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### [Biomedical Engineering]

## Sectioned imaging of DMD-based structured illumination fluorescence microscopy<sup>\*</sup>

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Abstract: In our novel structured illumination microscope (SIM), a programmable digital micromirror device (DMD) was used instead of piezo activated grating to produce stable and reliable structured illumination. An optical low-pass spatial filter was applied to block the diffraction order higher than  $\pm 1$  st order. The illumination pattern was not a sinusoidal grid with a single spatial frequency, so the algorithm used in the traditional SIM is not proper here. An improved algorithm based on the five raw images with different phase angles was introduced and discussed in detail. The results have demonstrated that the sectioned image can be reconstructed from five raw images with different phase angles, with resolving some fine lateral structure that was blurred in traditional microscopic image. Key words: structured illumination microscopy; digital micromirror device; sectioned imaging; resolution CLC number: TN 247 Document code: A

Structured illumination (SI) was proposed and utilized in biological imaging by Neil et al. in 1997<sup>[1-2]</sup>. Originally, it was used in improving the sectioning ability of traditional microscopes. Because of its comparable sectioning ability with confocal microscopy<sup>[3]</sup>, the method is attractive for biologists<sup>[4-5]</sup>. Actually, compared with confocal microscope, SIM has many advantages. Firstly, SIM is a wide-field imaging method, so the imaging speed is potentially faster than the point scanning method. Secondly, SIM can be realized with a conventional microscope after some convenient alternation, so it is a more economical method, and practically, the commercial device for upgrading traditional microscope to SIM is also available, such as OptiGrid, supplied by Olympus.

grating in illumination path, and the grating pattern was projected on the focal plane of the objective and formed the so-called SI. The movement of the grating was controlled by a piezo-activator, which made the phase of grating shift  $2\pi/3$  each time. Three images of the sample were collected when the phases of the grating were 0,  $2\pi/3$  and  $4\pi/3$ , respectively. Based on these raw images, the sectioned image was reconstructed by a simple algorithm<sup>[1]</sup>, and with another algorithm, the lateral resolution in the direction perpendicular with the strip of the grating could even be double improved<sup>[6]</sup>.

In such grating-based SIM, the grating has to be movable during the experiment, and such mechanical movement in the setup would probably affect the stability of the microscope. Thus, some programmable devices, for example, DMD (digital

SI was originally formed by utilizing a sinusoidal patterned

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micromirror device) was used to replace the grating and obtain better stability<sup>[7]</sup>.

In this paper, an optical low-pass spatial filter was used to block the diffraction order higher than  $\pm 1$ st because of the pixel structure of DMD and the algorithm used under this condition was discussed in detail. The results obtained have demonstrated that the sectioned image can be reconstructed from five raw images with different phase angles.

## 1 Materials and methods

#### 1.1 Appratus

The setup for DMD-based SIM is shown in Fig 1. Light from an Argon ion laser (L) is expanded and collimated through lenses pair ( $L_1$  and  $L_2$ ), and then projected on DMD (Shanghai Lige electronics and science company, China). The pattern displayed on DMD is imaged on the focal plane through lens ( $L_3$ ) and the objective(Obj,  $10 \times$ , Olympus, Japan), after spatially filtered by a low pass spatial filter (SF) which block the diffraction orders except zeroth and  $\pm 1$ st orders. The fluorescence of the sample is collected by the objective (Obj) and lens ( $L_4$ ) and then imaged on CCD (Coolsnap EZ, Photometrics, USA). In order to keep the structured illumination and fluorescence collection synchronized, a delay device is used in the setup. The sample (As321c, Medical & Science Media, Australia) used in this paper is a slice of the rhizome of Convallaria (lily of valley) with concentric vascular bundles, stained by fluorescent dyes.





L: argon ion laser, with the excitation wavelength 488 nm (氫离子激光器,激发波长 488 nm);  $L_1 + L_2$ : collimator (准 直器); DMD: digital micromirror device (数字微镜装置); SF: spatial filter (空间滤波器);  $L_3 \ L_4$ : relay lens (中继透 镜); Obj: objective (物镜);  $F_1 + DM + F_2$ : filter set (荧光 滤光片组); CCD: digital camera (数字照相机); delay: digital delay generator (数字延时器).

#### 1.2 SI illumination

The pattern displayed on DMD is controlled by the computer. The original pattern on DMD and its enlarged part are shown in Fig 2. It is a one dimensional sinusoidal grating. During the experiment, the pattern translated one-fifth of the grating period along the direction perpendicular to the stripe. There are two kinds of status for each micro-mirror of DMD, "on" and "off". The gray level of each pixel is accomplished by its duty ratio, which is defined as ratio between the duration of status "on" and "off". The phase-shift is accomplished by changing the duty ratio of each pixel of DMD. In the SI produced in this paper, one period of the grating pattern on DMD is performed with ten columns of pixels. The phase of the grating shift  $2\pi/5$ means the grating pattern translated one-fifth of the period. After the phase-shift, the duty ratio of the pixel in column i is same as the duty ratio of the pixel in column i + 2 before the phase-shift. In each step of phase-shift, when the pattern is stable, one raw image is acquired, so during one period, 5 raw images are acquired with five illumination patterns which are different in their phases.



## 图 2 DMD 显示的光栅样图案及部分区域的放大图案

#### 1.3 Data processing

Every image acquired consists of two parts, the defocused and the infocused part.

$$I(x,y) = I_{defocus}(x,y) + I_{ijfocus}(x,y)I_{grid}(x,y).$$
(1)

On the other side, the illumination grid pattern on sample is the result of the coherent effect of the three diffraction beam, zeroth and  $\pm 1$ st orders, and can be denoted by  $I_{\text{arid}}(x)$ .

$$I_{\text{grid}}(x) = I_0 \Big[ (2 + \alpha^2) + 2\cos\Big(2\pi \frac{x}{p_x} - 2\phi_x\Big) + 4\alpha \cos\Big(2\pi \frac{x}{2p_x} - \phi_x\Big) \Big], \qquad (2)$$

where  $\alpha$  is the ratio of amplitude between the zeroth order and the +1st or -1st order;  $p_x$  is the lateral period of the grid pattern projected on the object;  $\phi_x$  is the lateral position offset of the grating. The illumination pattern has two contribution terms with different lateral periods,  $p_x$  and  $p_x/2$ , which both give rise to the improvement of the resolution.

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So, for simpleness sake, function (2) can be written as  $I_{grid}(x) = I_{grid1}(x) + I_{grid2}(x)$  (3)

where

$$\begin{split} I_{\text{grid1}}(x) &= I_{m1} \big[ 1 + m_1 \cos(v_0 x - \phi_x) \big] \\ I_{\text{grid2}}(x) &= I_{m2} \big[ 1 + m_2 \cos(2v_0 x - 2\phi_x) \big). \end{split}$$

For a given value of  $\phi_x$ , the  $2v_0$  and  $v_0$  terms can be isolated from those five images with five different values of  $\phi_x$ .

I(x,y) in function (1) can be rewritten as

$$I(x,y) = I_{cons}(x,y) + I_{s1}(x,y)\sin\phi_{x} + I_{c1}(x,y)\cos\phi_{x} + I_{s2}(x,y)\sin2\phi_{x} + I_{c2}(x,y)\cos2\phi_{x}$$
(4)

where

$$I_{cons} = I_0 (2 + \alpha^2),$$
  

$$I_{s1} = 4I_0 \alpha sin \left(2\pi \frac{x}{2p_x}\right),$$
  

$$I_{c1} = 4I_0 \alpha cos \left(2\pi \frac{x}{2p_x}\right),$$
  

$$I_{s2} = 2I_0 sin \left(2\pi \frac{x}{p_x}\right),$$
  

$$I_{c2} = 2I_0 cos \left(2\pi \frac{x}{p_x}\right).$$

So, the infocus information can be recovered from the following two functions that

$$I_{\rm infocus} = \frac{1}{m_1} \sqrt{I_{s1}^2 + I_{c1}^2},$$
 (5)

or 
$$I'_{infocus} = \frac{1}{m_2} \sqrt{I'_{.2} + I'_{.2}}.$$
 (6)

It means that, from the five raw images, with fluorescence intensity  $I_i(i = 1, 2, \dots, 5)$ , we can get two sectioned images simultaneously, but with different sectioned ability. The unknown values,  $I_{s1}$ ,  $I_{c1}$ ,  $I_{s2}$ , and  $I_{c2}$  in function (5) and (6) can be derived by

$$\begin{bmatrix} I_{1} \\ I_{2} \\ I_{3} \\ I_{4} \\ I_{5} \end{bmatrix} = X \begin{bmatrix} I_{cons} \\ I_{s1} \\ I_{c1} \\ I_{s2} \\ I_{c2} \end{bmatrix},$$
(7)

where

$$X = \begin{bmatrix} 1 & \sin\phi_{1} & \cos\phi_{1} & \sin2\phi_{1} & \cos2\phi_{1} \\ 1 & \sin\phi_{2} & \cos\phi_{2} & \sin2\phi_{2} & \cos2\phi_{2} \\ 1 & \sin\phi_{3} & \cos\phi_{3} & \sin2\phi_{3} & \cos2\phi_{3} \\ 1 & \sin\phi_{4} & \cos\phi_{4} & \sin2\phi_{4} & \cos2\phi_{4} \\ 1 & \sin\phi_{5} & \cos\phi_{5} & \sin2\phi_{5} & \cos2\phi_{5} \end{bmatrix}$$
  
So, 
$$\begin{bmatrix} I_{cons} \\ I_{c1} \\ I_{c2} \\ I_{c2} \end{bmatrix} = (X^{T}X)^{-1}X^{T} \begin{bmatrix} I_{1} \\ I_{2} \\ I_{3} \\ I_{4} \\ I_{5} \end{bmatrix}$$

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In this paper, five values of  $\phi_x$  are set as 0,  $2\pi/5$ ,  $4\pi/5$ ,  $6\pi/5$ , and  $8\pi/5$ , so we use function (5) as the reconstruction algorithm.

## 2 Results and discussion

The comparison of the reconstructed sectioned image and the traditional image is performed with their images and the fluorescence intensity distribution along some selected lines. The reconstructed sectioned image and the traditional image are shown in Fig 3 (a).

In order to compare the reconstructed image and the traditional image, two kinds of structures in the sample (shown as dotted lines and dashed lines in Fig 3 (a) and Fig 3 (b)) are chosen to plot the intensity distribution. Along the dotted line, there is only one layer of membrane-like structure, while along the dashed one, there are several finer structures. The intensity distribution plots along the dotted and dashed lines are shown in Fig 3 (c) ~ Fig 3 (f). Fig 3 (c) and Fig 3 (e) are plotted according to the traditional image (Fig 3 (a) and Fig 3 (d) and Fig 3 (f) are plotted according to the reconstructed sectioned image (Fig 3 (b)).

The single membrane-like structure can be resolved by a peak in Fig 3 (c) and Fig 3 (d). FWHM of the peak in the left plot is almost twice of that on the right side, which means that the structure can be resolved with higher resolution in the reconstructed image.

Result of intensity distribution along the dashed line approved it more powerfully (Fig 3 (e) and Fig 3 (f)). The peak corresponding to one fine structure (pointed with an arrow Fig 3 (f)) can be resolved clearly in the reconstructed image, but almost irresolvable in traditional image (pointed with an arrow in Fig 3 (e)).

## 3 Conclusion and outlook

Upon comparing the reconstructed sectioned image with the traditional image (Fig 3), it is clear that the structure of the sample in reconstructed sectioned image can be resolved with higher resolution. Some fine structure that cannot be resolved in traditional image can also be distinguished in the reconstructed sectioned image. The improvement of the resolution should be attributed to the removed blurred signal from the structure defocused in the reconstructed sectioned image.

Replacing the grating with a DMD in SIM has many advantages, but on the other side, the pixel structure of DMD will also bring some unexpected questions. Practically, the pattern displayed on DMD is not the perfect sinusoidal pattern, but the one modulated by the pixel structure of DMD. In order to decrease such effect, an optical low-pass spatial filter was used to block the diffraction order higher than ±1st. The illumination pattern is the result of the coherence effect of the diffraction orders of zeroth and ±1st. Because the illumination pattern is not a simple sinusoidal grid, but the sum of two grids with different periods, so the algorithm introduced in the traditional SI microscopy no longer fits here. Under this condition, sectioned image can also be derived from five raw images with a similar algorithm shown in the paper.

The preliminary results show that DMD-based SIM is of ability to get the sectioned image, however, the characteristics of



20

(e) normalized fluorescence intensity distribution along

the left dashed lines in (a)

fluorescence location in the labeled line

10

30

40

50

0.4

0.2

0

pattern, such as contrast ratio and period, will also affect the sectioning ability. What's more, DMD is able to display pictures at the refresh frequency as high as 200 Hz, which means that if reconstruction processing can be accomplished in quasi-real-time, and the image collecting speed of the detector can be matched with the changing speed of illumination patterns, the sectioned images can be acquired with the frequency as high as 40 Hz. So, such DMD-based SIM is also ideal in dynamic imaging. More work need be done to explore the potential of DMD-based SIM in the future.







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# 基于数字微镜装置的结构光照明层析成像

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摘 要: 在结构光显微系统中使用可编程数字微镜装置代替压电驱动的光栅,产生稳定可靠的结构光 照明.为避免数字微镜装置本身的像素化结构对成像的影响,系统中引入一个低通的空间光调制器,以滤 除经过数字微镜装置后产生的高级(±2nd 及以上)衍射光.此时在样品上形成的结构光不再是单一频率 的余弦光栅图案,传统结构光显微成像中通过光栅3步相移成像获得层析图像的方法不再适用.新算法通 过5步相移得到5幅源图像,也可重构出层析图像.结果表明,传统荧光图像中几乎不能分辨的某些精细 结构,在重构的层析图像中能够清晰地分辨出来.

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