Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2014, 6(3):537-542

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

Relationship between point spread function layers and biological microscopic image restoration based on the same space size

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ABSTRACT

In the three-dimensional biological microscopic image restoration processing, the selection of three dimensional point spread function (3D-PSF) space will determine the restoration effects and restoration time. Based on the theoretical analysis about the effect of 3D-PSF sampling interval and layers on biological image restoration effects and restoration time, through the simulation experiments, the relationship among 3D-PSF layers and biological image restoration effects and restoration time under the same 3D-PSF space size can be obtained. Experimental results showed that, for 3D-PSF with the same space size, the image restoration effect is improved with the increase of 3D-PSF layers, the degree of improvement is gradually slowed down with the increase of layers. Restoration time also increases with the increase of layers, and it shows an approximately linear increasing relationship. Based on the experimental results, Matlab fitting toolbox was used to construct the relationship model among 3D-PSF layers and normalized restoration time and Improved Signal-to-Noise Ratio (ISNR). The establishment of these models provided the theoretical basis and selection methods for 3D-PSF layer selection in the practical application of cell slice acquisition and three-dimensional biological microscopic image restoration.

Key words: Biological Image Restoration; Point Spread Function (PSF); Improved Signal-to-Noise Ratio (ISNR); Restoration Time; Model

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INTRODUCTION

Three-dimensional biological microscopic image restoration studies of digital confocal microscopy involve the establishment of point spread function model [1] and the selection of its parameters. Professors of Washington University and Oklahoma State University proposed the 3D-PSF (Three Dimensional Point Spread Function) model with the changes based on the depth [2]. Professors of Sichuan University presented the Gaussian 3D-PSF approximation model, and studied the depth-based 3D-PSF and its corresponding restoration [3], [4], [5]. Professors of Beijing University of Aeronautics and Astronautics studied the theoretical estimates of 3D-PSF and verified its validity [6]. Exploration experimental studied on 3D-PSF with different space size as well as the image restoration effects and restoration time, and the study showed that, the selection of 3D-PSF space size is closely related to the image restoration effects and restoration time [7]. Based on constant 3D-PSF space size, this article studied the relationship among 3D-PSF layers and image restoration effects and restoration time, and constructed the corresponding 3D-PSF mathematical model.

STRUCTURE OF 3D-PSF

3D-PSF, as the mathematical tools to describe the optical system, can be obtained from inverse Fourier transform of defocused optical transfer function OTF. Stokseth derived an approximate form of small defocus OTF of circular aperture optical systems:

$$H(w,q) = \frac{1}{\pi}(2\beta - \sin 2\beta) \times jinc[4kw(1 - \frac{|q|}{f_c})]\frac{q}{f_c} \tag{1}$$

Wherein, *w* is the defocus amount, *q* is the frequency, and f_c is the cutoff frequency of the system. 3D-PSF is the double funnel model as shown in Figure 1.

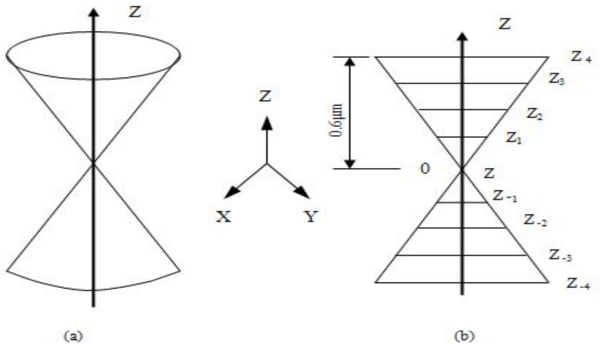

Fig.1 3D-PSF

3D-PSF can be viewed as a three-dimensional matrix in discrete spatial. Its different cross-sections (x-y) along the central axis (optical axis or z-axis) of double funnel are corresponding to radial 2D-PSF (Two Dimensional Point Spread Function) with a series of different defocus amount, wherein the middle section at z=0 is the focal plane 2D-PSF. The space size of 3D-PSF includes the radial size (x-y) and axial size z.

During the three-dimensional biological microscopic image restoration, theoretically, the larger for the selection of 3D-PSF space size, the more close to 2 times of sample radial and axial dimensions [8] and the better the restoration effects, but the longer the restoration time. Since most energy of 3D-PSF is concentrated in the area close to the cone top of double cone in the center, thus when studying the image restoration, only a small part of space area in the center of 3D-PSF can be selected, while ignoring most part of energy scarce areas around 3D-PSF.

For the selected small part of space area in the center of 3D-PSF, the axial (z direction) discrete sampling was conducted to obtain a series of radial 2D-PSF, as shown in Figure 1 (b). It can be predicted that, the higher the sampling frequency, the smaller the axial spacing Δz between 2D-PSF, and more 2D-PSF are needed to compose the required layers of 3D-PSF with the same space size, but it can more accurately reflect the energy distribution of 3D-PSF, thus when processing the three-dimensional cell slice image with corresponding interval through 3D-PSF being composed hereby, the restoration effect is better, and the restoration time is longer. Based on the above analysis, this article selected a small part of area in the center of 3D-PSF as the fixed space area, and in this area with the same space size, the sampling and restoration tests with the different axial spacing were conducted for 3D-PSF.

EXPERIMENT SECTION

Simulation images and 3D-PSF production

1. Production of Three-dimensional Simulation Sample Images

With the clear image (151×151, 256 gray level) shown in Figure 2 (a) as the initial 2D sample, 401 interconnected two-dimensional images were produced through the method of rotating, zoom in and zoom out, and then these 401 images were used to construct three-dimensional simulation image *f*, with he size of (151×151×401).

2. 3D-PSF Production

The parameters of microscope objective lens: the magnification is 40 times; the numerical aperture is 0.65; the mechanical tube length of objective lens is 160mm; the light source wavelength is 550nm; and CCD parameter is 1/4.

1) Production of Optical System 3D-PSF for Microscope Objective Lens

According to Formula (1), the optical system 3D-PSF *h* (71×71×71) was produced, with the diameter of 71 (71×71), the axial sampling interval (hereinafter referred to as "layer distance") of 0.05um, and 71 layers.

2) Production of Six 3D-PSF Used for the Restoration Processing

The space size of 3D-PSF in the Z axis used for restoration was set at 1.2μm, with z=0 as the center, with 0.6μm on each side, and all diameters were 11 (11×11). The layer distance of six 3D-PSF with different layer distances were respectively: $\Delta z_3 = 0.6\mu m$, $\Delta z_5 = 0.3\mu m$, $\Delta z_7 = 0.2\mu m$, $\Delta z_9 = 0.15\mu m$, $\Delta z_{13} = 0.1\mu m$ and $\Delta z_{25} = 0.05\mu m$, the corresponding number of layers was respectively 3 layers, 5 layers, 7 layers, 9 layers, 13 layers and 25 layers, which were named as h_3, h_5, h_7, h_9, h_{13} and h_{25}.

3. Production of Microscope Simulation Slice Image Used for Restoration

1) The convolution of h and f were used to obtain three-dimensional simulation slice image g (the size is 151×151×401, with the layer distance of0.05um), and Figure 2 (b) is an intermediate image g (:,:, 201) of g.

2) The corresponding layer distances of $\Delta z_3 = 0.6\mu m$, $\Delta z_5 = 0.3\mu m$, $\Delta z_7 = 0.2\mu m$, $\Delta z_9 = 0.15\mu m$, $\Delta z_{13} = 0.1\mu m$ and $\Delta z_{25} = 0.05\mu m$ for h_3, h_5, h_7, h_9, h_{13} and h_{25} were used for equally spaced axial sampling for g, and with z=0 as the center, taking 16 layers on each side, then 6 simulation slice images with the same layers (all 33 layers) but different layer distances can be obtained, respectively g_3, g_5, g_7, g_9, g_{13} and g_{25}, and the sizes were all 151×151×33.

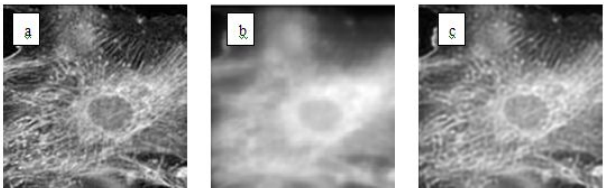

Fig.2.(a) clear image (b) blurred image (c)restoration image

Simulation experiment and result analysis

1) Biological Microscopic Image Restoration

3D-PSF h_3, h_5, h_7, h_9, h_{13} and h_{25} with different layers were used for deconvolution restoration for the simulation slice image g_3, g_5, g_7, g_9, g_{13} and g_{25} with corresponding layer distances respectively, the restoration algorithm used the maximum likelihood method, the number of iterations was set as 600 times, then the restoration time was recorded, and the restoration time by h_3 was used as the benchmark for normalization. Restoration result image was expressed as $\hat{f}_3$, $\hat{f}_5$, $\hat{f}_7$, $\hat{f}_9$, $\hat{f}_{13}$ and $\hat{f}_{25}$, and the sizes were all 151×151×33, wherein an intermediate image of $\hat{f}_9$ was $\hat{f}_9$ (:,:, 17), as shown in Figure 2(c).

2) *ISNR* and Normalized Restoration Time of Different Point Spread Function Restoration Image

The improved signal-to-noise ratio (*ISNR*) was calculated for an image in the middle of six three-dimensional restoration result images of $\hat{f}_3$, $\hat{f}_5$, $\hat{f}_7$, $\hat{f}_9$, $\hat{f}_{13}$ and $\hat{f}_{25}$, and the calculation was based on Formula (2).

$$ISNR = 10\log(|f\text{-}g|^2 / |\hat{f}\text{-}f|^2) = PSNR_{\hat{f}} - PSNR_g \tag{2}$$

Wherein, f, g, and $\hat{f}$ are respectively the intermediate image (151×151) of three-dimensional clear image, three-dimensional blurred slice image and three-dimensional restoration image. The higher *ISNR* value indicates the better restoration effect of images.

Table 1 shows the experimental data of *ISNR* and normalization restoration time t when using the 3D-PSF with the same space size but different layers for image restoration under the iteration number of 600 times.

Table 1. Relationship among 3D-PSF Layer and ISNR and t

3D-PSF	h_3	h_5	h_7	h_9	h_{13}	h_{25}
PSF Layer	3	5	7	9	13	25
ISNR	1.9004	1.9355	1.9503	1.9583	1.9662	1.9839
ISNR Normalization Improvement Rate	1	1.85%	2.63%	3.05%	3.465%	4.39%
t	1	1.62	2.29	3.22	5.02	10.23

Figure 3 is the relationship curve between *ISNR* and restoration time *t* respectively with 3D-PSF layer *m*

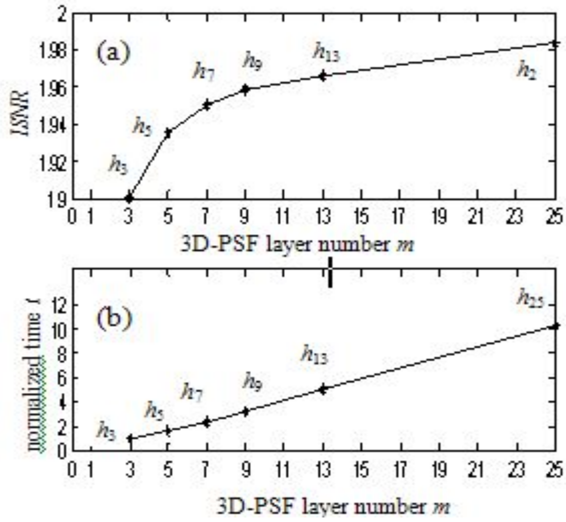

Fig. 3. Relationship between *ISNR* and *t* with PSF

The data in Table 1 was analyzed: when using h_3 to restore the image, the *ISNR* of restoration image is 1.9004, and with h_3 as the comparison benchmark, *ISNR* improvement rate normalization is 1. When using h_5 to restore the image, the *ISNR* improvement rate of restoration image is 1.85%, i.e. *ISNR* is increased by 1.85% than using h_3, and the restoration time *t* is 1.62 times of h_3; when using h_7 for restoration, *ISNR* is increased by 2.63% than using h_3, and the restoration time *t* is 2.29 times of h_3; when using h_{25} for restoration, *ISNR* is increased by 4.39% than using h_3, and the restoration time *t* is 10.23 times of h_3.

From the above experimental data, and in conjunction with Figure 3, it can be seen that, when 3D-PSF has more layers, *ISNR* is the greater, the image restoration effect is the better, and the time being used is the longer. In case of less layers, for example 5 or 7 layers, *ISNR* increases more rapidly, i.e. the increasing of restoration effect improvement degree is more rapidly. With the increase of 3D-PSF layers, *ISNR* increasing degree is reduced, and when 13 layers and 25 layers, *ISNR* tends to be flat, and the increasing degree has been reduced to very low. And the time used for restoration shows the approximate linear increase with the increase of 3D-PSF layers. When 3D-PSF has 25 layers, the restoration time *t* is 10.23 times of 3 layers.

Therefore, how to select the number of layers for 3D-PSF is an important issue for the restoration effect and time involved in the image restoration applications.

CONSTRUCT THE MATHEMATICAL MODEL

In various applications of three-dimensional microscopic image restoration processing for biological cells, there are the requirements of various conditions and different degrees, such as the quick view and accurate analysis, which are corresponding to different restoration effects and restoration processing time. Accordingly, in the practical operation of slice acquisition and image restoration for the cells, the selection of axial sampling interval is required for 3D-PSF, so as to obtain the corresponding 3D-PSF layers.

According to the above simulation experiments, Matlab fitting toolbox was used to build three relationship models among 3D-PSF layer *m* and normalized restoration time *t* and *ISNR* under the corresponding set parameter conditions.

Formula (3) is the mathematical model relationship between *ISNR* and 3D-PSF layer *m*:

$$ISNR = 1.948*\exp(0.0007259*m) - 0.2203*\exp(-0.4809*m) \tag{3}$$

Formula (4) is the mathematical model relationship between normalized restoration time *t* and 3D-PSF layer *m*:

$$t = 6.191 - 5.862*\cos(0.07995*m) + 2.106*\sin(0.07995*m) \tag{4}$$

Formula (5) is the mathematical model relationship between *ISNR* and restoration time *t*, and Figure 6 is the relationship curve between *ISNR* and *t*.

$$ISNR = 1.95*\exp(0.001652*t) - 0.2573*\exp(-1.589*t) \quad (5)$$

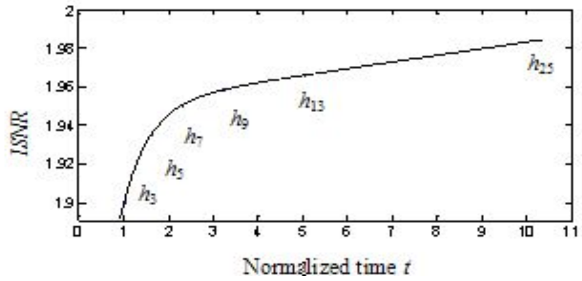

Fig. 4. Relationship Curve between *ISNR* and Restoration Time *t*

Through these three relationship expressions, the relationship data among *ISNR* and normalized restoration time and 3D-PSF layers can be calculated, as shown in Table 2.

Table 2. Relationship among *ISNR*, Normalized Restoration Time and 3D-PSF Layers Calculated through Fitted Model

3D-PSF	h_3	h_5	h_7	h_9	h_{11}	h_{13}	h_{17}	h_{19}	h_{21}	h_{23}	h_{25}
ISNR	1.9004	1.9353	1.9513	1.9586	1.9628	1.9662	1.9727	1.9759	1.9789	1.9817	1.9843
ISNR Improvement Rate	1	1.84%	0.83%	0.37%	0.21%	0.17%	0.16%	0.16%	0.15%	0.14%	0.13%
ISNR Normalization Improvement Rate	1	1.84%	2.68%	3.06%	3.28%	3.46%	3.80%	3.97%	4.13%	4.28%	4.41%
t	1	1.61	2.34	3.17	4.08	5.04	7.02	7.99	8.92	9.77	10.54

Through Table 2 above, according to the requirements of different degree about quick view and accurate analysis and other conditions, *ISNR* and normalized restoration time can be appropriately selected, and then after selecting the 3D-PSF layers, 3D-PSF axial sampling interval can be calculated.

The establishment of mathematical relationship model provides the possibility for quantitative analysis of the relationship among *ISNR*, normalized restoration time and 3D-PSF layers from a mathematical point of view, which can reduce the artificial randomness during 3D-PSF parameter selection, thereby providing the theoretical basis for 3D-PSF parameter selection. Meanwhile, it also provides the selection methods of 3D-PSF axial sampling interval and layer number for cell slice acquisition and biological microscopic image restoration processing under different parameter conditions.

CONCLUSION

Based on the theoretical analysis about the effect of 3D-PSF sampling interval and layers on image restoration effects and restoration time, and through the simulation experiments, the relationship among 3D-PSF layers and image restoration effects and restoration time under the same 3D-PSF space size can be obtained. Experimental results showed that, for 3D-PSF with the same space size, the image restoration effect is improved with the increase of 3D-PSF layers, the degree of improvement is gradually slowed down with the increase of layers. Restoration time also increases with the increase of layers, and it shows an approximately linear increasing relationship. The establishment of these models provided the theoretical basis and selection methods for 3D-PSF layer selection in the practical application of cell slice acquisition and three-dimensional biological microscopic image restoration.

Acknowledgements
This research was supported by The National Natural Science Foundation of China（No. 61164019）and Guangxi Natural Science Foundation (No.2012GXNSFAA053216).

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