The Influence of Noise in the Super-Resolution Reconstruction of Structured Illumination Microscopy

Jinxi Bai, Zhendong Shi, Hua Ma, Lijia Liu and Lin Zhang*

Laser fusion research center of China Academy of Engineering Physics, No.64, Mianshan Road, Mianyang, Sichuan Province, 621900, China
E-mail: 1169418081@qq.com

Abstract. As one of the mainstream super-resolution imaging technologies, structured illumination microscopy (SIM) is popular for its fast imaging speed and simple optical path structure. Spectrum separation is a key step in the reconstruction of super-resolution images. However, in the process of imaging, the unavoidable noise will seriously affect the accuracy of frequency spectrum separation. This paper carries out a simulation study on the influence of noise in the process of frequency spectrum separation. The results show that although noise can cause distortion of low-frequency information in frequency spectrum separation results, it has little influence on high-frequency information. Therefore, a super-resolution image reconstruction method is proposed to effectively suppress the influence of noise. Both simulation and experimental results are shown the method can suppress the influence of noise without losing the details of super-resolution.

Keywords. Structured illumination microscopy; super-resolution; noise; image reconstruction.

1. Introduction
The diffraction limit objectively existing in the optical system has always been the main reason that limits the further improvement of the resolution of the microscopic imaging system. Until the end of the 20th century, there have been significant scientific research progresses in the physics one after another. Many attempts to break the diffraction limit to obtain super-resolution imaging have achieved great breakthroughs. Researchers have successively developed several super-resolution microscopy imaging techniques, such as stimulated emission depletion(STED), structured illumination microscopy(SIM), stochastic optical reconstruction microscopy(STORM) etc[1-7], bringing the optical microscopy imaging technology to the nanometer level. Among these super-resolution detection methods, SIM technology is widely used due to its fast imaging speed and simple optical path structure.

Since the SIM technology was proposed, it has attracted wide attention from scholars at home and abroad, and a lot of research results have been obtained in the theory and application of the technology. Many scholars found in the structured illumination super-resolution imaging and image reconstruction process that the noise generated by the detector response and the noise formed by dust and dirty spots in the imaging system will seriously affect the quality of the super-resolution reconstructed image[8,9]. In microscopic imaging technology, the methods usually used to suppress noise are mainly divided into two categories in principle: one is to directly filter the image, but this method will seriously affect the detailed information of the microscopic image while suppressing the noise; the other is to suppress or eliminate the information of the specific position of the microscopic image after the location of the noise is determined, so as to achieve the effect of suppressing the noise while maintaining the image
quality. This paper systematically analyzes the influence of noise in the SIM reconstruction process during the imaging process through computer simulation, and based on the simulation results, combined with the spectral characteristics of the SIM, a method is proposed to effectively suppress the noise during the imaging process. This method can reduce the impact of noise on the reconstruction process under the premise of ensuring the quality of super-resolution image reconstruction.

2. Basic Principles

2.1. Theoretical Foundation of SIM
SIM is also called frequency domain processing technology. The basic principle is that utilizing moiré fringe moves high-frequency information that could not pass through the system to the spectrally detectable by the optical imaging system. After post-data processing, the high-frequency information is decoded and moved to the correct position, so that the spectral range of the optical microscopy system is expanded, thereby breaking the diffraction limit and realizing an improvement in resolution [5]. Assuming that the surface information of the sample is \( S(r) \) and the structured illumination light intensity distribution is \( I(r) \), the image distribution function obtained after passing through the optical imaging system is:

\[
p(r) = [S(r) \cdot I(r)] \ast h(r)
\]

(1)

Where \( h(r) \) denotes the microscope system’s point spread function (PSF), its Fourier transform is optical transfer function (OTF), \( \ast \) is the convolution operator and the structured illumination light intensity \( I(r) \) satisfies the sinusoidal distribution:

\[
I(r) = I_0 \left[ 1 + m \cos \left( 2\pi k_0 r + \varphi \right) \right] / 2
\]

(2)

Where \( I_0 \) is the mean illumination intensity of sample, \( m \) is the modulation factor, which indicates the contrast of sinusoidal illumination. \( k_0 \) and \( \varphi \) are the spatial frequency of structured illumination in reciprocal space and the initial phase of sinusoidal illumination. The Fourier transform is performed on the image \( p(r) \) collected by the detector to obtain the frequency spectrum \( P(k) \),

\[
P(k) = I_0 \left[ S(k) + m / 2 \cdot \exp(i\varphi) \cdot S(k - k_0) + m / 2 \cdot \exp(-i\varphi) \cdot S(k + k_0) \right] \cdot H(k)
\]

(3)

From the above equation, the frequency spectrum information has already carried misplaced sample high-frequency information \( S(k-k_0)H(k) \) and \( S(k+k_0)H(k) \), as long as the two are accurately separated and moved back to the correct position, structured illumination light super-resolution imaging can be achieved.

To simplify the operation and better understand SIM theorem, we denote the three unknown components of \( S(k)H(k) \), \( S(k-k_0)H(k) \) and \( S(k+k_0)H(k) \) as \( D_z \), \( D_n \), and \( D_p \), respectively. From equation (3), we can know that in order to achieve the precise separation of the three parts of information \( D_z \), \( D_n \) and \( D_p \), it is necessary to collect three sample images with different initial phase structure light modulation, and construct the following equations[7,10]:

\[
\begin{align*}
P_z(k) &= I_0 \begin{bmatrix} 1, m / 2 \cdot \exp(i\varphi_1), m / 2 \cdot \exp(-i\varphi_1) \\ 1, m / 2 \cdot \exp(i\varphi_2), m / 2 \cdot \exp(-i\varphi_2) \end{bmatrix} \begin{bmatrix} D_z \\ D_n \end{bmatrix} \\
P_n(k) &= I_0 \begin{bmatrix} 1, m / 2 \cdot \exp(i\varphi_1), m / 2 \cdot \exp(-i\varphi_1) \\ 1, m / 2 \cdot \exp(i\varphi_2), m / 2 \cdot \exp(-i\varphi_2) \end{bmatrix} \begin{bmatrix} D_z \\ D_p \end{bmatrix}
\end{align*}
\]

(4)

Where \( \varphi_1 \), \( \varphi_2 \) and \( \varphi_3 \) denotes the initial phase values of the illumination light of each image structure respectively. Without the interference of noise, equation (4) can accurately separate high-frequency information.
2.2. The Principle of Noise Suppression

However, in the actual experiment process, there are usually a lot of random noise and fixed pattern noise in the obtained image due to the uneven dark current and uneven photoelectric response of the image sensor. In addition, dust or dirty spots on the imaging system will also cause a lot of fixed noise on the microscopic image. According to the imaging principle, noise can be introduced into equation (4) [12, 13] as an unmodulated item, then equation (4) can be rewritten as:

\[
\begin{bmatrix}
P_1(k) \\
P_2(k) \\
P_3(k)
\end{bmatrix} = I_0 \begin{bmatrix}
1, m / 2 \cdot \exp(i\phi_1), m / 2 \cdot \exp(-i\phi_1) \\
1, m / 2 \cdot \exp(i\phi_2), m / 2 \cdot \exp(-i\phi_2) \\
1, m / 2 \cdot \exp(i\phi_3), m / 2 \cdot \exp(-i\phi_3)
\end{bmatrix} \begin{bmatrix}
D_z \\
D_n \\
N_p
\end{bmatrix} + \begin{bmatrix}
N_1(k) \\
N_2(k) \\
N_3(k)
\end{bmatrix}
\]

(5)

Where \(N_1(k), N_2(k), N_3(k)\) denotes the noise in the three images. Under such condition, the \(D_z, D_n\) and \(D_p\) obtained by solving this equation will have large deviation, and the inaccurate spectrum separation result will seriously affect the reconstruction quality of the super-resolution image. Respectively use the first and second terms in the equation group of equation (5) to subtract the third term, the following equations are obtained,

\[
\begin{bmatrix}
P_1(k) - P_3(k) \\
P_2(k) - P_3(k)
\end{bmatrix} = C \begin{bmatrix}
\exp(i\phi_1) - \exp(i\phi_2), \exp(-i\phi_1) - \exp(-i\phi_2) \\
\exp(i\phi_2) - \exp(i\phi_3), \exp(-i\phi_2) - \exp(-i\phi_3)
\end{bmatrix} \begin{bmatrix}
D_n \\
D_p
\end{bmatrix} + \begin{bmatrix}
N_1(k) - N_3(k) \\
N_2(k) - N_3(k)
\end{bmatrix}
\]

(6)

Where \(C = I_0 m/2\). According to the fixed point noise position is relatively stable [9, 11], and the original SIM image is continuously collected in a short period of time. Therefore, the position of the noise can be approximated not jumped. The equations (6) can be approximated as,

\[
\begin{bmatrix}
P_1(k) - P_3(k) \\
P_2(k) - P_3(k)
\end{bmatrix} \approx C \begin{bmatrix}
\exp(i\phi_1) - \exp(i\phi_2), \exp(-i\phi_1) - \exp(-i\phi_2) \\
\exp(i\phi_2) - \exp(i\phi_3), \exp(-i\phi_2) - \exp(-i\phi_3)
\end{bmatrix} \begin{bmatrix}
D_n \\
D_p
\end{bmatrix}
\]

(7)

It can be clearly seen from equation (2)–(6) that the solution results of \(D_n\) and \(D_p\) are almost unaffected by noise, and a large amount of errors caused by the noise term \(N(k)\) remain in the low-frequency information \(D_z\), so \(D_z\) can be processed with low-pass filtering and noise reduction. Although this process will attenuate the higher-frequency information in \(D_z\) at the same time, due to the particularity of the super-resolution image reconstruction process, the existence of high-frequency components \(D_n\) and \(D_p\) makes the reconstructed image not lose high-frequency information due to the filtering process of \(D_z\). In summary, the following equations can be used to reconstruct the super-resolution image to complete the suppression of noise in the imaging process:

\[
\begin{align*}
D_n &= \text{fft} \left[ \exp(+ik_0r) \cdot \text{iff} \left( D_n \right) \right] \\
D_p &= \text{fft} \left[ \exp(-ik_0r) \cdot \text{iff} \left( D_p \right) \right] \\
I_w &= \text{iff} \left[ \text{filter} \left( D_n \right) + D_n + D_p \right]
\end{align*}
\]

(8)

Where \(I_w\) is the reconstructed super-resolution image, \text{filter} denotes the low-pass filter model in the frequency domain. \text{fft} and \text{iff} stand for Fourier transform and inverse Fourier transform, respectively.

2.3. The Design of Filter

From the analysis in section 2.2, it can be seen that to reconstruct a high-quality super-resolution image, it is necessary to perform low-pass filtering on the low-frequency information \(D_z\) to reduce noise, and the selection of filter parameters is particularly important, because this is not only related to the effect of noise suppression in the reconstruction process, but seriously affects the percentage of high-frequency and low-frequency information. Unbalanced proportions will cause distortion of the reconstructed image. The relationship between the spatial frequency \(k\) and the spectral gravity \(M\) in the
horizontal direction of $D_z$, $D_n$, and $D_p$ can be shown as follows.

**Figure 1.** The specific gravity of each part of the spectrum after separation.

Figure 1 shows the respective proportions of $D_z$, $D_n$ and $D_p$ in the frequency spectrum. In order to ensure that the proportion of the low frequency part after the superposition of the spectrum is not lower than $M'$, the cut-off frequency $k_{cf}$ of the low-pass filter should be selected according to the spatial frequency value of the sinusoidal illumination fringe.

$$k_{cf} = \begin{cases} 
0, & k_0 \leq k_{cf} (2 - M') / 2 \\
(M' - 1) k_{cf} + k_0, & k_0 > (2 - M') / 2,
\end{cases} \quad (9)$$

Where $k_{cf}$ denotes the cut-off frequency of optical microscopy system. The above equation shows that when the spatial frequency of the sinusoidal illumination fringe is lower than a threshold of $k_{cf} (2 - M') / 2$, the low frequency parts of $D_n$ and $D_p$ are superimposed to satisfy the spectrum proportion not lower than $M'$, so the cut-off frequency of the low-pass filter is selected as 0, and $D_z$ can be directly discarded. Although the noise suppression effect is the best in this case, the lower spatial frequency of structured light will result in an insignificant increase in the resolution of super-resolution images, which is obviously contrary to the purpose of using SIM to improve the resolution of the microscopy system. Therefore, on the one hand, it should be as small as possible to enhance the noise suppression effect without causing image distortion. On the other hand, in order to improve the resolution, sinusoidal illumination spatial frequency should be increased as much as possible. Comprehensively consider the filter function as shown in the following equation,

$$\text{filter}(k) = \begin{cases} 
0, & k \geq k_{cf}\text{-filter} \\
M' \frac{k_0 - k}{k_{cf} - k}, & k < k_{cf}\text{-filter}.
\end{cases} \quad (10)$$

3. Simulation Verification and Analysis

Based on the above theoretical analysis, the MATLAB program is used to verify and analyze the influence of noise and the suppression method in the process of super-resolution image reconstruction. In the simulation system, the numerical aperture of the objective lens is set to 0.75, the fluorescence wavelength detected by the system is 630nm, and the width of each pixel of the object surface sample is 100nm. The sample surface information and the optical transfer function of the system are shown in the figure below.
3.1. The Influence of Random Noise on the Process of Spectrum Separation

In order to verify the theoretical analysis in section 2.2, a computer simulation analysis was carried out for the influence of random noise on the SIM spectrum separation process. Firstly, the result of spectrum separation obtained without noise is shown in the figure below.

![Figure 2. Microsystem model.](image)

(a) The sample surface information. (b) The optical transfer function of the system.

Figure 3. Ideally frequency spectrum separation results.

In the three images collected by the detector, Gaussian noise and salt & pepper noise are introduced, and use equation (5) to obtain $D_z$, $D_n$, and $D_p$. And the residuals are processed separately with the separation results introduced by noiseless, and the root mean square values of the residuals are compared. The root square values are denoted as $R_z$, $R_n$, and $R_p$ respectively. Table 1 shows the separation results of $D_z$, $D_n$ and $D_p$ after introducing Gaussian noise with different variances and salt & pepper noise with different densities.

| Gaussian noise | Variance | 0.04  | 0.08  | 0.12  | 0.16  | 0.2   | 0.24  |
|----------------|----------|-------|-------|-------|-------|-------|-------|
| $R_z$          |          | 15.18 | 21.50 | 26.14 | 29.98 | 33.01 | 35.50 |
| $R_n$          |          | 0.2876| 0.3534| 0.4078| 0.4549| 0.5002| 0.5382|
| $R_p$          |          | 0.2876| 0.3534| 0.4078| 0.4549| 0.5002| 0.5382|

Table 1. Separation accuracy under random mode noise
It can be seen from the final residual data that the $D_z$ separation error is two orders of magnitude higher than $D_n$ and $D_p$ in value, which fully shows that the influence of noise on the low-frequency information part is much higher than the high-frequency part. The separation accuracy of $D_z$ further decreases with the increase of the noise intensity. Although the separation accuracy of $D_n$ and $D_p$ also increases with the change of the random noise position, judging from the magnitude of the error data, a large amount of noise still remains in $D_z$, which is consistent with the theoretical analysis in section 2.2. In addition, because $D_n$ and $D_p$ always have a conjugate symmetric relationship, the separation accuracy of the two is equal.

3.2. The Effect of Fixed Pattern Noise on the Process of Spectrum Separation

Different from random noise, the distribution of fixed pattern noise can be described as a random process of Poisson distribution [14], so three images with Gaussian noise and salt & pepper noise are introduced into three images with relatively fixed Poisson noise. Compared $R_z$, $R_n$, $R_p$, as shown in table 2.

| Variance | 0.04 | 0.08 | 0.12 | 0.16 | 0.2 | 0.24 |
|----------|------|------|------|------|-----|------|
| $R_z$    | 18.23| 23.74| 27.98| 31.64| 34.44| 36.85|
| $R_n$    | 0.2876| 0.3525| 0.4072| 0.4556| 0.4974| 0.5401|
| $R_p$    | 0.2876| 0.3525| 0.4072| 0.4556| 0.4974| 0.5401|

Comparing table 1 and table 2, it is clearly find that after introducing Poisson noise with a relatively fixed position, $R_z$ continues to increase compared to the introduction of Gaussian noise and salt & pepper noise, but $R_n$ and $R_p$ do not fluctuate significantly. It shows that a large amount of fixed pattern noise will remain in $D_z$ in the process of spectrum separation, which also provides ideas for the process of optimizing the quality of SIM super-resolution image reconstruction.

3.3. The Results of Super-Resolution Image Reconstruction

In order to further verify the noise suppression effect in the image reconstruction process. We use the sinusoidal fringe with spatial frequency $k_0=0.15\text{pixel}^{-1}$ to illuminate the sample and enter the different mode noises discussed in Section 3.1 and Section 3.2 at the same time. Using equation (6) to separate the frequency spectrum, $D_z$, $D_n$ and $D_p$ are obtained as shown in figure 3.
By comparing figure 3 and figure 4, it can be clearly found that after the introduction of noise interference, the separation of $D_z$ has a large error, and there is a lot of noise in the frequency spectrum. Although $D_n$ and $D_p$ also have some noise pollution, the spectrum information of the sample still accounts for a relatively high proportion. Use the noise suppression method proposed in section 2.3 to reconstruct the super-resolution image, set $M'=1$ to construct the filter, and perform the filtering process in the super-resolution image reconstruction process, and the results shown in figure 5 and figure 6 are obtained. Figure 5(d) and figure 6(d) show the super-resolution reconstructed image after noise suppression in the middle, compared with the ordinary image in figure 5(a)~(b) and figure 6(a)~(b) with wide-field microscopy images, the reconstructed image has been significantly improved in resolution. Compared with the unprocessed super-resolution reconstructed image in figure 5(c) and figure 6(c), the noise interference in figure 5(d) and figure 6(d) is significantly reduced, making the super-resolution details clearer.

Figure 4. Frequency spectrum separation results after noise introduced.

Figure 5. Sample information under microscopic system. (a). Wide-field imaging. (b). wide-field
imaging under noise pollution. (c). Unoptimized SIM reconstruction image. (d). Filtered SIM reconstruction image.

Figure 6. Sample details. (a). Wide-field imaging. (b). Wide-field imaging under noise pollution. (c). Unoptimized SIM reconstruction image. (d). Filtered SIM reconstruction image.

4. Experimental Results
In order to verify the effectiveness of the filtering method proposed in this paper, the structured light microscopy images of 100nm gold nanoparticle spheres collected in the laboratory were used for super-resolution reconstruction. The microscope system use ±1 diffraction order interference to generate structured illumination. We used a high power coherent laser as a light source (Coherent, $\lambda = 632.8 \pm 0.002$ nm). The ±1 order diffracted light is generated by diffracting the illuminating laser by the spatial light modulator (SLM, X13138-01, Hamamatsu). The numerical aperture of the objective lens is 0.8(Nikon, 100X/0.8), and the pixel size of the image detector is 4.4μm (acA1600-20um, basler).

Figure 7. Imaging results of 100nm gold nanoparticles. (a). Wide-field imaging. (b). Unoptimized SIM reconstruction image. (c). Filtered SIM reconstruction image.
As shown in figure 6, the SIM reconstruction image has a significant improvement in resolution compared to the traditional microscopic wide-field image. However, comparing figure 7.(b) and figure 7.(c), the difference between the two does not seem to be as obvious as the simulation result. In order to compare the imaging effects of the two more accurately, the light intensity changes at the arrows in figure 7 were respectively analyzed quantitatively, and the results shown in figure 8 below were obtained.

![Diagram of strength variation at arrow. (a) Wide-field imaging intensity distribution. (b) Unoptimized SIM reconstruction imaging intensity distribution. (c) Filtered SIM reconstruction imaging intensity distribution.](image)

Figure 8. Diagram of strength variation at arrow. (a) Wide-field imaging intensity distribution. (b) Unoptimized SIM reconstruction imaging intensity distribution. (c) Filtered SIM reconstruction imaging intensity distribution.

Figure 8(a) shows that the FWHM of 100nm gold nanoparticles under wide-field imaging is 385.9nm. The intensity changes in figure 8(b) and figure 8(c) show that the filtered structured light reconstructed image effectively retains the high-frequency information of the sample, and its FWHM is 284.6nm. However, by observing the intensity changes of the edges in figure 8(b) and figure 8(c), this position corresponds to the background part of the image. It can be clearly observed that the
background part of the filtered structured light image has been smoothed to a certain extent, which suppresses high-frequency noise in the image. Therefore, it can be proved that the filter model designed in section 2.3 can effectively suppress the noise generated in the imaging process while retaining the high-frequency information of the sample.

5. Conclusions
This paper analyzes the effect of noise during the reconstruction of SIM, and concludes that the effect of noise on the separation result is mainly concentrated in the low-frequency information part in the process of spectral separation, and the reliability of the conclusion is verified by simulation. Aiming at this feature, a reconstruction method that effectively suppresses the noise interference is proposed. By controlling the spectral weight of low-frequency information, while ensuring the quality of super-resolution reconstruction, it also enhances the anti-noise ability in the image reconstruction process.

Acknowledgments
This research is supported by Youth Research Fund (RCFCZ4-2020-1) of Laser Fusion Research Center of China Academy of Engineering Physics.

References
[1] Hell S W, Wichmann J. 1994 Breaking the diffraction resolution limit by stimulated emission: fluorescence microscopy [J] Opt. Letters 19 (11) 780-782.
[2] Klar T A, Jakobs S, Dyba M et al. 2000 Fluorescence microscopy with diffraction resolution barrier broken by stimulated emission[J] Proceedings of the National Academy of Sciences 97 (15) 8206-8210.
[3] Rust M J, Bates M, Zhuang X. 2006 Sub-diffraction-limit imaging by stochastic optical reconstruction microscopy (stom) [J] Nature Methods 3 (10) 793-796.
[4] Bates M, Huang B, Dempsey G T et al. 2007 Multicolor super-resolution imaging with photo-switchable fluorescent probes[J] Science 317 (5845) 1749-1753.
[5] Heintzmann R, Cremer C G. 1999 Laterally modulated excitation microscopy: improvement of resolution by using a diffraction grating [J] Proc. SPIE 3568 185-196.
[6] Gustafsson M G L, Agard D A, Sedat J W. 2000 Doubling the lateral resolution of wide-field fluorescence microscopy using structured illumination [J] Proc. SPIE 3919 141-150.
[7] Gustafsson M G L. 2000 Surpassing the lateral resolution limit by a factor of two using structured illumination microscopy [J] Journal of Microscopy 198 (2) 82-87.
[8] Zhu L, Zheng W B. and Zhang T. 2017 Research on Multi-Phase Structure Illumination Microscopy Reconstruction Simulation Technology [J] Optics & Optoelectronic Technology 15 (1) 81-85.
[9] Wang Y G, Jiang G Y, Yu M, Peng Z J, Fan S L and Shao F. 2011 Fixed Noise Locating Algorithm Based on Quasi-Gaussian Model for Microscope Image [J] Opto-Electronic Engineering 38 (11) 106-112.
[10] Gustafsson M G L, Shao L, Canton P M et al. 2008 Three-dimensional resolution doubling in wide-field fluorescence microscopy by structured illumination [J] Biophysical Journal 94 (12) 4957-4970.
[11] Nakamura J. 2005 Image Sensors and Signal Processing for Digital Still Cameras [M] CRC Press Inc.
[12] Zhou X, Dan D, Qian J, Yao B L and Lei M. 2017 Super-Resolution Reconstruction Theory in Structured Illumination Microscopy [J] Acta Optica Sinica 37 (003) 1-12.
[13] Lal A, Shan C, Xi P. 2016 Structured illumination microscopy image reconstruction algorithm [J] IEEE Journal of Selected Topics in Quantum Electronics 22 (4)50-63.
[14] Xia Z Y. 2005 Noise Processing and Sub-pixel Feature Extraction for Microscopic Images [D].