 3**	**Sample 4**	**Sample 5**	**Sample 6**
1	22.6255	31.1791	20.8908	8.3525	11.0595	43.7163
2	22.4838	30.1113	35.7760	7.0933	16.5616	40.4506
5	21.9430	26.9641	32.1599	3.2432	26.6556	33.8005
10	20.8010	23.1698	27.4417	-3.3539	25.8080	28.0298
15	19.5560	20.4871	24.2312	-10.1915	22.9631	24.5648
20	18.3351	18.4292	21.8843	-25.3550	-4.4466	22.0833
25	17.1897	16.7638	19.9718	-23.9359	9.3490	20.1567

From Table 1, one may observe that variations keep fluctuating based on intensity of input images. This Denoising provides stable performance for all variance

7 Conclusion

Finally results from the various denoising algorithms showed that the BLS_GSM denoising and PURE-LET denoising exhibited stable performance when compared to BM3D and OWT-SURELET for almost all fibroblast images. OWT-SURELET approaches provided higher ISNR when the low sigma value. As the sigma value increased there was a sharp decrease in the signal to noise ratio. BM3D has showed variations which kept back fluctuating based on intensity of input images. They did not provide significant improvement in the SNR and showed poor performance as sigma increased. All algorithms showed deterioration in SNR as sigma increased. However The BLS_GSM and OWT-SURELET showed improvement while using the exact unbiased transform when compared to asymptotic inverse transform. The performance improvement gets slow when there is increase in sigma values. The total comparison of results shows that the PURELET BLS_GSM or OWT SURELET strategies can be used for low sigma values. As standard deviation increases it is better to stick on to BLS_GSM or PURELET strategy.

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LIST OF PUBLICATIONS

A part of the material given in the thesis has been published in reputed Journals presented in conferences.

Journals

1. **Sampathkumar, K** and Arun, C 'Poisson Noise Removal from Fluorescence Images Using Optimized Variance-Stabilizing Transformations and Standard Gaussian Denoising Strategies', European Journal of Scientific Research, , Vol. 84, No.3, pp.336-344, June 2012,ISSN 1450-216X.

2. **Sampathkumar, K** and Arun, C 'An Improved Image Denoising approach using Optimized Variance-Stabilizing Transformations', International Review on Computers & Software, Vol.8, No.8, pp. 1991-1996, August 2013.

3. **Sampathkumar, K** and Arun, C 'Validation of Various Standard Strategies for Fluorescence Image Denoising of Cardiac Fibroblast Cells', Research Journal of Information Technology, Vol.6, pp. 110-123, May 2014.

4. **Sampathkumar, K** and Arun, C 'Fluoresence Image Denoising Using Diverse Strategies and their Performance Evaluation', Research JournalofInformationTechnology', Vol.7, No.23, pp.5072-5081, June 2014.

5. **Sampathkumar, K** and Arun, C 'A Novel Wavelet –Based Fluorescence image Denoising Approach with Optimized variance-Stabilizing Transformations', International Journal of Applied Engineering Research, Vol.10, No 3, pp.6975-6986, March 2015.

Conferences

1. **Sampathkumar, K** and Karthikeyan, D 'A Novel Approach for Image Denoising using Fractal Compression Technique', ISBN 978-1-4675-1449-1, April 2012.

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3. **Sampathkumar, K** and Jagadeswari, D 'Robust Secure Scan Design against Scan-Based Side Channel Attack on Cryptography', NCRTECS-2K12, September 2012.

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