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Research Article

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Studies on three dimensional point spread function energy distribution of biological microscopic imaging system

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ABSTRACT

This paper conducted the theoretical analysis on 3D-PSF constitution, 3D-PSF energy distribution of different defocus amount and radial amount of focal-plane of digital biological confocal microscopic imaging system at first. In the study of 3D-PSF energy distribution of biological microscopic imaging system, it proposed the concept of energy ratio, conducted simulation experiments and calculated the energy ratio values of three groups with different magnifications, finally established its energy ratio spatial distribution model. Results showed that, 3D-PSF axial and radial energy rapidly decays with the axial and radial increasing, and the greater the magnification, the faster the energy attenuation, the more concentrated the energy.

Key words: Biological Microscopic Imaging System; 3D-PSF; Space Size; Energy Ratio; Energy Distribution

INTRODUCTION

In the Digital Confocal Microscopy Technology, the three-dimensional biological specimen are moved along the optical axis to obtain two-dimensional slice images of the sequence through the objective lens focal plane of bio-optical microscope, which are then processed through three-dimensional microscopic image restoration algorithm to obtain the clear two-dimensional image sequence and three-dimensional images [1]. The space size of 3D-PSF (Three Dimensional Point Spread Function) used for biological image restoration processing determines the restoration effect and restoration time [2]. Since most energy of 3D-PSF is concentrated in the area close to the cone top of double cone in the center, how much the space area in the center of 3D-PSF should be selected, and what kind of relationship exists between the restoration effect and restoration time of images are still the problems to be studied and solved.

This article studied the 3D-PSF energy distribution in biological optical microscopic imaging system, identified its characteristics, and established its energy spatial distribution models, thereby laying the foundation for the studies of relationship among 3D-PSF energy ratio and image restoration effect and restoration time as well as for the establishment of 3D-PSF optimization selection model.

BIOLOGICAL MICROSCOPIC IMAGING SYSTEM 3D-PSF

Proposal of 3D-PSF Energy Ratio Concept

3D-PSF space area of biological optical microscope imaging system is the image space area of entire imaging. The 3D-PSF space size involved in three-dimensional biological microscopic image restoration processing is 2 times of the size of biological samples [3], which is a very large area, while most energy of 3D-PSF is concentrated in the area close to the cone top of double cone in the center. In this paper, 3D-PSF energy ratio (ER) is defined as the ratio of the energy in cone top space area of double cone in the center of 3D-PSF accounting for the energy of entire area.

Construction of 3D-PSF

In the optical slice analysis, it is required to firstly consider 2D-PSF with different defocus amount. Circular

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defocusing optical transfer function is expressed as follows [4]:

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

Wherein, w is the defocus error, w= $\Delta z \cdot NA^2 / 2$, Δz is the defocus amount; q is the frequency, f_c is the cut-off frequency of the system, $q^2 = u^2 + v^2$, $f_c = \frac{2NA}{\lambda}$, $\beta = \cos^{-1}\frac{q}{f_c}$, $k = \frac{2\pi}{\lambda}$, $\operatorname{jinc}(x) = 2\frac{J_1(x)}{x}$.

cut-off frequency of the system, q = u + v, $z \in \lambda$, f_c , $x \in \lambda$, $x \in X$. Through calculating the optical transfer function with different defocus amount, a series of corresponding defocus 2D-PSFs can be obtained, thereby constituting the biconical 3D-PSF [4, 5].

Theoretical Analysis of 3D-PSF Energy Distribution

Under the narrowband incoherent illumination of central wavelength λ , the focal plane 2D-PSF of circular aperture lens with the diameter a can be expressed as [4]:

$$h(r) = \left[2 \frac{J_1(\pi[\frac{r}{r_0}])}{\pi[\frac{r}{r_0}]} \right]^2$$
(2)

Wherein, the constant r_0 is a scale factor with the dimension, $r_0 = \frac{\lambda d_i}{a}$, r is the radial distance from the optical axis of image plane, $r = \sqrt{x_i^2 + y_i^2}$.

The relationship between the energy E of a function f(r) in the infinite space with this function f(r) is an integral [6]:

$$E = \int_{0}^{1} |f(r)|^{2} dr$$
 (3)

The energy Er of this function f(r) in the limited discrete space can be expressed as:

$$E_{r} = \sum_{0}^{r} |f(r)|^{2} \Delta r$$
(4)

Through the calculation of Formula (1) and (4), the energy ratio of ERN for 2D-PSF with different defocus amounts can be obtained. Through the calculation of Formula (2) and (4), the energy ratio ERr of focal plane 2D-PSF at different radial distances can be obtained, as shown in Fig.1 (a) and (b) below.



Fig. 1 Impact of Defocus Amount and Radial Distance on the PSF Energy

Fig.1 shows the energy gradually decays with the increase of defocus amount and radial distance.

SIMULATION EXPERIMENT AND ANALYSIS

3D-PSF Spatial Energy Distribution

1) Simulation Production of Optical System 3D-PSF

Three groups of microscope objective lens parameters with different magnification are set: (1) the magnification M=40x, the numerical aperture NA=0.6; (2) the magnification M=20x, the numerical aperture NA=0.45; (3) the magnification M=10x, the numerical aperture NA=0.25.

The mechanical tube length of microscope is 160mm; the light source wavelength is 550nm; CCD parameters: 1/4 inch, the pixel value 640×480 .

3D-PSF space size is set as $r \times r \times N$, r is 3D-PSF diameter, and N is the number of layers. 3D-PSF layer distance L is taken as $0.2 \mu m$.

Three 3D-PSF with the magnifications of 40x, 20x and 10x respectively and with the same space size of $101 \times 101 \times 101$ are produced according to Formula (1). The diameter r is 101, the number of layers N is 101 layers, and the layer distance is taken as $0.2\mu m$, which are all expressed as h $101 \times 101 \times 101$.

2) Simulation Production of 3D-PSF with Different Space Size

In each h101*101*101, the diameter r is taken from the center as 3, 5, 7, ..., 101, the number of layers N is 3, 5, 7, ..., 101, and then it obtains three groups each containing 2500 3D-PSF and where the space size is $3\times3\times3$, $3\times3\times5$, ..., $3\times3\times101$, $5\times5\times3$, $5\times5\times5$, ..., $5\times5\times101$, ..., $101\times101\times101$.

3) 3D-PSF Space Size and Energy Ratio

By the use of 3D-PSF spatial point energy value accumulating method, each of the three groups containing 2500 3D-PSFs' energy is calculated respectively, and conducted with its respective h101*101*101 energy as 1, obtaining the energy ratio ER of these three groups 3D-PSF with different space size. Table 1 shows the ratio of energy of three groups of some 3D-PSF with different space size, and Fig.2 is the curve between energy ratio and space size.

Table 1 Relationship between Energy Ratio and Space Size of Some 3D-PSF under Three Kinds of Magnification

Magnification	40x	20x	10x
h(3×3×3)	0.1321	0.0851	0.0164
h ₂ (5×5×5)	0.3370	0.2130	0.0539
h ₉ (7×7×7)	0.4949	0.3148	0.1041
h(9×9×9)	0.5974	0.4074	0.1540
h (11×11×11)	0.6767	0.4902	0.1970
h(13×13×13)	0.7411	0.5629	0.2364
h(15×15×15)	0.7889	0.6245	0.2737
h ₈ (17×17×17)	0.8211	0.6757	0.3089
h(19×19×19)	0.8447	0.7179	0.3430
htt(21×21×21)	0.8662	0.7529	0.3761
hu(101×101×101)	1	1	1



Fig. 2 Diagram between Energy Ratio and Space Size of Some 3D-PSF under Three Kinds of Magnification

Table 1 and Fig. 2 show the situation that 3D-PSF energy ratio changes with the increase of space size.

It can be seen from Fig. 2 that, in the stage where the 3D-PSF layers (N axis) and the diameter (r axis) are relatively

small (areas close to the origin of coordinates), its energy ratio shows the relatively rapid-growing trend with the increase of space size, and the greater the magnification, the faster the energy ratio increase, such as 40x. When its space size increases to a certain extent, the degree of this growth is slowed down. This indicates that, for 3D-PSF with the greater magnification, its energy is more concentrated in the central area; while for 3D-PSF with the smaller magnification, its energy is more spread over a wide space area.

Axial Energy and Radial Energy

1) Energy Attenuation in Optical Axis and Central Layer of 3D-PSF

For the above three $101 \times 101 \times 101$ 3D-PSF, we respectively calculated the energy ratio value in its optical axis with the axial size N and the energy ratio value of focal plane 2D-PSF in central layer (N is 0) with the radial size r, Fig. 3 and Fig. 4 are respectively the corresponding curve diagram between axial N and radial r with energy values.



Fig. 3 3D-PSF Axial Energy Attenuation Diagram of Optical Axis (a) M=40 (b) M=20 (c) M=10



Fig. 4 3D-PSF Radial Energy Attenuation Diagram of Central Layer (a) M=40 (b) M=20 (c) M=10

As seen from Fig. 3, and Fig. 4, 1) The axial energy along the optical axis and the radial energy in the central layer of 3D-PSF decay rapidly with the axial and radial increase. 2) The greater the magnification, the larger the energy in 3D-PSF center point and nearby areas, and the energy at 40x center point is nearly 10 times of the energy at 10x center point. This also proves that, for 3D-PSF with the greater magnification, its energy is more concentrated in the central areas.

2) Energy Distribution along the Optical Axis Cross-section and Center Layer of 3D-PSF

The axial cross-section is taken along the optical axis for three 3D-PSF h101*101*101 respectively, and the energy ratio values for the points in cross section are calculated. Energy distribution in the cross section is shown in Fig. 5 (in order to show the energy distribution, the logarithmic conversion processing is conducted for energy values in Fig. 5).



From Fig. 5, it can be seen that, the energy of 3D-PSF spreads from the center to the right and left sides, the shape is similar to the shape of double cone, and the brightest place in the center is the top of double cone. Comparing Fig. 5(a), Fig. 5(b) and Fig. 5(c), it can be seen that, with the reduction of magnification, the cone angle gradually becomes smaller, and under the same defocus amount, the radial spread degree of energy is reduced; and the bright area in the central area of 3D-PSF spreads towards the left and right sides in the axial direction, the energy of 3D-PSF also spreads along both sides of optical axis.

The focal plane 2D-PSF is taken respectively for three 3D-PSF h101*101*101, and the energy value of each point is calculated. The energy distribution of focal plane 2D-PSF is shown in Fig. 6 (in order to show the energy distribution, the logarithmic conversion processing is conducted for energy values in Fig.6).



As seen from Fig. 6, the energy of focal plane 2D-PSF shows the annular wave spreading from the center to the surrounding. Comparing Fig. 6(a), Fig. 6(b) and Fig. 6(c), it can be seen that the central bright spot of 40x focal plane 2D-PSF is the smallest, and the central bright spot of 10x focal plane 2D-PSF is the largest, indicating that, with the decrease of magnification, the energy spread degree of focal plane 2D-PSF from the center to the surrounding increases.

3D-PSF ENERGY RATIO SPATIAL DISTRIBUTION MODEL

In order to clarify the change relationships between 3D-PSF energy and space size under the conditions of fixed parameter variables, this article conducted the fitting on the spatial distribution of energy ratios of three groups containing 2500 3D-PSFs, three 3D-PSF energy distribution models were obtained, which are respectively Formula (5), (6) and (7).

$E_{40} = 0.333 + 0.01027r + 0.02177N - 0.00019r^{2}$	
$+3.7 \times 10^{-5} rN - 0.00035 N^2 + 1.045 \times 10^{-6} r^3$	(5)
$-2.5 \times 10^{-7} r^2 N - 1.8 \times 10^{-9} r N^2 + 1.7 \times 10^{-6} N^3$	
$E_{20} = 0.0727 + 0.0083r + 0.034N - 0.00016r^2$	
$+2.94 \times 10^{-5} rN - 0.00052N^2 + 9.47 \times 10^{-7} r^3$	(6)
$-2.63 \times 10^{-7} r^2 N + 6.8 \times 10^{-8} r N^2 + 2.5 \times 10^{-6} N^3$	
$E_{10} = -0.061 + 0.0027r + 0.022N - 4.65 \times 10^{-5}r^{2}$	
+ $1.79 \times 10^{-5} rN - 0.00018N^2 + 2.5 \times 10^{-7} r^3$	(7)
$-7.398 \times 10^{-8} r^2 N - 4.16 \times 10^{-8} r N^2 + 5.3 \times 10^{-7} N^3$	

The fittings were conducted by using the least squares polynomials, the highest power of independent variable (r, n) was taken as 3, 6-order was the polynomial form, and the fitting confidence took the default value of 0.95. The fitting square error (SSE) of Formula (5) was 0.3798, the root mean square error (RMSE) was 0.01235; the fitting square error (SSE) of Formula (6) was 0.2942, the root mean square error (RMSE) was 0.01087; the fitting square

error (SSE) of Formula (7) was 0.2711, and the root mean square error (RMSE) was 0.01043.

6-order polynomial fitting surface plot of 3D-PSF with 40x magnification is shown in Fig. 7.



Fig. 7 6-order Polynomial Fitting Surface Plot of 3D-PSF with 40x Magnification

In Fig. 7, the lattice is the actual energy ratio distribution, and the surface is the fitting surface. Through the 3D-PSF energy ratio spatial distribution model, each 3D-PSF energy ratio of different space size can be obtained, thereby laying the foundation for the studies of 3D-PSF energy ratio and image restoration effect and restoration time as well as for the optimization selection of 3D-PSF.

CONCLUSION

In the study of 3D-PSF energy distribution of biological microscopic imaging system, this paper proposed the concept of energy ratio. The simulation experiments were conducted for 3D-PSF of three optical biological imaging system with 40x, 20x and 10x magnification, the energy ratio values of three groups each containing 2500 3D-PSF with different magnifications and different space size were calculated, the energy distribution status and its characteristics were identified, and its energy ratio spatial distribution model was established. Simulation experiment results showed that, the axial and radial energy of 3D-PSF rapidly decays with the axial and radial increasing, the greater the magnification, the faster the energy attenuation. Moreover, for 3D-PSF with the greater magnification, its energy is more concentrated in the central area; while for 3D-PSF with the smaller magnification, its energy is more spread over a wide space area.

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