Tilt illumination for structured illumination imaging

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Abstract

To achieve super-resolution imaging, the information in higher frequency of the observed sample is collected by illuminating with a structure beam for a limited optical transfer function. In this paper, tilt illumination mode is introduced to structured illumination microscopy (SIM) for enhancing lateral resolution. More sample spectrum more than traditional SIM, can be obtained by detector. Thus, SIM with tilt illumination can be improved at the aspect of lateral imaging resolution.

Keywords Tilt illumination · Structured illumination · Super-resolution imaging

1 Introduction

Structured illumination microscopy (SIM) is a tool of super-resolution imaging. In 2000, Gustafsson optimized the generation mode of sinusoidal structured light for illumination Gustafsson (2000), which successfully has a double resolution in some imaging systems Shao et al. (2012); Gustafsson et al. (2008); Shao et al. (2011); Chen et al. (2013); Grebenyuk and Ryabukho (2018). SIM has been widely used in optical sectioning, phase imaging, and 3D surface measurement Saxena et al. (2015). As an expanded case, digital grating was introduced into virtual structured detection Lu et al. (2013); Jin et al. (2019); Ni et al. (2017), for modulating measured patterns.

In SIM, a sinusoidal modulated intensity pattern is used by moving spectrum for capturing the high frequency data. A twofold resolution imaging can be achieved by SIM Lee et al. (2018); Huttunen et al. (2017). Here the incident laser beam is parallel to the optical axis Chowdhury and Izatt (2013). The collimated light restricts the range of spatial resolution.
Fourier ptychographic microscopy is also a super-resolution imaging technique by expanding frequency range Dong et al. (2005). Nonlinear SIM Refo et al. (2012); Mou et al. (2020); Cheng et al. (2014) was invented to promote resolution. Coincidentally, the saturated structured illumination microscopy (SSIM) Gustafsson (2005); Cao et al. (2017) was introduced to improve resolution by utilizing the nonlinear effect of fluorescent molecules. SSIM, however, is limited by fluorescent labelling. Some samples in SSIM are easily damaged by high intensity laser Gao et al. (2011); Kner et al. (2009); Wicker et al. (2013); Jones et al. (2011).

In this work, tilt illumination is introduced to SIM (tiSIM) for enhancing resolution. The mathematical model of tilt illumination is an exponential function in object reconstruction. This function is used to shift spectrum. The immeasurable frequency components are moved into the passband of OTF. The proposed method can make the resolution enhancement as a nonlinear SIM. The major contributions of our work consist of two aspects. The first one is that the tilt illumination is introduced to SIM for enhancing lateral resolution. Here more sample frequency spectrum can be received. The second contribution is to increase noise robustness for SIM. The theoretical analysis and simulation results are given to validate the performance of tiSIM.

2 Method

In SIM, the reconstruction of high-resolution image requires multiple measured patterns with different initial phases along carrier frequency directions. The grating centers on the optical axis and rotates specific angles, such as 0°, 45°, 90°, and 135°. The sinusoidal stripes have different orientations due to the grating rotation at different angles. At each rotation angle of the grating, 0, and ±1 orders of diffraction light are used to illuminate the sample simultaneously. Here ±1 order diffraction beam illuminating sample is equivalent to moving the sample spectrum in two opposite directions. To realize super-resolution imaging, it is necessary to decouple the spectra from 0, ±1 orders of diffraction patterns Gao et al. (2011); Kner et al. (2009); Wicker et al. (2013); Jones et al. (2011).

In Fig. 1, a schematic diagram of tiSIM is illustrated. It should be pointed out that Fig. 1 is a scenario diagram rather than a true experimental setup diagram. Two lenses

![Fig. 1](A schematic of experimental system for tiSIM. CM: collimating mirror; L1, L2, L3: lens; RM: rotatable mirror. RM1 and RM2 achieve the tilt illumination. γ is the tilt angle between the incident light and the optical axis marked with chain line. FT and FT⁻¹ represent Fourier transform and its inverse)
(L1 and L2) constitute a 4f system. P1, P2 and P3 are object plane, frequency plane and imaging plane, respectively. The incident light is divided into two parts by the prism. Two beams interfere on the surface of the mirror to form the sinusoidal stripe as the illumination pattern playing the role of the grating. The illumination pattern of the sinusoidal stripe reflected by RM1 has a tilt angle with the optical axis and projected onto the grating with modulated transmittance. In this way, the sample is irradiated by the sinusoidal stripe. The sinusoidal stripe as the illumination pattern can be located on object plane P1. The tilt angle $\gamma$ can be adjusted by rotating RM1. RM2 is applied to ensure that the output beam can propagate vertically to CCD.

Several pictures are generated by moving a grating for reconstructing a high resolution image Cai et al. (2020); Hong et al. (2019); Righolt et al. (2013); Gao et al. (2020). In SIM, the illumination light is sinusoidal pattern modulated by the grating. The distribution of the illumination light can be expressed as the grating equation. The grating function is expressed as follows

$$m(x, y) = \cos[2\pi k_0 (x \cos \theta + y \sin \theta) + \phi],$$

(1)

where $\theta$ is the rotation angle of sinusoidal stripe. In SIM, the grating requires multiple rotations to realize the illumination light imaging the sample in different directions. The sinusoidal stripe has different orientations during rotating grating. In the general operation of SIM, $\theta$ varies as the grating rotates centring on the optical axis. The initial phase $\phi$ is the most appropriate modulation parameter. In tiSIM, the controlling method of $\theta$ is the rotation of prism, because the actual grating is replaced by two beams interference. The orientation $\theta$ of the sinusoidal stripe varies as the rotation of prism. $\phi$ represents the initial phase of the grating function. The carrier frequency $k_0$ is equal to the cutoff frequency $f_c$. The $k_0$ represents the spatial frequency of the grating. The imaging model of SIM is expressed as

$$g(x, y) = [s(x, y) \cdot m(x, y)] \otimes h(x, y),$$

(2)

where $h(x, y)$ represents the point spread function (PSF) as follows

$$h(x, y) = \text{circ} \left( \frac{\sqrt{x^2 + y^2}}{f_c} \right).$$

(3)

The imaging process of tiSIM is written as

$$g(x, y) = [s(x, y) \cdot \exp(i\omega x \cos \gamma) \cdot m(x, y)] \otimes h(x, y).$$

(4)

where the exponential term $\exp(i\omega x \cos \gamma)$ is from tilt illumination. Here $\omega = 2\pi / \lambda$ denotes wave number. $\lambda$ is the illumination wavelength. tiSIM in frequency domain is written as

$$\begin{bmatrix}
I_1 \\
I_2 \\
I_3
\end{bmatrix} = \frac{1}{2} M_\phi M_H M_S,$$

(5)

where

$$M_\phi = \begin{bmatrix}
2 e^{-i\phi_1} e^{i\phi_2} \\
2 e^{-i\phi_2} e^{i\phi_1} \\
2 e^{-i\phi_1} e^{i\phi_2}
\end{bmatrix}.$$
\[ M_H = \begin{bmatrix} H & 0 & 0 \\ 0 & H & 0 \\ 0 & 0 & H \end{bmatrix}, \] (7)
and
\[ M_S = \begin{bmatrix} S(k - \cos \gamma / \lambda) \\ S(k + k_0 - \cos \gamma / \lambda) \\ S(k - k_0 - \cos \gamma / \lambda) \end{bmatrix}, \] (8)

where \( I_n \) represents the spectrum of image received by CCD. The symbol \( S \) indicates the spectrum corresponding to 0, and \( \pm 1 \) orders of diffraction light. \( H \) is OTF. The parameters \( k \) and \( \phi_n \) denote the spatial frequency and the different initial phases. \( \phi_1, \phi_2 \) and \( \phi_3 \) are three different initial phases of \( \phi \) in Eq. (6). Three independent equations are needed to be constructed for obtaining the exact solutions of unknown spectral components, since Eq. (8) contains three unknown spectral information. Three different initial phases \( (\phi_1, \phi_2 \) and \( \phi_3) \) are selected to construct matrix equations as shown in Eq. (5) and Eq. (6). Three different initial phases \( \phi_1, \phi_2 \) and \( \phi_3 \) of Eq. (6) are set as 0, \( 2\pi / 3 \) and \( 4\pi / 3 \), respectively. The phase shifts from \( \phi_1 \) to \( \phi_2 \) and from \( \phi_2 \) to \( \phi_3 \) are both \( 2\pi / 3 \). The parameter \( k_0 \) in Eq. (8) is the Fourier transform of \( k_0 \) in Eq. (1). Selecting different values of \( \phi_n \), a system of linear matrix equations can be obtained to solve the sample spectrum.

The images in Fig. 2 are obtained by wide field (WF) imaging and SIM. The resolution of wide field imaging can be calculated as \( \sigma_{WF} = 0.61\lambda / \text{NA} = 3.86 \mu m \). Here the wavelength of incident light is 632.8 nm. Numerical aperture (NA) is 0.1. The resolution of SIM is \( \sigma_{SIM} = 1.93 \mu m \), since strutured illumination has 2-fold resolution. The distance between the vertical lines specified by blue and red lines in Fig. 2a and b is 2 \( \mu m \). Here the three vertical lines can only be resolved by SIM rather than wide field imaging because 2 \( \mu m \) is between \( \sigma_{WF} \) and \( \sigma_{SIM} \). The resolution of WF and SIM can be validated by blue and red lines in Fig. 2c.

In Fig. 3, three images are the sample frequency spectrums of WF, SIM and tiSIM. The spectrum from tiSIM in Fig. 3c is larger than the one in Fig. 3b. The more infromation of spectrum can be carried to OTF of tiSIM, which is beneficial for high-resolution reconstruction. The operations of tiSIM are divided to two parts. Firstly, the incident light is parallel to the optical axis. Secondly, tilt illumination is performed to rich the spectrum.

In Fig. 4a and b, the images recovered by SIM and tiSIM (10°), are displayed. The blue and green structures specified by the same color lines in reconstructed images are the diffraction limits of SIM and tiSIM (10°). From Fig. 4, tiSIM can enhance the resolution of reconstructed image.

In SIM, the numerical aperture, \( \text{NA}_{SIM} \) is defined as
\[ \text{NA}_{SIM} = n \cdot \sin \beta = \lambda \cdot k_0, \] (9)
where \( n \) is the refractive index of the medium. \( \beta \) is the half of the aperture angle in Fig. 5(a). The grating equation is \( (\sin \beta) / k_0 = k\lambda \), \( (k = 0, \pm 1, \pm 2, \ldots) \). The period is defined as \( d = 1 / k_0 \) and \( k \) is the diffraction order. In tiSIM, \( \text{NA}_{tSIM} \) is defined as
\[ \text{NA}_{tSIM} = n \cdot \sin \beta_1 = \lambda \cdot k_0 + \sin \gamma. \] (10)

Thereby, the resolution ratio between SIM and tiSIM is expressed as
where \( k_0 = 0.1/(0.61\lambda) \).

Considering the aperture of 4f system, the angle \( \gamma \) is limited as follows

\[
- \frac{D}{4f\lambda} \leq -k_0 + \frac{\cos \gamma}{\lambda} \leq \frac{D}{4f\lambda},
\]

(12)
where \( D \) is the minimum aperture radius of 4f system.

Noise robustness of image reconstruction is tested by tiSIM and SIM. Here Gaussian white noise is added for simulating experimental case. In Fig. 6, two retrieved images by SIM and tiSIM (10°) are shown. Despite the influence of Gaussian white noise, the three vertical lines specified by the green lines can still be differentiated in Fig. 6b. From Fig. 6, tiSIM has higher noise robustness than SIM.
To improve the imaging resolution of tiSIM, multi-angle tilt illumination is employed. Here NA of the optical system is enhanced. In Fig. 7, the spectrum of the recovered images by tiSIM (10°) and tiSIM (10°, 15°, 20°, 30°) is illustrated. In Fig. 7b, a bigger area of spectrum is obtained and can be used for high-resolution reconstruction.

The flowchart of tiSIM with multi-angle illumination is displayed in Fig. 8. At each angle, the spectrum is calculated by Eq. (5). In Fig. 8a–c, the spectra obtained

![Image](image-url)

**Fig. 6** The noise robustness with Gaussian white noise: a by SIM, b by tiSIM (10°), c the blue, purple, green and red curves are normalized intensity of the area specified by the same color lines in (a) and (b)

### 3 Multi-angle tilt illumination

To improve the imaging resolution of tiSIM, multi-angle tilt illumination is employed. Here NA of the optical system is enhanced. In Fig. 7, the spectrum of the recovered images by tiSIM (10°) and tiSIM (10°, 15°, 20°, 30°) is illustrated. In Fig. 7b, a bigger area of spectrum is obtained and can be used for high-resolution reconstruction.

The flowchart of tiSIM with multi-angle illumination is displayed in Fig. 8. At each angle, the spectrum is calculated by Eq. (5). In Fig. 8a–c, the spectra obtained

![Image](image-url)

**Fig. 7** The frequency spectrums of the recovery images by tiSIM (10°) and tiSIM (10°, 15°, 20°, 30°)
by SIM, tiSIM ($\gamma_1$), and tiSIM ($\gamma_k$) are given. In Fig. 8d the integrated spectrum with several angles is shown. From Fig. 8, more data in high frequency range of spectrum is moved into the pass band of OTF by tiSIM with more angles. In Fig. 9, the images are calculated by tiSIM with multi-angle illumination. From Fig. 9c, the resolutions are 0.9

![Flowchart of tiSIM with multi-angle illumination](image)

**Fig. 8** A flowchart of tiSIM with multi-angle illumination. (a–c) represent the spectrum generated by SIM, tiSIM ($\gamma_1$) and tiSIM ($\gamma_k$). d is an integrated spectrum

![Reconstructed images](image)

**Fig. 9** The reconstructed images: a by tiSIM ($10^\circ$), b tiSIM ($10^\circ$, $15^\circ$, $20^\circ$, $30^\circ$), c normalized intensity profiles from specified range
\[ \mu m \text{ and } 0.5 \mu m \text{ for the two cases. It shows that multi-angle tilt illumination can improve resolution by collecting spectrum from more directions.} \]

In tiSIM, inclination angle error is considered for experiment. In Fig. 10a, b are the images made by tiSIM under the condition that all inclination angle errors of tilt illumination are 2°. The three vertical lines specified by the blue lines are not able to be differentiated in Fig. 10a under the influence of the errors by compared with the same area in Fig. 4a. The different contrast between blue and green curves in Figs. 4c and 10c also proves the different angular errors robustness.

### 4 Comparison with nonlinear SIM

Nonlinear SIM (NSIM) is put forward by applying photoswitchable protein instead of fluorescence saturation for super-resolution imaging Heintzmann et al. (2002); Hassanien et al. (2020); Lin et al. (2016); Hu et al. (2020). The sample frequency spectrum and the recovery image in Fig. 11 are obtained by WF, SIM, and third-order NSIM. The third-order NSIM can obtain much more high frequency spectrum. Compared with nonlinear SIM, tiSIM is by rotating illumination beam to detect high frequency without high density laser. Thus, the advantage of tiSIM compared with nonlinear SIM is that the damage to biological samples can be reduced.

![Fig. 10](image-url)  
**Fig. 10** The recovery images by tiSIM under the condition that the inclination angle error: a single angle, b four angles, c normalized intensity curves from the area specified by the same color lines.
5 Conclusion

In SIM, the parallel incident light restricts the high frequency information carried to OTF. Tilt illumination is employed to SIM for expanding the spectrum detection range. More data of high frequency can be integrated for super-resolution imaging. The proposed method has higher noise robustness. In addition, SIM with multi-angle illumination has higher angular error robustness. tiSIM can be applied for super-resolution biological cell imaging, 3D surface measurement, and optical sectioning. The imaging speed of tiSIM is affected and is limited for quick measurement, because of tilt illumination. It could be improved by high-speed hardware or parallel illumination.

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Declarations

Conflict of interest The authors declare that they have no conflict of interest.

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