Enhancement of Cell Visibility and Contrast for Fluorescence Microscope Images by Subjective and Objective Analysis of Several Visual Aspects

Abed Alrahman Mkayes, Nicolas Walter, Naufal M. Saad, Ibrahima Faye, Sok Ching Cheong and Kue Peng Lim

Abstract Automated detection and identification of abnormal cells in the human body is a critical application for medical image computing. Enhancement and de-noising of images remain challenging tasks and imperative steps for image analysis algorithms. Indeed, due to its early role in the process, the results of advanced operators for feature extraction will highly depend on the quality of enhanced image produced. Depending on the presence of different noise types, particular algorithms will respond better. This paper presents a comprehensive comparison between several linear and non-linear filters applied on fluorescence microscope images for the localization and counting of specific cancer phenotypes from mouth cell samples. The objective analysis proposed is evaluating the PSNR and Delta-SNR (the SNR to SNR measure between original images and filtered ones) for blood sample sequences taken from Cancer Research Malaysia. Thirty Fluorescence microscope images with low contrast and non-uniform illumination have been tested and analysed. Non-linear algorithms seem to show improved contrast and background removal abilities compared to linear blurring and approximating filters.

Keywords Fluorescence microscope images · Image enhancement algorithms · Linear and non-linear filters · PSNR and Delta-SNR · Mouth cancer

A.A. Mkayes (✉) · N. Walter · N.M. Saad · I. Faye
Center for Intelligent Signal and Imaging Research (CISIR), Universiti Teknologi
PETRONAS, 32610 Bandar Seri Iskandar, Perak, Malaysia
e-mail: abdulrahman.mkaies@gmail.com

N. Walter
e-mail: walter.nicolas.pro@gmail.com

S.C. Cheong · K.P. Lim
Cancer Research Malaysia, 47500 Selangor, Malaysia

1   Introduction

In the last decades, image processing has been an active research field and played an important role in applications such as medical aided systems, geographical information, computer vision and a lot of areas in our daily life due to developments in computer and microscopy hardware [1].

In the medical applications, there are several types of medical imaging system, such as Magnetic Resonance Image (MRI), X-Ray and Position Emission Tomography (PET). However, we have to take into account that it is quite hard to get a clear image, because, in reality, an images are often corrupted by noise due to several factors and parameters such as illumination and Data acquisition [2, 3].

Nowadays, fluorescence microscope images are a primary source of information and have became an essential tool in biology and biomedical sciences. The main problems faced with fluorescence microscopy images are blurring and non-uniformity of contrast [4]. Hence, enhancement and de-noising are the most critical stages in image processing. The primary aim of the pre-processing stage is to reduce noise and highlight the interested information simultaneously. The removal of noise has become one of the main focus and challenging topics for image processing researchers. The main target is to reduce the noise while protecting the useful information and highlight it.

From the literature, there are some several approaches for image enhancement and de-noising that are dealing with different kind of images [5, 6]. These approaches show the difference in nature of the different images to be analyzed. Molecular MRI imaging can provide an imaging solution to the detection of cells, but the technology is expensive and the works found were only from simulation results. Some works have been done on the detection of lymphocytes in optical microscope images [7, 8]. These works are interesting as the cell images present low contrast. The authors addressed the problem by applying a median filter. The overall method seems efficient for optic microscope images but may not be suited for fluorescence images as the contrast is non-uniform in the immunotherapy image sequences and the cells to be detected are not lymphocytes.

In their work for immunotherapy [9], authors used very high resolution images. The cells are clear with very high contrast for some of the samples. Other approaches illustrate the variety of cell images and the difference in nature of these original images [10, 11]. Similarly, the contrast and resolution seem very high and will not need a pre-processing step. To fulfil this target, measurement of visual quality is a critical step for numerous images for judging and evaluating images to identify cells and count them to follow the process of healing.

This paper presents several linear and non-linear filters for image enhancement and de-noising applied on fluorescence microscope images. To objectively realize a comprehensive comparison between these filters and evaluate the best method, the Peak Signal to Noise Ratio (PSNR) and Delta Signal to Noise (SNR) values between original and filtered images have been used.

The remaining of this paper is organized as follows: Sect. 2 presents the methods and techniques that have been applied on microscopy images. The results will be shown and discussed in Sect. 3. Section 4 will conclude the paper by summarizing the findings and propose future works.

2 Enhancement Methods

In this paper, various filters have been implemented with a different set of parameters on the fluorescence microscope images. The filters are divided them into two main groups. The first group is composed of linear Filters and the second group of non-linear filters. We have analyzed the quality of the different filtering methods based on PSNR and Delta-SNR values.

2.1 Linear Filter

2.1.1 Mean Filter

Moving average or "mean" filter is a linear filter. It regularises the image by averaging the pixel values contained in a specify neighbourhood. This filter is one of the most commonly used for reducing white Gaussian noises and given by [12]:

$$Y(i,j) = \frac{1}{m.n} \sum_{i=1}^{m} \sum_{j=1}^{n} p(i,j) \tag{1}$$

where; m and n are the image size and (p) is the pixel that localized at (i, j) position. When the mask of this filter is going bigger, the resulting image will be more blurred and smoothed.

2.1.2 Gaussian Filter

The Gaussian filter is a type of "low pass filter" and is used to regularize images by averaging details to reduce the noise [13]. The Gaussian filter equation is given by:

$$G(x,y) = \frac{1}{2\pi\sigma^2} . e^{-\frac{x^2+y^2}{2\sigma^2}} \tag{2}$$

where x, y are the distance from origin in the horizontal and vertical axis respectively and σ^2 is the standard deviation of Gaussian distribution. This filter is more preferment to filter white Gaussian noises.

2.2 Non-linear Filter

2.2.1 Median Filter

The Median filter is a non-linear filter used to reduce speckle noise in an image. The median value is computed from pixels in a specified neighbourhood. The pixels are sorted in function of their respective values to obtain an increasing order vector. The value of the central pixel is then replaced by selecting the middle value of the ordered vector [12]. It is formulated as follows:

$$G(x, y) = Median\{f(x-k, y-l), (k,l)\epsilon W\} \tag{3}$$

where x and y are position indices of the sample point considered, k and l characterizing the size of neighbour window W taken into account. $f(x-k, y-l)$ is the vector of increasing ordered pixel values contained in the window W, $G(x, y)$ is the point value of output image at position (x, y). This filter is very efficient in removing speckle and salt and pepper noises. As well, it is efficient for removing white Gaussian noise if the noise level is not too high.

2.2.2 Wavelet Transformation

Wavelet transform (WT) is a powerful tool and widely used in image de-noising and enhancement due to its energy compaction and multi-resolution characteristics. It overcomes some of the limitations of Fourier transform with its ability to represent a function simultaneously in time and frequency domain. Discrete Wavelet Transform (DWT) critically sampled form of WT provides most compact representation. We have applied the DWT based on Haar wavelet for noise removal [14]. Indeed, Haar wavelet transform is famous due to its simplicity and speed of computation. However, Haar wavelet transform decomposes complex signals into sum of basic functions. It consists of high pass and low pass filter. The decomposition of images consists of two parts; detailed coefficient part (high frequency component) and approximation part (low frequency component).

2.2.3 CLAHE

Contrast-Limited Adaptive Histogram Equalization [15], often abbreviated CLAHE, is a computer image processing technique used to enhance and improve the contrast of the image. CLAHE functions on small regions in the image, called tiles, rather than the entire image. Each tile's contrast is enhanced, so it differs from traditional histogram equalization that is working on the entire image. This technique proposes advanced noise removal capabilities compared to the other filters presented.

2.3 PSNR and Delta-SNR

The PSNR and the Delta-SNR are commonly used as to measure of the quality of an image after the reconstruction. The Peak Signal to Noise ratio (PSNR) is the maximum possible power of a signal and the power of corrupting noise that effects the fidelity of its representation. The PSNR equation is given as follows:

$$PSNR = 10.\,log_{10}\left(\frac{Max_I^2}{MSE}\right) \tag{4}$$

where, mean squared error (MSE) is given as follows:

$$MSE = \frac{1}{m\,n}\sum_{i=0}^{m-1}\sum_{j=0}^{n-1}\left[I(i,j)-K(i,j)\right]^2 \tag{5}$$

Here, Max_I is the maximum possible pixel value of the image, I and K are the original and filtered images respectively. On the other hand, The Signal to noise ratio (SNR) is used as a measure of sensitivity. It is formulated as follows:

$$SNR = 10.log_{10}\left(\frac{P_{signal}}{P_{noise}}\right) \tag{6}$$

where; P are average power for signal and noise respectively. Both signal and noise must be measured at the same.

Hence, Delta-SNR is the SNR to SNR measure between original and filtered images.

3 Results

Cancer Research Malaysia team provides us with digital immunofluorescence image database for oral cancer research (see Fig. 1).

The final goal of the proposed research is to extract the beneficial information for detection and identification of the different cell types. 30 fluorescence microscope images has been analysed with the different filters outlined in Sect. 2.

First, linear filters have been applied, i.e. Mean and Gaussian, with different sizes: 3×3, 5×5, 7×7, and 9×9. The PSNR and Delta-SNR between original and enhanced images are resumed in Tables 1 and 2. The Gaussian filter seems to achieve better noise filtering compared to Mean filter (see highlighted PSNR and Delta-SNR values in Tables 1 and 2). On one hand, for the Mean filter, the bigger the mask, the more blurred the result images (see Fig. 2). Hence, a mean filter size of 3×3 has been chosen as best results due to the preservation of the edge locations that correspond to the border of the cells.

326 A.A. Mkayes et al.

Fig. 1 Original fluorescence microscopy image
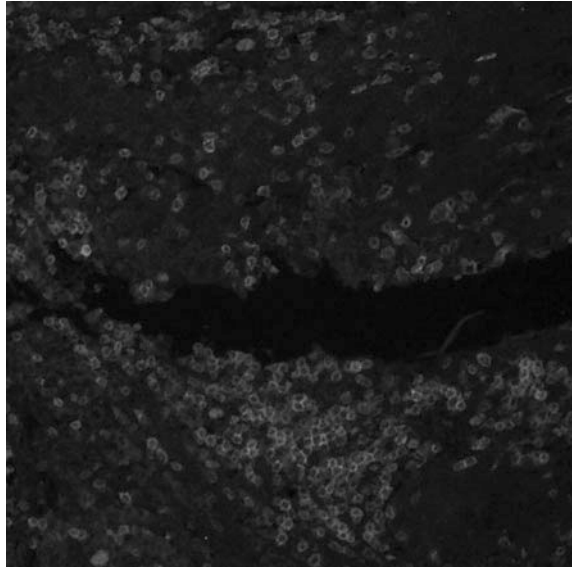
Table 1 Average for 30 images of PSNR and Delta SNR values for mean filter

Mean filter	3 × 3	5 × 5	7 × 7	9 × 9
Delta SNR (dB)	0.7	1.04	1.35	1.64
PSNR (dB)	36.39	35.14	34.14	33.32

Table 2 Average for 30 images of PSNR and Delta SNR values for Gaussian for all mask sizes

Gaussian size	Standard deviation (σ)	0.5	0.6	0.7	0.8	0.9
3 × 3	Delta-SNR	0.38	0.54	0.62	0.66	0.64
	PSNR	43.93	40.72	39.22	38.42	37.95
5 × 5	Delta-SNR	0.38	0.55	0.65	0.69	0.73
	PSNR	43.91	40.66	39.08	38.18	37.6
7 × 7	Delta-SNR	0.38	0.55	0.65	0.71	0.74
	PSNR	43.91	40.66	39.08	38.17	37.59
9 × 9	Delta-SNR	0.41	0.55	0.64	0.71	0.78
	PSNR	44.6	41.33	39.73	38.81	38.2

On the other hand, Gaussian filter (for all mask sizes and parameters) exhibits very small Delta-SNR values. This highlight the weakness of the Gaussian filtering for enhancement of immunofluorescence images (see Fig. 3). As a conclusion for linear filters, the Delta-SNR values obtained are very small, highlighting a very slight increase in image quality, despite very high PSNR values.
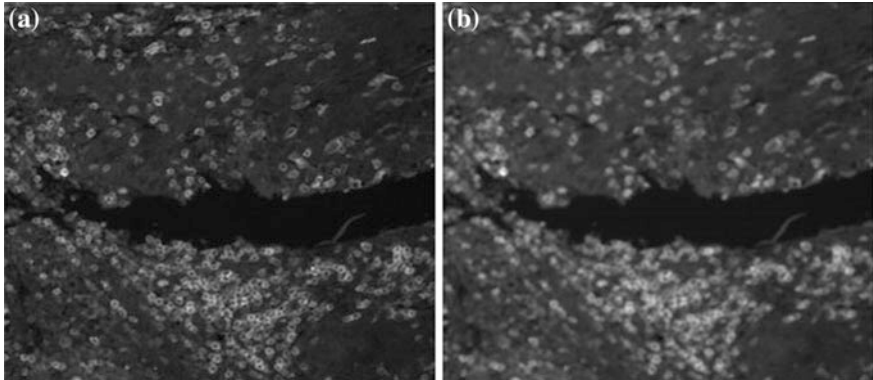
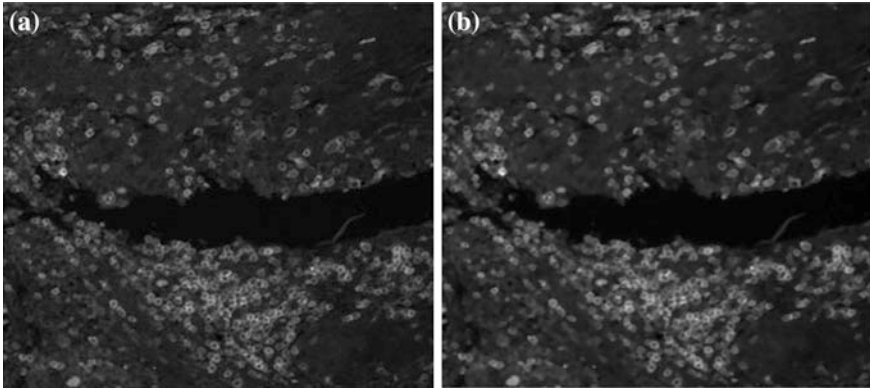**Fig. 2** Filtered images **a** using mean filter [3 × 3] and **b** [9 × 9]

Fig. 3 Filtered image using Gaussian filter [9 × 9], sigma = 0.9
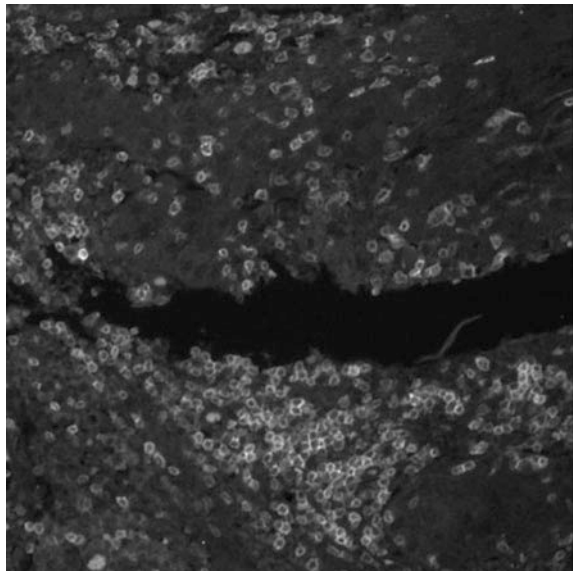
Table 3 Average for 30 images of PSNR and Delta SNR values for wavelet transform when Haar = 1, 2, 3

| Wavelet	*Dec 1 Haar*	Dec 2 Haar	Dec 3 Haar
Delta SNR (dB)	*0.49*	0.9	1.56
PSNR (dB)	*36.39*	35.14	34.14

In contrast, Wavelet de-noising gives us reasonable values of PSNR and Delta-SNR while using a one level Haar decomposition (see Table 3). For two and three levels Haar decompositions, the result images are more blurred despite increased values for the PSNR and Delta SNR (see Fig. 4).

Fig. 4 Filtered image using wavelet transform **a** Haar = 1 and **b** Haar = 2

Fig. 5 Filtered image using
median filter [3 × 3]
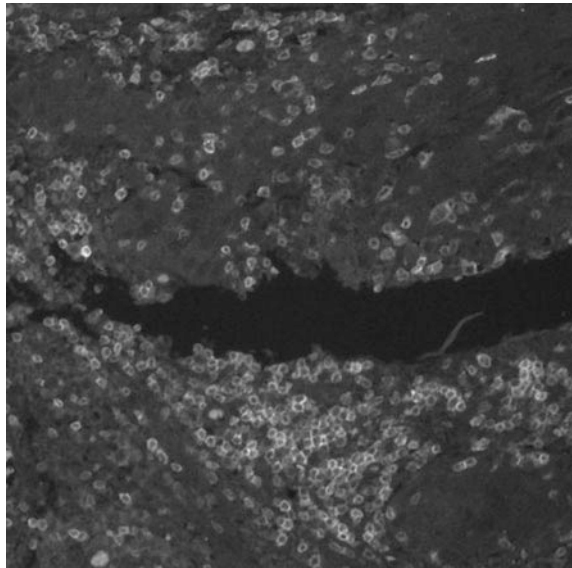
Non-linear filters (i.e. Median and CLAHE) give us better results. As a comparison, the difference between Median and Mean Delta-SNR values are very small, but from the PSNR values, the median filter (see Fig. 5) seems to produce slightly better enhancement (see Table 4). The results obtained with the CLAHE filter (see Fig. 6), Delta SNR and PSNR values indicate a high increase in image quality (see Table 5). Indeed, the Delta-SNR values exhibit an increase superior to 3 dB, highlighting a power increase in resulting enhanced image superior to two times.

Table 4 Average for 30 images of PSNR and Delta SNR values for median filter

Median filter	*3 × 3*	5 × 5	7 × 7	9 × 9
Delta SNR (dB)	*0.69*	1.12	1.53	1.92
PSNR (dB)	*37.56*	36.47	35.49	34.62

Fig. 6 Filtered image using CLAHE for ClipLimt = 0.01 and Alpha = 0.5
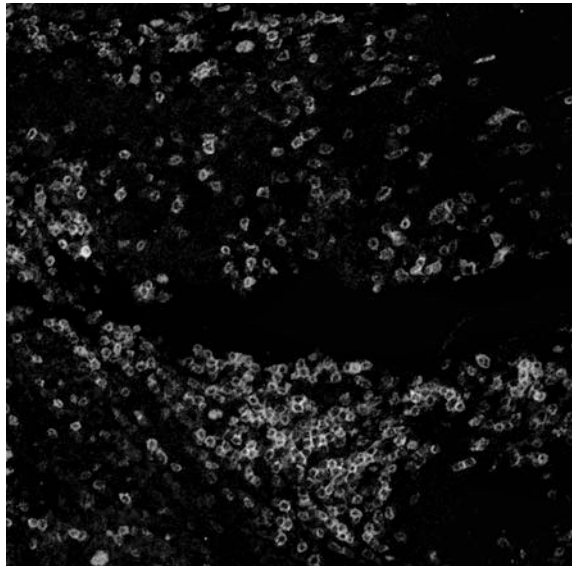
Table 5 Average for 30 images of PSNR and Delta SNR values for CLAHE with different parameters

Alpha	ClipLimit 0.01		ClipLimit 0.02	
	Delta SNR (dB)	PSNR (dB)	Delta SNR (dB)	PSNR (dB)
α = 0.1	17.11	21.05	14.92	21.46
α = 0.2	11.09	22.24	9.19	22.47
α = 0.3	7.589	23.57	5.62	23.62
α = 0.4	5.28	24.96	3.32	24.95
α = 0.5	*3.8*	*26.19*	1.86	26.07
α = 0.6	*2.86*	*27.15*	0.93	26.88
α = 0.7	2.25	27.87	0.34	27.43

CLAHE filter have been implemented with all alpha values ranging from 0.1 to 1 with steps of 0.1, where α specify the Rayleigh distribution parameter. From Table 5, the higher the α value, the higher the PSNR values. "ClipLimit" is another parameter specifying the contrast enhancement limit between tiles. A lower cliplimit value seems to produce better enhancement.

4　Conclusion

In this paper, several linear and non-linear filters are applied for enhancement and de-noising of immunofluorescence images. The extended comparative study based on visual results, Delta-SNR and PSNR values shows the superiority of non-linear filters in terms of de-noising and enhancement. However, the results obtained from CLAHE filtering seems to be suitable for immunofluorescence images as it provides a better background removal, edge preservation and contrast enhancement as compared to the methods discussed above. Thus, this research work concludes that, CLAHE is best pre-processing image enhancing technique to be used for other post processing steps, i.e., automatic segmentation, identification and counting of the different cell types.

References

1. Wienert S et al (2012) Detection and segmentation of cell nuclei in virtual microscopy images: a minimum-model approach. Sci Rep 2
2. Nejad ARM, Hossein-Zadeh, G-A, Zadeh HS (2007) Evaluating effects of imaging parameters on single cell detection in molecular MRI via simulation. In: ICSPC 2007. IEEE international conference on signal processing and communications 2007. IEEE
3. Wang Y et al (2008) Medical image processing by denoising and contour extraction. In: 2008 international conference on information and automation ICIA 2008. IEEE
4. Wilson SM, Bacic A (2012) Preparation of plant cells for transmission electron microscopy to optimize immunogold labeling of carbohydrate and protein epitopes. Nat Protoc 7 (9):1716–1727
5. Shitong W, Min W (2006) A new detection algorithm (NDA) based on fuzzy cellular neural networks for white blood cell detection. Inf Technol Biomed IEEE Trans 10(1):5–10
6. Ke C. (2008) White blood cell detection using a novel fuzzy morphological shared-weight neural network. In: International symposium on computer science and computational technology, ISCSCT'08. IEEE
7. Cheng ED, Challa S, Chakravorty R (2009) Microscopic cell segmentation and dead cell detection based on cfse and pi images by using distance and watershed transforms. In: Digital image computing: techniques and applications, DICTA'09. IEEE
8. Massoudi A, Semenovich, D, Sowmya A (2012) Cell tracking and mitosis detection using splitting flow networks in phase-contrast imaging. In: 2012 Annual international conference of the IEEE Engineering in Medicine and Biology Society (EMBC). IEEE
9. Chiang, A-S et al. (2010) Automatic detection of antinuclear autoantibodies cells in indirect immunofluorescence images. In: 2010 3rd international conference on biomedical engineering and informatics (BMEI). IEEE
10. Malakooti MV, Tafti AP, Naji HR (2012) An efficient algorithm for human cell detection in electron microscope images based on cluster analysis and vector quantization techniques. In:

2012 second international conference on digital information and communication technology and it's applications (DICTAP). IEEE

11. Li Y et al (2011) An improved detection algorithm based on morphology methods for blood cancer cells detection. J Comput Inf Syst 7(13):4724–4731

12. Griffin LD (2000) Mean, median and mode filtering of images. In: Proceedings of the royal society of london A: mathematical, physical and engineering sciences. The Royal Society

13. Nixon M (2008) Feature extraction & image processing. Academic Press

14. Ruikar S, Doye D (2010) Image denoising using wavelet transform. In: 2010 2nd international conference on mechanical and electrical technology (ICMET). IEEE

15. Bhat M, Patil T (2014) Adaptive clip limit for contrast limited adaptive histogram equalization (CLAHE) of medical images using least mean square algorithm. In: 2014 International conference on advanced communication control and computing technologies (ICACCCT). IEEE