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Cell Recognition of Microscopy Images of TPEF (Two Photon Excited Florescence) Probes

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Abstract

The behavior of a cell can be described through tissue morphogenesis, which involves the migration, division or death of tissue, and is regulated with the molecular scale. Automated cell detection from microscopy image has become an important step in cell-based experiments. We have developed a method to detect abnormal behavior of the cell through real-time images. Our method consists of pixel classification using K-Means and Bayesian classification. It is based on the combination of gray level threshold. Furthermore, Fast Fourier Transfer (FFT), covariance coefficient and verification of cell variation were applied to investigate the cells after drug injection. We have considered different types of confocal microscopy images. The images were taken after every five min. The NL1 compound has high fluorescent, which selectively targets the mitochondria, and mitochondria are sensitive to the environmental changes. Identification of cells in a TPEF probe test is very important for determining cell abnormalities. Detection of abnormal cells is very crucial in the early stage diseases, and it is beneficial for mitochondrial microenvironment related diseases.

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Keywords: Image comparison; two photon excited florescence probes; cell detection; thresholding method.

1. Introduction

Convenient and small design water-soluble molecular two-photon excited fluorescence (TPEF) probes always remain a challenge for biological application [3]. The drug is injected into TPEF probes and then the images of this probe were taken. The behavior and type of cell can be investigated using these images. It selectively targets mitochondria and monitors their viscosity. The cell images showed that the Bayesian and K-Mean classifications are not able to detect accurately the inflammatory cells and nuclei [17], which were based on a combination of gray level threshold technique. Image recognition based on optical correlation performs the optical Fourier transform of the two-dimensional image by using light characteristic of interference, refraction, and diffraction [1]. The advantages of optical correlation recognition are high speed, parallel processing, large information, high discrimination and real-time, etc [2]. Mitochondrial diseases and disorders are caused by dysfunctional mitochondria, which are the source of energy in the cells. Mitochondria are found in every cell of a human body except in red blood cell. The main function of these cells is to convert the energy of food molecules into the adenosine triphosphate (ATP), which is the power of most cells.

In this article, we have measured the behavior of the water-soluble TPEF probe, and this probe bears N-methyl pyridinium moiety, and it is very sensitive to the local viscosity. After comparison of the compound images, it showed a significant improvement in the monitoring of viscosity. Additionally, in this paper, we have compared different cell images and check their behavior after injection of nystatin drug, and it targets the selective mitochondria. In this article, we proposed a method to recognize the TPEF (Two-Photon Excited Fluorescence Probes) images using the FFT (Fast Fourier Transfer) and covariance coefficient. The correlation between normal and drug images, and the intensity of the single cell suggested that there is a significant improvement in the monitoring of the mitochondrial viscosity, with a little change in the correlation (Fig. 6).

2. Related Work

The investigation of macular biology (cellular dynamics) from microscopy image describes and tracks the cells. The watershed algorithm [4] is a region growing method, and it is used as cell segmentation from fluorescence microscopy image [5- 9]. Single seed point segmentation in each cell is the success of the watershed-based method to avoid any potential error, which is manually corrected by polygons and is a time-consuming process. Tracking cells are linked as a frame to frame, which is based on cell overlap [5, 7, 10]. To find the overlap of cells, the first identity of a cell neighbor can be used [11]. Tacking method heavily depends on segmentation accuracy, and cell tracking can be carried out through segmentation autonomous by matching image sub-regions instead of individual cells. Optic flow uses the cross-correlation of two images to calculate local similarities [12], and also tracks the cell-based matching fluorescence pattern [8, 13]. The independent method of segmentation is computationally expensive. Temporal sampling or fine spatial is necessary for a rapid and cost-effective process. Thus, integrating segmentation and tracking can be beneficial for the effectiveness of both approaches [14-15].

3. Method

In the object recognition, we have used TPEF to recognize specific cell details. Mitochondria are enclosed by two membranes- the inner membrane, outer membrane, cristae, and matrix (Fig. 2). In the general, nucleus (plural, nuclei) is the central part of the cell; the inner membrane is the site of the electron transport chain; the outer membrane is a thin layer, which forms the outer boundary of living cells; the inward projection of the inner membrane, which increases the surface area of the chemical reaction; and the matrix contains soluble enzymes, which catalyze organic molecules and oxidize pyruvate. Mitochondria also contain ribosomes and DNA [16]. Figure 1 is a proposed research design. The research design consists of several stages, including nuclei and inflammatory cell detection based on pixel classification using K-Means and Bayesian classifications as well as the combination of gray level threshold. In biology, the nucleus has the genetic materials (DNA). The auto covariance coefficient is expressed in equation 1. The x (i) is a given signal, and k is the shift of x(i) signal with the size of k samples, and N is the length of x(i). Equation 2 shows the xs value. The steps involved in the grayscale method include converting the image from RGB to grayscale, improving the image with adjustment and Unsharp filter, segmenting the image by applying global threshold of 0.65 to obtain a crystal black and white image of cytoplasmic candidate, calculating the cytoplasm feature, namely centroid, area, and bounding box. Cropping cytoplasm and nucleus automatically carried out with bounding box> 200x200 pixels. Subimage segmentation was done for k = 1,2,3, ..., n; where n is the subimage. The subimage was converted from RGB to grayscale. Global threshold of 0.25 was subsequently applied to get a black and white image of nucleus candidate. The nucleus candidate features, such as the centroid, area and bounding box were calculated. The candidate nucleus was cropped automatically with bounding box (13x13 pixels), and the Cn was the nucleus candidate.

$$\Gamma(k) = \frac{1}{N-1}\sum_{i=0}^{N-K}(x(i+k)-x_s)(x(i)-x_s) \quad (1)$$

$$X_S = \frac{\sum_{i=0}^{N} x(i)}{N} \quad (2)$$

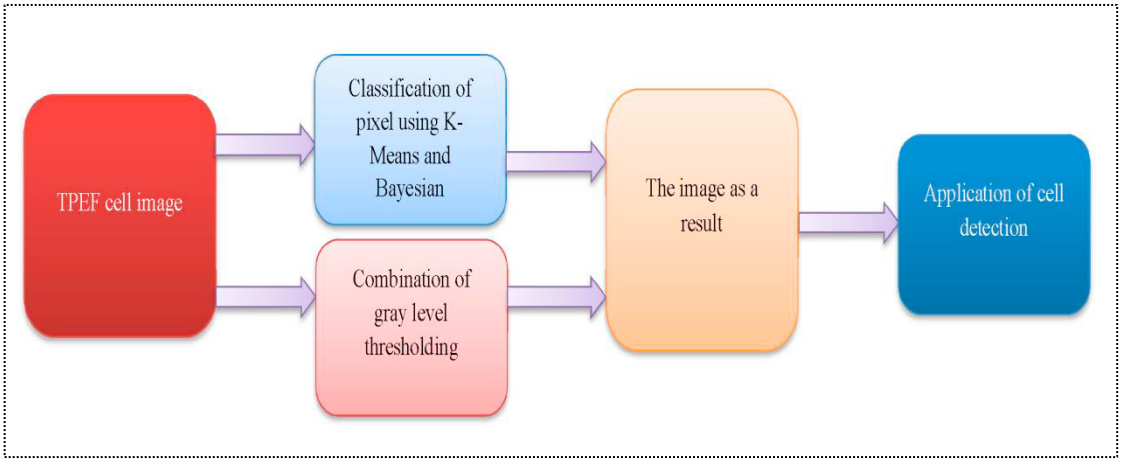

Figure.1: TPEF cell detection

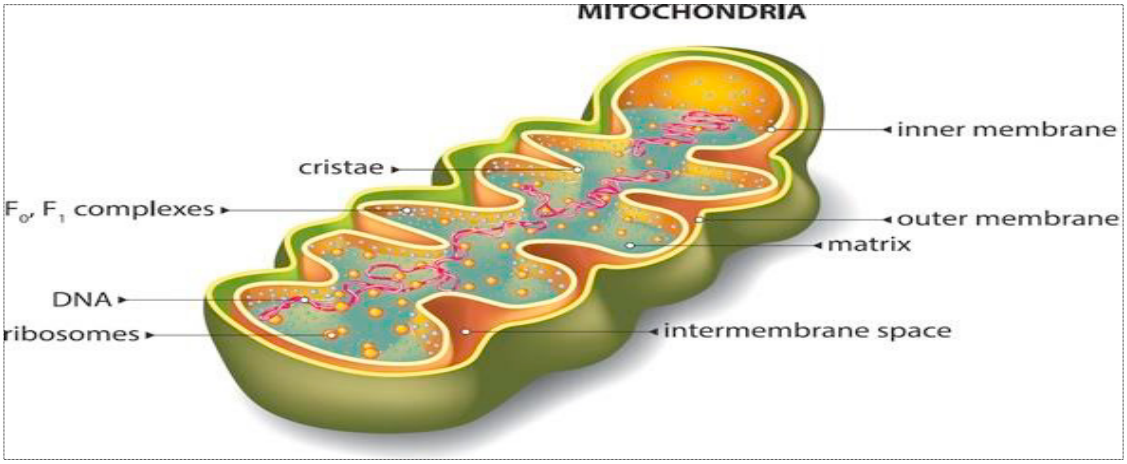

Figure.2: Mitochondrial inner structure

4. Results and Discussion

4.1. Data Set

Through confocal laser scanning microscopy, images of cells were taken on a (ZEISS LSM 710 META), with oil lens 63x. A (CCP) Coherent Chameleon pulsed infrared multiphoton laser for two-photon imaging at wavelength of 900nm. For real-time cell imaging, an incubation chamber was connected to a ZEISS temperature control unit at 37 °C and CO_2 controller with proper humidity (1-2 h before the experiment was allowed for the stabilization of temperature and CO_2 concentration). In the NL1, the excitation wavelength was 900 nm, and the emission wavelength range was 560-620 nm. Co-staining was performed by incubating cells with 1 μM Mitotracker (λex = 579 nm, λem = 585–610 nm) and 10 μM NL1 (λex = 488 nm, λem = 560–620 nm) 142 for 30 min. The following tools were used for data collection, image J, LSM Image Expert and Zeiss LSM Image Browser [3]. Total twelve pictures were taken (normal and durg) and here we have shown six pictures (normal and drug injected) at different time intervals in a consecutive order 5, 10, 15, 50, 55 and 60 min (Fig.3).

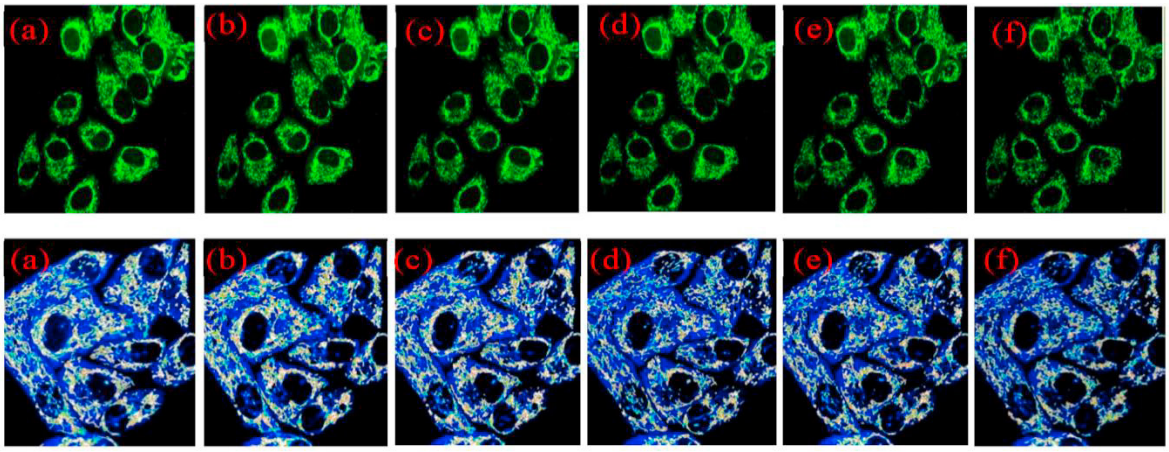

Figure.3 (a,b,c,d,e and f). Real -time images

4.2. Results

In this process, three stages were performed, which are: the image simplification, image conversion into grayscale image and cell detection. In this Bayesian and K-mean methods, background cleaning and classification of each pixel were applied; Figure 4 shows the training image (Real image) for this method. In the first image the region is specified, and the other images are masked by fuzzy threshold. Prior to the thresholding process, it is necessary to eliminate the deformation via subtraction and compensating deformation surface to the previous phase image. The method of cell tracking is based on the cellular nuclear identification, and it is possible with help of center of gravity, and previous image phase. The main reason of cell association is based on nuclei, and the nucleus represents the phase difference. It is distinguished from background. Nucleus shape is more compact as compared to the cell body. It shows that the center of gravity is the center of a nucleus, and it reliably points for identification of the nucleus from the following image. Figure 4(b) is the result of cell detection and K-means. Figure 4(c) is the cell detection by Bayesian, Figure 4(d) is the detection through the threshold, Figure 4(e) is nucleus detection, white part shows the nucleus, thresholding method give better result as compared to other methods, and Figure 4(f) is the FFT (Fast Fourier Transfer) image.

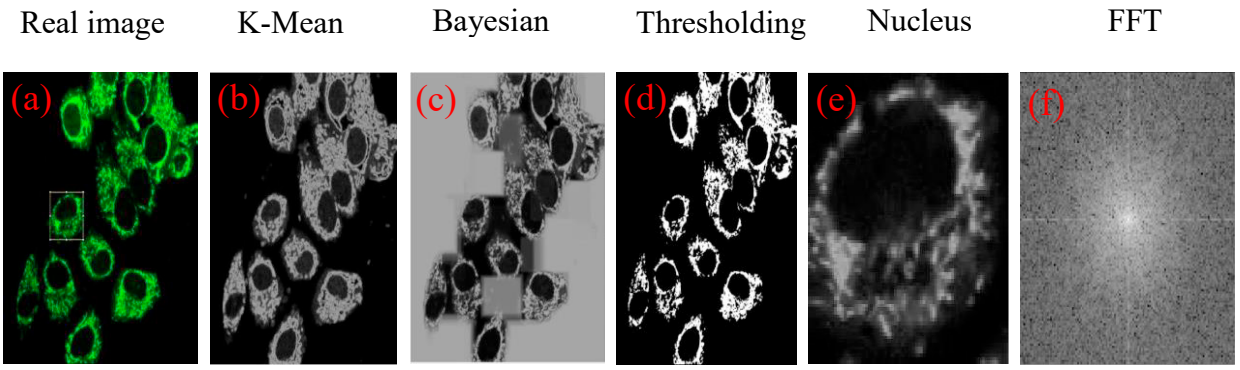

Figure.4 (a, b, c, d, e and f) Cell detection

The correlation between the drug treated and drug untreated cell images are shown in Figure 5 (a and b). The drug- treated cell images have greater value of the coefficient of correlation compared to the untreated cell images, which means they are more similar. All values are positive, and the relationship between the images is linear positive. If the value is closer to 1, it has a more positive linear relation. Thus, the value of the maximum similarity of an image is 1. The cell images (one and eight) are very similar as they have the correlation value of 0.8. The cell images (seven and eight), and the cell images (nine and eleven) also show similarity as they have the correlation value above 0.7. The cell images (one and twelve), (seven and twelve), (five and six) and (four and twelve) are also reasonably similar as they have a correlation value of above 0.6. Less similarity is found between cell images (eleven and twelve), (nine and twelve), (five and ten), (five and elven), and (five and twelve) as they have a correlation value around 0.3 (Fig. 5a and b). It means the after injecting the drug into a compound with the passage of time not too much effect, a slight difference between the images and the viscosity resulting in a great change. Figure 6(a) shows the variance value between all images after drug treatment. In Figure 5(b), the intensity means the numerical value of pixel or the amount of light. Figure 6(b) shows the image intensity of Two-Photon Excited Fluorescence Probes. Figure 6(b) shows the slight difference overtime and the intensity of pixels will be reduced. The viscosity is increased. Each column has a mean value and plots the mean as SD (standard deviation).

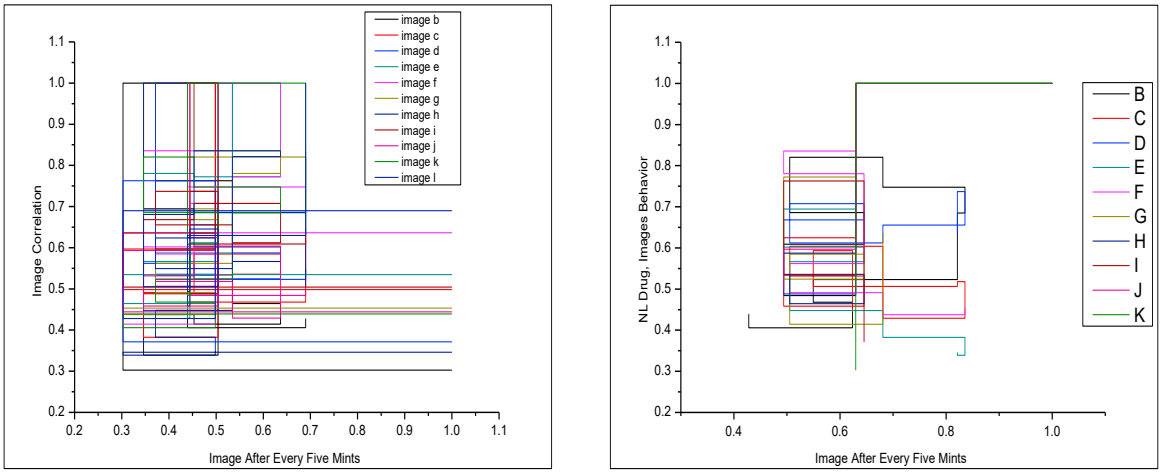

Figure 5: Images correlation and image behavior (a, b)

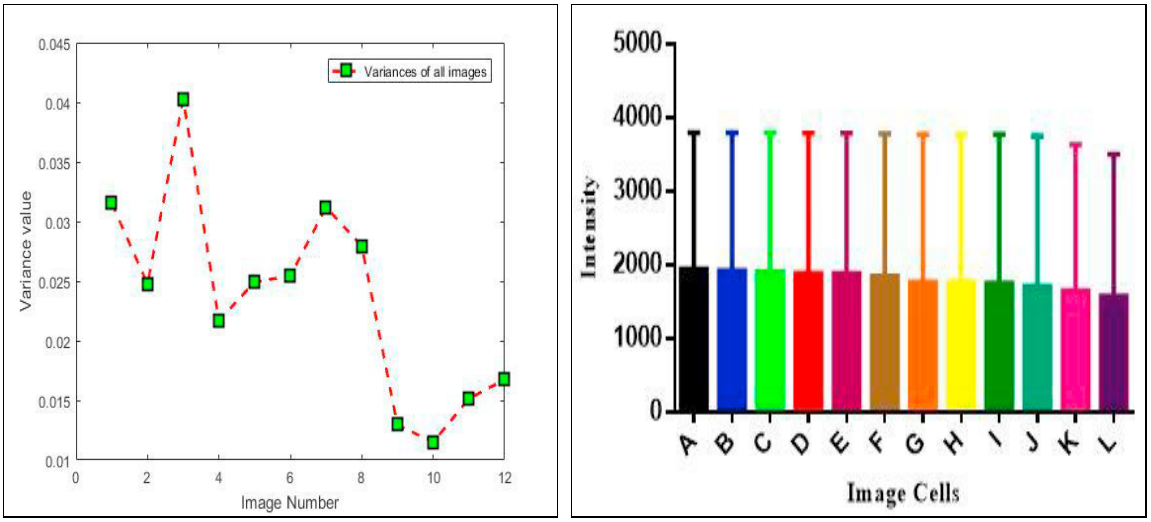

Figure 6: Variance value of all images (after drug) (a) and cell intensity (b)

Conclusion

In this research, we compared the TPEF images, detected the viscosity and intensity changes in the compound images. The viscosity has a significant improvement. For cell detection, the best technique is to properly interpret the mitochondrial cell confocal microscopic image and precisely analyze the derivation deductions. It is beneficial for mitochondrial microenvironment related diseases. Moreover, the grouping of gray level thresholding technique is appropriate for the recognition of nuclei and microscopic cell in Two-Photon Excited Fluorescence Probes. This study is an initial study in an effort to find the recognition approach of nuclei and cells in Two-Photon Excited Fluorescence Probes. In our future work, we extend our method and accurately detect the nuclei, as well as the cell overlapping. Furthermore, we will consider extracting the cell overlap in both normal and drug images and finding the mitochondrial movement, such as fusion and fission.

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