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Image visualization of photon counting confocal microscopy using statistical estimation

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ABSTRACT

In this paper, we propose image visualization method of photon counting confocal microscopy using statistical estimation. Since high power coherent light source is used to record sectional images from micro-objects in conventional confocal microscopy, it may cause damage for a structure of micro-objects. Thus, low power coherent light source may be required. However, in low light level environment, it is difficult to capture sectional images of micro-objects. On the other hand, photon counting imaging technique can detect sectional images of micro-objects in photon-starved conditions. Therefore, in this paper, we apply a photon counting imaging technique to conventional confocal microscopy for visualization of micro-objects. Photon counting detection can be modeled by statistical process. To visualize micro-objects under photon-starved conditions, statistical estimation methods such as maximum likelihood estimation can be used. In addition, we present color photon counting imaging system which considers different carried photon energy with each basic color channels. To prove and evaluate our method, we show simulation results for image visualization of photon counting confocal microscopy and calculate mean square error.

1. Introduction

There have been various microscopy techniques for visualization of micro-objects; optical microscopy [1], electron microscopy [2], scanning probe microscopy [3], ultraviolet microscopy [4], infrared microscopy [5], digital holographic microscopy [6], virtual microscopy [7], laser microscopy [8], confocal microscopy [9], and so on. Especially, confocal microscopy can record multiple sectional images of micro-objects via their depth direction. Applying integral imaging to confocal microscopy, 3D visualization has been implemented [10]. Since it uses high power coherent light source to capture sectional images, it may cause damage for a structure of micro-objects. Thus, low power coherent light source may be required. However, in photon-starved conditions or low light level environment, it is difficult to detect sectional images of micro-objects using conventional confocal microscopy. On the other hand, since photon counting imaging technique [11–14] can be used to obtain images under photon-starved conditions, it can record sectional images without any damage for the structure of micro-objects. In addition, using statistical estimation methods such as maximum likelihood estimation (MLE) [11] or maximum a posterior (MAP) [12], it can visualize micro-object under photon-starved conditions. Since physical photon counting detectors have a cost problem, we model photon counting detector by statistical process [15]. Also, we introduce a color photon counting imaging technique which considers different carried energy from micro-objects under photon-starved conditions with each basic color channel such as red (R), green (G), and blue (B), respectively [15]. Therefore, in this paper, we apply a statistical model of photon counting detector for each basic color channel to conventional confocal microscopy and use MLE for visualization of micro-objects. The paper is organized as follows: We present a statistical full color photon counting model and visualization technique of photon counting confocal microscopy using MLE. Then, to prove and evaluate our method, we show simulation results for photon counting confocal microscopy and calculate mean square error (MSE). Finally, we conclude our results with summary.

2. Image visualization of photon counting confocal microscopy

Using confocal microscopy as depicted in Fig. 1(a), uniformly magnified sectional images of micro-objects as shown in Fig. 1(b) can be obtained via different depths. However, since it may not

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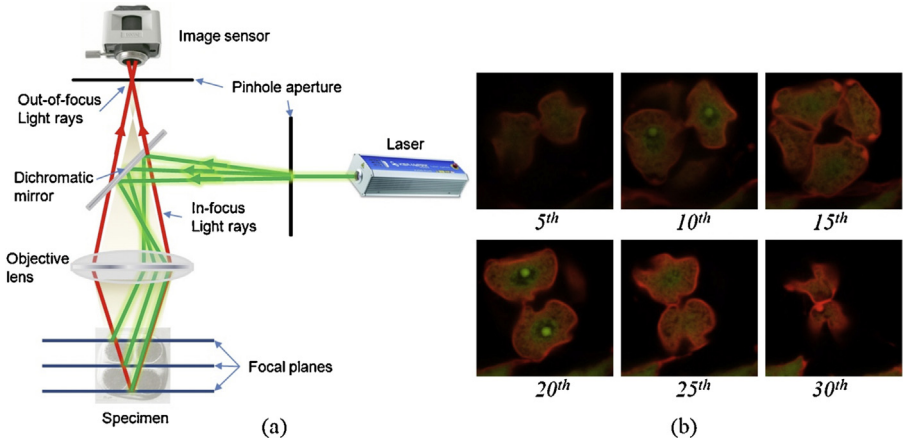

Fig. 1. (a) Conventional confocal microscopy and (b) sectional images via depth direction using confocal microscopy.

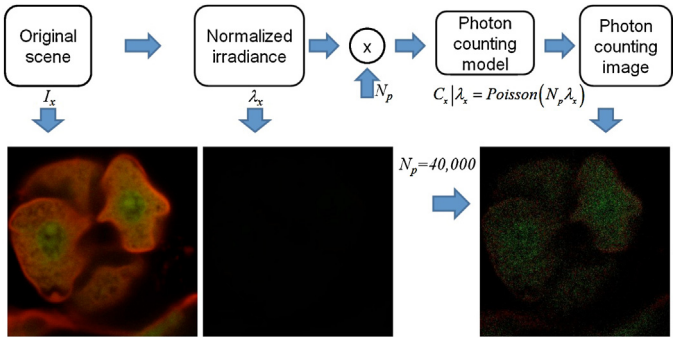

Fig. 2. Statistical model of photon counting imaging.

capture sectional images under photon-starved conditions, photon counting imaging technique is required. Photon counting detector can be modeled by Poisson distribution because this statistical distribution can be applied when events occur rarely in unit time and space [15].

A statistical model of photon counting detector is shown in Fig. 2. For computational simplicity, we consider one-dimensional only. Thus, photon counting process for sectional images by confocal microscopy can be implemented by the following [11]:

$$C_k(x)\,|\,\lambda_k(x) \sim \text{Poisson}\left(N_p\lambda_k(x)\right) \tag{1}$$

$$\lambda_k(x) = \frac{I_k(x)}{\sum_{x=1}^{N_x} I_k(x)} \tag{2}$$

where $C_k(x)$ is the kth photon-limited sectional image, $\lambda_k(x)$ is the normalized irradiance of the kth sectional image, N_p is the expected number of photons in the sectional image, $I_k(x)$ is the kth sectional image by confocal microscopy, and N_x is the total number of pixels of the sectional image, respectively.

Using Eqs. (1) and (2) with N_p = 40,000, multiple photon-limited sectional images for confocal microscopy can be obtained as shown in Fig. 3. However, each sectional image cannot be recognized well due to lack of the number of photons. In addition, it is difficult to visualize micro-objects from each sectional image.

To visualize micro-objects, we can assume that all sectional images stack up through depth direction as shown in Fig. 4(a).

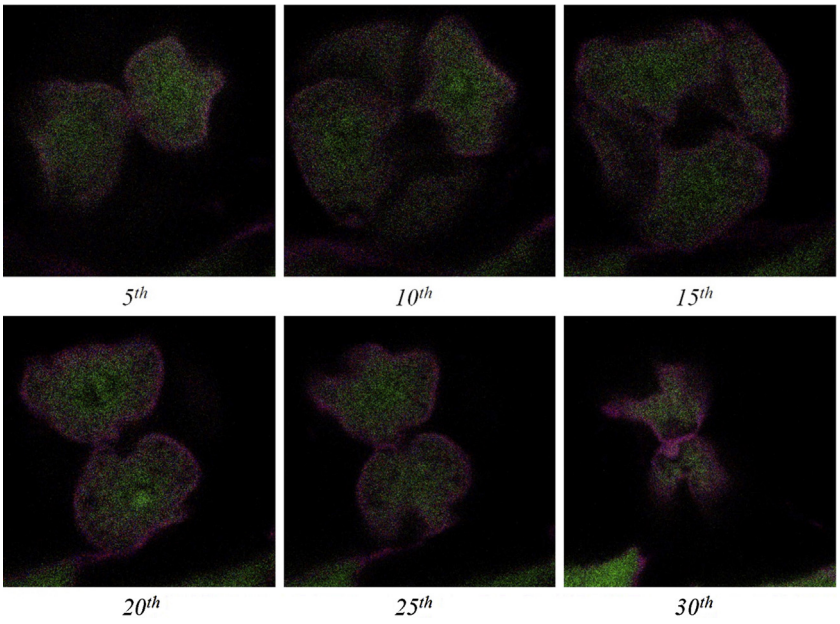

Fig. 3. Photon-limited sectional images using statistical photon counting model.