A STATISTICAL APPROACH FOR INTENSITY LOSS COMPENSATION OF CONFOCAL MICROSCOPY IMAGES

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ABSTRACT

In this paper a probabilistic technique for compensation of intensity loss in the confocal microscopy images is presented. Confocal microscopy images are modeled as a mixture of two Gaussians, one representing the background and another corresponding to the foreground. Images are segmented into foreground and background by applying Expectation Maximization (EM) algorithm to the mixture. Final intensity compensation is carried out by scaling and shifting the original intensities with help of parameters estimated for the foreground. Since foreground is separated to calculate the compensation parameters, the method is effective even when image structure changes from frame to frame. As Intensity Decay Function (IDF) is not used, complexity associated with estimation of IDF parameters is eliminated. Also, images can be compensated out of order as only information from the reference image is required for compensation of any image. These properties make our method an ideal tool for intensity compensation of confocal microscopy images which can suffer intensity loss due to absorption/scatteing of light as well as photobleaching and can change structure from optical/temporal section to section due to change in the depth of specimen or due to a living specimen. The proposed method was tested with number of image stacks and results for one of the stacks are presented here to demonstrate the effectiveness of the method.

***Index Terms*—** Image compensation, Biomedical image processing, Biomedical microscopy

1. INTRODUCTION

Images produced by confocal microscope tend to decrease in intensity with time as an effect of photobleaching when conventional fluorescence tags are used and with depth due to absorption or scattering of excitation and fluorescence. These effects make analysis of the images without intensity correction a complicated problem. Methods used to compensate this intensity loss can be categorized into two types,

- *Pre-processing methods*: Ones that correct the intensity loss with modified optics as the images are being captured.
- *Post-processing methods*: These methods compensate the images after they are captured. IDF methods model intensity loss in the images as parametric decay function of depth and time. The decay parameters are estimated and compensated for in these methods. Another family of methods rely on matching histogram profiles of image stacks. These methods however cannot handle change in image structure along optical axis.

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Optics based methods assume that the majority of the intensity loss is due to absorption and scattering of light as it travels through the specimen. As rate of photobleaching can be different for different types of specimens, intensity loss caused by it cannot be compensated by the optics alone. For this reason, we concentrate on post processing methods to correct the intensity loss. As factors contributing to the intensity loss cannot be modeled accurately for practical images [1], it poses a problem when IDF is used for intensity compensation. Also combination of intensity loss due to photobleaching and depth can give rise to a complicated IDF function. Our method is motivated by histogram matching, however it deals with continuous domain by modeling image as mixture of two Gaussians and matching profiles of foreground Gaussian. By matching foreground only, our method avoids the problems arising due to change in structure of the image.

Different approaches to correct the intensity variations in images can be found in the present literature. In [2], the authors apply a general model in which the horizontal and vertical flow fields as well as additive and multiplicative intensity relationships are estimated for every pixel. According to [3], this approach is computationally expensive. A least square optimization based approach, in which brightness and contrast are the parameters to be optimized, is proposed in [4, 5]. These techniques are highly sensitive to outliers. In [5] reweighed least square method is used to correct the disadvantage of [4]. The method discussed in [5] is not only sensitive to noise which can be eliminated by median filtering, but also to the dynamic movement of objects in neighboring optical sections. This gives erroneous and unstable results even in the presence of a very few outliers in optical sections [3]. In [6] intensity variations are corrected based on histogram warping, but it is restricted to the case where a global, spatially invariant, non linear, monotonically increasing relationship exists between the intensities of the two images. Ĉapek *et al.* [3] combines the approaches of [5] and [6] and attempts to give a general and fully automatic method of correcting intensity loss in confocal microscopy images. The proposed method manipulates the image histogram as in Ĉapek *et al.* [3], but it focuses on a continuous domain of probabilities to filter the foreground information to calculate the correction parameters. Before we present our approach, we shortly discuss approach by Ĉapek *et al.* [3].

The approach proposed in [3] consists of two stages. In the first stage, a standard histogram is constructed with the help of histograms of optical sections in the image stack. In the second stage, the individual histograms are warped according to the standard histogram to achieve the brightness and contrast of the standard histogram. The construction of standard histogram is adopted from [7]. The approach is based on landmarks chosen in the image histogram. The landmarks chosen are the minimum and maximum intensities

and percentiles of the foreground mode of the image.

Bimodal image histograms are frequently observed for medical images captured with confocal microscope. In case of the bimodal histograms, one mode corresponds to the background while the other mode corresponds to the foreground object. Hence, it is selected as the base for calculating other landmarks in histogram. The disadvantage of method [3] is that only a heuristic method is presented to calculate the foreground mode which is the basis for setting the scale for the construction of standard histogram. The foreground mode is calculated by removing the background mode by thresholding. The overall mean intensity of the image is set as the threshold. The choice of the actual landmark configuration is also an important factor. This mode based method is not appropriate for images in which foreground and background modes are very close to each other. A better approach suggested in [7] is to choose the median of the histogram of the foreground as the landmark. This method too does not guarantee best fit mode in all cases [7]. Apart from the foreground mode, minimum and maximum intensities are also set as landmarks in the creation of standard histogram. The minimum and maximum intensity of image are highly sensitive to noise. In order to eliminate the influence of noise concentrated around minimal and maximal intensity values, the upper and lower boundaries of the standard histogram must be set which requires interaction with the user. The proposed method aims to overcome the disadvantages of [3]. The main idea is to filter the foreground information from a given image by modeling it as a mixture of Gaussians and use this information to compensate the intensity loss of the photobleached images. The foreground mean and standard deviation is used to transform the pixel intensities of the original image relative to the intensity parameters of a reference image.

The paper is organized as follows: Section 2 explains the proposed approach in detail. Section 3 presents experimental results. The paper concludes in Section 4.

2. PROPOSED APPROACH

In many statistical applications, Gaussian Mixture Model (GMM) is used as a general tool for modeling a large heterogeneous population. Detailed introduction to GMM can be found in [8]. GMM is a semi-parametric estimation approach that provides good flexibility and precision in modeling the statistics of unlabeled sample data. In our case, the image data can be assumed to be generated from two components, one forming the background of the image and the other pertaining to the foreground of the image. However, it is not known that which pixel belongs to which component. As a result of this the problem can be considered to have missing data, i.e., background/foreground membership information.

Each component can be considered to have its own parameters θ, which define the probability density function $P_i(x)$. These parameters can be estimated through the Expectation Maximization (EM) algorithm which is the widely used approach to solve the missing data problem. It devises appropriate parameters for the chosen model with respect to the data points generated by individual components. In the EM algorithm, initial estimates for the parameters are chosen arbitrarily. As the selection of initial estimates affect the final results, they must be chosen carefully. The iterative parameter estimation process consists of two steps, the Expectation (E) step and the Maximization (M) step. In the expectation step, the expected value of the missing data is calculated. In the Maximization step, the resulting value of the expectation is maximized by selecting new set of parameters. The E and M steps are iterated until a stopping criterion such as a number of iterations is met or until there is no change in the mixture model parameters.

As discussed previously, most of the images captured with confocal microscope are bimodal, one mode each for background and foreground. Hence, the image data is modeled as two-component GMM. Based on the assumption that the loss of intensity increases relatively with time or depth or both, the first image of the stack will have minimal loss of intensity and can be considered as the reference image. The reference image should have good visual information of the object or specimen to be studied. Initially, mean intensity and standard deviation for the foreground and background are estimated with EM algorithm. Then the parameters of the foreground component are used to warp the each pixel of the image to its relative reference intensity. Following subsections explain individual steps taken during this process in detail.

2.1. Parameter estimation

For a two component GMM of j^{th} image in the stack, there are six unknown parameters,

$$\theta^j = \{(w_1^j, \mu_1^j, \sigma_1^j), (w_2^j, \mu_2^j, \sigma_2^j)\}, \tag{1}$$

where w_1^j,w_2^j are mixture weight constants, μ_1^j,μ_2^j represent mean intensities, and σ_1^j,σ_2^j give the standard deviation corresponding to foreground and background Gaussian distribution respectively for j^{th} image in the stack.

First step is to estimate the membership probability for each n^{th} pixel of j^{th} confocal microscopy image. Intensity of this pixel is given by x_n^j. The membership probability can be calculated as,

$$F_i^j(n,j) = \frac{w_i^j \cdot P_i^j(x_n^j)}{w_1^j \cdot P_1^j(x_n^j) + w_2^j \cdot P_2^j(x_n^j)}, \tag{2}$$

where,

$$P_i^j(x_n^j) = \frac{1}{\sigma_i^j\sqrt{2\pi}} \exp\left\{\frac{-(x_n^j - \mu_i^j)^2}{2(\sigma_i^j)^2}\right\}. \tag{3}$$

In the above equations $i = 1, 2$ and $n = 1, 2, \ldots, N \times M$, where $N \times M$ is the dimension of the image and $j = 1, 2, \ldots, K$ where K is the number of the image slices.

In the second step, the Gaussian mixture parameter values can be estimated from the above membership probability.

$$w_i^j = \frac{1}{M \times N} \sum_{n=1}^{M \times N} F_i^j(n,j), \tag{4}$$

$$\mu_i^j = \frac{\sum_{n=1}^{M \times N} F_i^j(n,j) \cdot x_n^j}{\sum_{n=1}^{M \times N} F_i^j(n,j)}, \tag{5}$$

$$\sigma_i^j = \sqrt{\frac{\sum_{n=1}^{M \times N} F_i^j(n,j) \cdot (x_n^j - \mu_i^j)^2}{\sum_{n=1}^{M \times N} F_i^j(n,j)}}. \tag{6}$$

Above two steps are iterated until the all the parameters converge. The iterative process is repeated for each image in the stack.

2.2. Image warping

Once the mixture parameters for the image are known, image can be compensated easily. Generally, compensation for foreground and background parameters requires different transformation. These transformation can be applied individually to the pixels by classifying them as background or foreground pixel. This can be done based on the membership probability. If $F_1^j(n,j) > F_2^j(n,j)$, the pixel belongs to the background, otherwise the pixel belongs to the foreground. To carry out the compensation, for the most of the images there is no need to separate background and foreground as

background of confocal microscopy images has intensity close to zero. Also, one is very rarely interested in background information. Hence, the compensated intensity for n^{th} pixel in j^{th} image can be calculated from original intensity x_n^j as:

$$(x')_n^j = \frac{(x_n^j - \mu_2^j)}{\sigma_2^j}\sigma_r + \mu_r \tag{7}$$

Generally, foreground mean and standard deviation of the first image in the stack should be set as μ_r and σ_r, i.e., the reference parameters for the image stack restoration. However, in a case where first image in the stack does not have enough details or is not the brightest, one of the other images can be chosen to be the reference.

3. EXPERIMENTAL RESULTS

The proposed approach was implemented in MATLAB and was tested on several sets of image in Biovision lab database. The experimental image sequences were acquired by Zeiss LSM 510 META confocal laser scanning microscope. Before proceeding to the experimental results, we briefly talk about the initialization used for the experiment.

Initialization is crucial for EM algorithm. As parameters from reference frame are needed for image restoration, EM is carried out on the reference image first. The mixture weights for the reference frame m can be initialized as,

$$w_1^m = w_2^m = 0.5.$$

Since background mean should be lower than overall image mean and foreground mean should be higher, one can select their initial values arbitrarily to follow above restriction.

$$\mu = \frac{1}{M \times N}\sum_{n=1}^{M\times N} x_n^m, \;\; \mu_1^m = \frac{\mu}{2}, \mu_2^m = \frac{3\mu}{2}.$$

Good initial value for the mixture standard deviation is the overall standard deviation of the image.

$$\sigma_1^m = \sigma_2^m = \sqrt{\frac{1}{(M \times N) - 1}\sum_{n=1}^{M\times N}(x_n^m - \mu)^2}.$$

These values can be improved upon by randomly using various initializations and then choosing the one that maximizes the membership probabilities. However reasonable fixed values as stated above were used for repeatability of the experiment.

After successful completion of the EM procedure for the reference frame m, reference parameters are set as,

$$\mu_r = \mu_2^m, \;\; \sigma_r = \sigma_2^m.$$

As any image in the sequence is very similar its previous image, parameters of previous image after EM are used to initialize EM procedure for the next image.

$$w_1^j = w_1^{j-1}, \mu_1^j = \mu_1^{j-1}, \sigma_1^j = \sigma_1^{j-1},$$

$$w_2^j = w_2^{j-1}, \mu_2^j = \mu_2^{j-1}, \sigma_2^j = \sigma_2^{j-1}.$$

This also helps to reduce the computational burden, by reducing number of EM iterations.

The sequence tested here, is a temporal 3D sequence with resolution $336 \times 256 \times 12$. 45 instances of the specimen were captured in 65 seconds. Figure 1(a) to (l) shows all the 12 optical sections at time $t = 1$. It can be observed from the images that the intensity of the optical sections varies significantly from one section to the other. The intensity rises from depth $z = 1$ to $z = 5$ and drops again till $z = 11$ before it rises in the final optical section at $z = 12$. Also, the structure of the image changes significantly with depth.

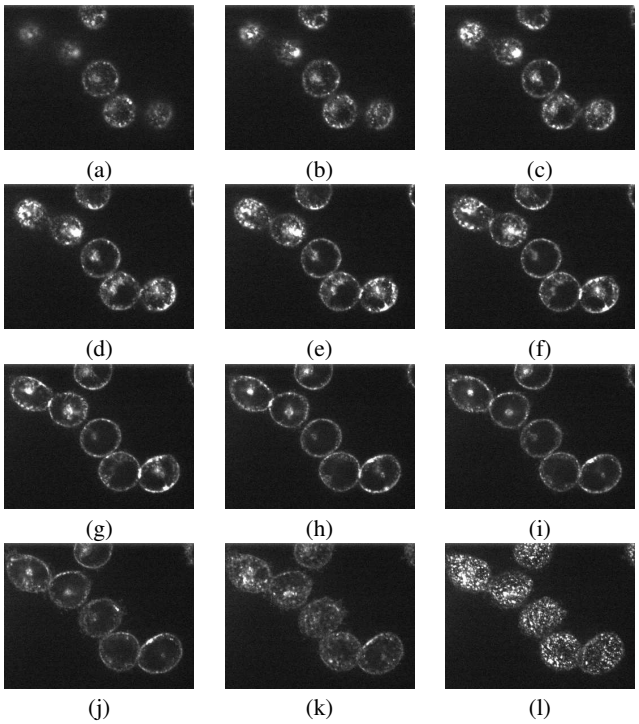


Fig. 1. Original optical sections at time $t = 1$ from (a) top ($z = 1$) to (l) bottom ($z = 12$) (bottom frame is used as the reference)

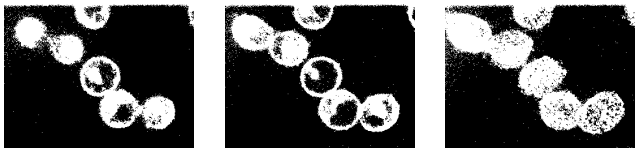
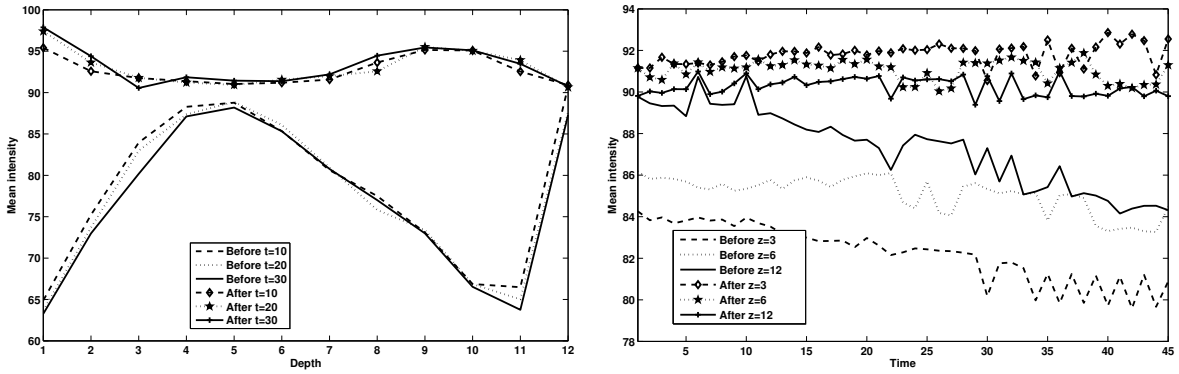


Fig. 2. Foreground and background classification for optical sections at time $t = 1$ (a) $z = 3$, (b) $z = 6$, (c) $z = 12$ (white region denotes foreground and black region denotes background)

GMM parameters were calculated with EM algorithm. For each frame, the iterative process was terminated when foreground and background mean values changed by less than 0.01. After estimating GMM parameters, the classification of image pixels into foreground and background gives Figure 2. Despite the structural changes and changes in intensity, the foreground regions are consistently detected. The success of the proposed method can be attributed to this consistency.

Based on the classification, mean intensities were calculated for the image foreground for analysis. Figure 3(a) shows the plot for the variation in the foreground mean with depth at time $t = 10, 20, 30$, which is in agreement with the visual observations made. However, variation of mean intensity with time plotted in Figure 3(b) for depth $z = 3, 6, 12$ reveal facts which are difficult to observe visually. Intensity of the foregrounds drops as the time progresses as expected due to effects of photobleaching. However, rate of the decay is different at different depth levels. At depth $z = 6$ the mean intensity

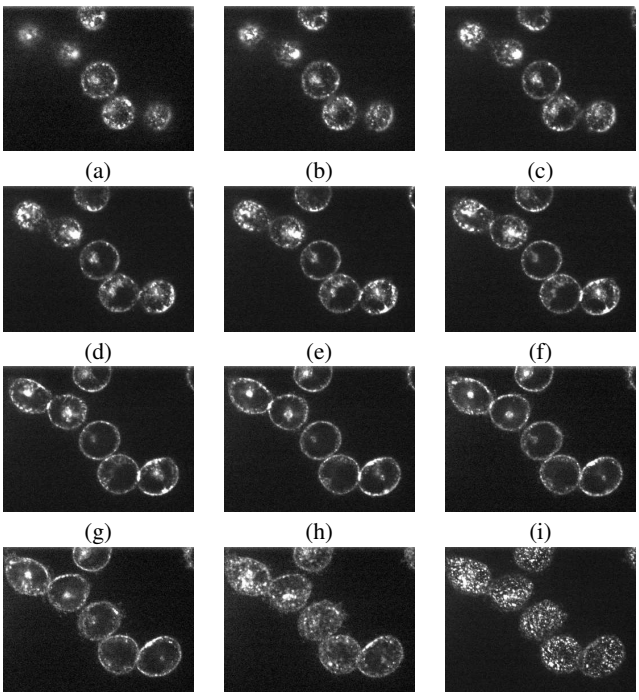


(a) (b)

Fig. 3. Variation in the mean intensity of foreground of original image stack and restored image stack (a) with depth (b) with time

drops from 86.2 to 84.5, while at depth $z = 12$ it drops from 89.8 to 84.5. Decay rate at $z = 12$ is almost 3 times the decay rate at $z = 6$.

As our method does not utilize IDF, estimation of complicated IDF which is required to model this image sequence becomes unnecessary. The image sequence was restored with reference values $\mu_r = 82.5$ and $\sigma_r = 51$ which were estimated from frame at $t = 1$ and $z = 12$. This frame was chosen as it has the maximum mean intensity. Restored images at $z = 1$ are shown in Figure 4. Steady values of the intensities can be observed in the plot which is also reflected in the restored images in Figure 4.



(a) (b) (c) (d) (e) (f) (g) (h) (i) (j) (k) (l)

Fig. 4. Restored optical sections at time $t = 1$ from (a) top ($z = 1$) to (l) bottom ($z = 12$)

4. CONCLUSION

For reliable analysis as well as visualization of cell dynamics, it is essential that the acquired images reflect the exact information of the specimen. The objective of the proposed method was to help regain the information lost due to various deteriorating factors such as scattering and absorption of the excitation, photobleaching of fluorescent images etc. Current approaches to solve this problem are computationally complex, time consuming, restricted to parametric decay models (IDF) and highly sensitive to noise. The proposed method provides a simple yet effective statistical approach to solve this problem. It overcame the disadvantages of current methods and at the same time increased the visual value of confocal microscopy images. The main idea was to filter the foreground information from a given image by modeling it as a mixture of Gaussians and use this information to compensate the intensity loss of the confocal microscopy images. When multiple fluorescence tags are used in a specimen, the proposed method can be simply applied individual tags or a multiple Gaussian mixture model can be used to handle the scenario.

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