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Invited paper

Parallel detecting super-resolution microscopy using correlation based image restoration

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HIGHLIGHTS

- Obtain detectors' relative location using correlation of images recorded.
- Resolution enhancement with a factor of 1.7 compared to confocal microscopy.
- Use deconvolution with maximum likelihood processing to increase SNR.

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ABSTRACT

A novel approach to achieve the image restoration is proposed in which each detector's relative position in the detector array is no longer a necessity. We can identify each detector's relative location by extracting a certain area from one of the detector's image and scanning it on other detectors' images. According to this location, we can generate the point spread functions (PSF) for each detector and perform deconvolution for image restoration. Equipped with this method, the microscope with discretionally designed detector array can be easily constructed without the concern of exact relative locations of detectors. The simulated results and experimental results show the total improvement in resolution with a factor of 1.7 compared to conventional confocal fluorescence microscopy. With the significant enhancement in resolution and easiness for application of this method, this novel method should have potential for a wide range of application in fluorescence microscopy based on parallel detecting.

1. Introduction

During the process of imaging, the resolution of conventional microscope is restricted by the diffraction nature of light [1]. The image of an ideal point will spread into a blurred disk with diameter relevant to wavelength of light. The distribution of intensity in the disk is a feature of the imaging system called point spread function (PSF), which determines the restricted resolution of imaging system. Three different approaches for resolution improvement have been proposed, including frequency shift, single molecule localization and PSF engineering. Frequency shift is a technique using the optical method to shift high frequency components into observable region. Structure Illumination Microscopy (SIM) is a typical example of frequency shifting microscopy [2]. However, application of such technique is limited owing to the complexity of equipment and image processing [2,3]. Another approach, single molecule localization, is based on the idea of detecting one fluorescence molecule each time, so the diffraction limit can be avoided. Photoactivated Localization Microscopy (PALM) [4] and Stochastic Optical Reconstruction Microscopy (STORM) [5] are two exemplary methods of single molecule localization. Despite the potential impressive resolution this method can achieve, the speed of imaging is an obstacle. Due to the necessity of capturing thousands of frames during imaging, the speed of this technique is too slow for real time imaging. PSF engineering is a technique in which the PSF of confocal microscopy is engineered to decrease the size of effective PSF and thus increase the resolution. This is also the approach we adopt.

The excitation PSF and the detection PSF together make up the overall PSF of the confocal microscopy, all the adaptations in PSF engineering aim to narrow these two PSFs. Conventional confocal

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microscopy generally use smaller pinhole to narrow the detection PSF to gain the enhancement in resolution. Theoretically, the smaller the pinhole, the better the resolution. However, the smaller pinhole also lead to lower intensity, decreasing the signal to noise ratio (SNR). The balance between resolution and SNR has become the dilemma in conventional confocal microscopy [1,6,7].

A variety of efforts have been done to narrow the system's PSF. The novel techniques, such as the Image Scanning Microscopy (ISM) [8], Stimulated Emission Depletion Microscopy (STED) [9], Fluorescence Emission Difference Microscopy (FED) [10], Virtual Fluorescence Emission Difference Microscopy (VFED) [11], Saturated Virtual Fluorescence Difference Microscopy (SVFED) [12], Virtual *k*-Space Modulation Optical Microscopy (VIKMOM) [13], all adopt different approaches to achieve a narrower PSF.

Among all the effective methods proposed for enhancement in resolution of confocal microscopy, one of the most practical and popular methods recently is the Airyscan introduced by Zeiss Company. In Fig. 1(a), Airyscan uses 32 detectors, each of which is a hexagon with the length of 0.2 Airy unit (AU, corresponding to 1.22λ/Numerical Aperture) for each side, forming a detector array with diameter of 1.25 AU. Such arrangement would record most of the information in the airy disk. While scanning, Airyscan uses parallel detecting for collection, each detector collects its own image. As for the process of image restoration, different detector's image is counted with different weight and linear deconvolution is used to obtain the final image. Such method for image processing is strongly based on the exact locations of detectors in detector array [8,14]. Owing to the decrease in the size of effective PSF and the increase in the SNR, Airyscan can achieve a total improvement in resolution by a factor of 1.7 in all spatial directions. Because of these benefits, the Airyscan has become one of the most mature and commercial methods in super resolution area [15,16].

However, during the process of image restoration, preinstalled relative locations of detectors in detector array are needed for all kinds of parallel detecting microscopy, including Airyscan. Because relative locations of detectors are used for image restoration. In Airyscan, relative location of each detector is used to generate PSF and perform linear deconvolution. Such necessity limits the usage of Airyscan as well as many other parallel detecting methods such as VIKMOM, ISM, FED.

Here, a novel approach for image processing in parallel detecting is proposed. Instead of using the preinstalled wavelength to calculate detectors' locations, we find the relative location of each detector by scanning part of the image captured by one detector on another detector to find the position with maximum correlation. Besides, in our method, deconvolution is performed twice for better SNR and resolution. In the first deconvolution process, linear deconvolution is performed for each image individually and then we add them up, to achieve image combination. In the second deconvolution process, we use maximum-likelihood estimation method for better SNR and prevent the false information introduced during the process of deconvolution. Maximum-likelihood estimation deconvolution is a method based on photons' Poisson distribution and can achieve a resolution better than conventional Richardson–Lucy deconvolution [17]. With our method, preinstalling relative locations is no longer a necessity, we can perform parallel detecting easily on different detector array settings, including those made with optical fiber bundle. Our method's accuracy and universality are proved by theoretical analyze as well as simulated and experimental samples.

2. Theory

In our novel method, images obtained from a randomly distributed detector array are needed and by performing the calculation for correlation of images obtained from different detectors, relative locations of detectors can be acquired. In this section, we discuss the imaging nature of confocal microscope to determine the theoretical feasibility of identifying the relative location in detector array of each detector.

During the imaging process of confocal microscopy, the light generated in the laser diode, called excitation light, is focused on the object, simulating fluorescence emission. The fluorescence light, called emission light, is then focused on the focal plane of system, where lays the pinhole or, in this case, the detector array. The overall PSF of the system can be written as follow [6]:

$$PSF_{confocal} = PSF_{excitation} \times \left(PSF_{emission} \otimes pinhole\right). \tag{1}$$

Ignoring the slightly difference in the wavelength between excitation and emission light, in conventional confocal microscopy, the $PSF_{excitation}$ and $PSF_{emission}$ are the same for all detectors:

$$PSF_{emission} = PSF_{excitation} = \left(\frac{J\left(\frac{1.2\pi \times NA \times r}{\lambda}\right)}{\frac{\pi \times NA \times r}{\lambda}} \right)^2. \tag{2}$$

According to the formula of the pinhole:

$$pinhole = \begin{cases} 1 & \left(\sqrt{(x-a)^2 + (y-b)^2} \le r_0\right) \\ 0 & \left(\sqrt{(x-a)^2 + (y-b)^2} > r_0\right) \end{cases}. \tag{3}$$

And the formula of 2-dimensional convolution:

$$convolution = \sum_x \sum_y I_1(x,y) \times I_2(p-x, q-y). \tag{4}$$

In the equation, I_1 and I_2 represent the intensity of pixels in two images being calculated, x and y represent the position of pixel in the image and integer p and q represent the deviation of two images.

The influence of different detectors on the convolution result of pinhole and $PSF_{emission}$ are the shift in location which is proportional to the relative position of two detectors. After convolution, the confocal PSF is the product of convolution result and the $PSF_{emission}$, which can also be considered as the product of two PSFs with different deviation.

The correlation formula is as follows:

$$correlation = \sum_x \sum_y I_1(x,y) \times I_2(x-p, y-q). \tag{5}$$

In the equation, I_1 and I_2 represent the intensity of pixels in two images being calculated, x and y represent the position of pixel in image and integer p and q represent the deviation of two images.

For detector array with size of 1.2 AU, the correlation coefficient of PSF in different detectors with PSF of center detector is still relatively large. Compared with center detector, correlation between detectors is bigger than 0.9. This result showed the adaptability of our novel method in acquiring relative location in detector array with different size.

The image formed on detectors after scan can be expressed as the following formula:

$$Image(x,y) = PSF_{confocal} \otimes Object(x,y). \tag{6}$$

From the formula, it is obvious that if $PSF_{confocal}$ of system are the same or alike, image formed by the system are also the same or alike. Accordingly, images formed by different detectors are highly alike because of the strong correlation of the PSFs in different detectors. Information from the same area of the object are the same in different images. Due to the shift of PSF is proportional to the deviation of the detector, scanning the image from one detector on another detector's image can find out the relative position of the detectors. Define α as the matrix of correlation in two images:

$$\alpha(x,y) = correlation(image1(a,b), image2(x+a, y+b)). \tag{7}$$

In the equation, image1 and image2 represent images captured by detectors, integer x and y represent the deviation of two images and integer a and b represent the position of image extracted in the detector's image. The coordinate of the maximum α represents the

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