Simulation of structured light illumination microscope imaging reconstruction algorithm

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Abstract: Based on the SIM imaging theory, I wrote the simplest MATLAB implementation algorithm to demonstrate the feasibility and effectiveness of SIM imaging technology to achieve super-resolution imaging, and introduced every detail of the SIM imaging technology reconstruction algorithm in detail. The relevant Matlab implementation code and data have been shared to my GITHUB repository https://github.com/crease123/-matlab-/blob/main/final_final_SIM.mlx

Introduction

The optical microscope plays a vital role in the research of life science. However, the spatial resolution of the optical microscope is limited by diffraction. In order to break through the diffraction limit of optical microscopy and achieve super-resolution optical imaging, in the past few decades, scientists have created many technologies, such as single molecule imaging, and structured light illumination imaging technology. In the super-resolution imaging technology, structured illumination microimaging (SIM) technology is a revolutionary innovation, because it reconstruct the image from the frequency domain to achieve super-resolution, while other technologies such as STED, PALM/STORM reconstructing the image from the real space to achieve super resolution. And SIM greatly reduces the sample requirements, any fluorescent sample used for wide-field fluorescence microscopy can be used for SIM imaging. However, due to the imperfection of the experiment and the inevitable uncertainty of the experimental parameters in the experiment, such as the phase and direction of the illumination mode, the reconstruction process of the structured light illumination microscope imaging technique is quite challenging.

In order to show the reconstruction algorithm of SIM imaging technology to the reader in the simplest way, this paper will ignore the uncertainty of the phase, direction and other parameters of the lighting mode in the actual experiment and the noise introduced in the actual imaging process.

In this paper, we first introduce the mathematical principle of SIM imaging to achieve super-resolution imaging, and then introduce the matlab implementation of SIM super-resolution imaging reconstruction algorithm and the result display in detail. Finally, the significance of SIM imaging technology is discussed.

Theory

Structural illumination microscope imaging technology is to use periodic structured light (sinusoidal structured light) to illuminate the samples labeled by flurescin to achieve the realization of moving high-frequency information that cannot enter the objective lens OTF into the OTF, and then restore and reconstruct the spectrum of the image obtained by the microscope through the algorithm. In other words, the frequency components that were moved into the support OTF are moved back to their original positions , and then the inverse Fourier transform is performed to achieve super-resolution imaging. The following is a detailed explanation of the structural light illumination microscope imaging reconstruction algorithm simulation steps.

1. **Generate sinusoidal structure illumination pattern**

$I_{\theta,\phi}(\vec{r})$ represent sinusoidal mode illumination, which is expressed as follows:

Time domain expression:

$$I_{\theta,\phi}(\vec{r}) = I_0\left[1-\frac{m}{2}\cos\left(2\pi p_\theta \cdot r+\phi\right)\right]$$

Frequency domain expression:

$$\widetilde{I_{\theta,\phi}}(k) = I_0\left[\frac{1}{2}-\frac{m}{2}\cdot\frac{1}{2}\left(\delta\left(k-p_\theta\right)e^{-i\phi}+\delta\left(k+p_\theta\right)e^{i\phi}\right)\right]$$

$r \equiv (x, y)$: Two-dimensional space position vector; I_0: Peak illumination intensity, In this simulation I_0 =1;$p_\theta \equiv \left(p\cdot\cos(\theta), p\cdot\sin(\theta)\right)$: The representation of the illumination mode in reciprocal space, In other word, this is the frequency shift generated by the structured light modulating the fluorescence sample;m: modulation coefficient, In this simulation m=1; θ: The orientation of the sinusoidal illumination pattern, The structured illumination patterns used in this simulation in three directions: $\theta_1 = 0^o$; $\theta_2 = 60^o$; $\theta_3 = 120^o$ or -60^o; ϕ: The phase of the sinusoidal illumination pattern, The structured illumination patterns used in this simulation have three directions: $\phi_1 = 0^o; \phi_2 = 90^o; \phi_3 = 180^o$.

The structured light generated in three directions according to the above formula is as follows:

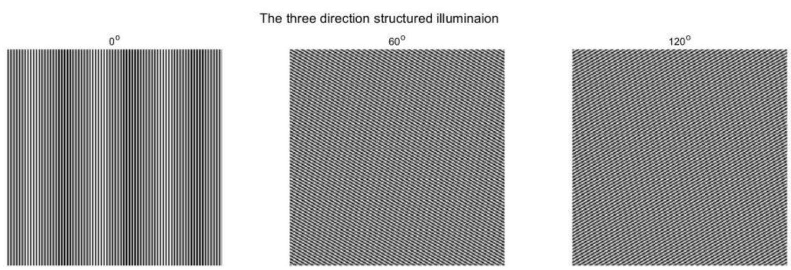

Fig. 1. The three direction structured illumination

2. Generate the optical transfer function of the optical system

In order to simplify the simulation, I define $\widetilde{H}(k)$ the OTF in which the frequency components in the circle, with the center of the spectrum as the origin and the diameter of the spectrum half, can pass through lossless, and the other frequency components are completely filtered out, as shown below:

$$\widetilde{H}(k) = circ\left(\frac{|k|}{D/2}\right)$$

So the image of a wide-field fluorescence microscope can be computed using follow fomula :

$$D_{WF}(r) = S(r) * H(r)$$

Where, $S(r)$: represents the distribution of fluorescent substances in the sample; $H(r)$: Represents the point spread function of an optical system.

The following figure shows the effect of OTF on the image processing from the real domain and frequency domain:

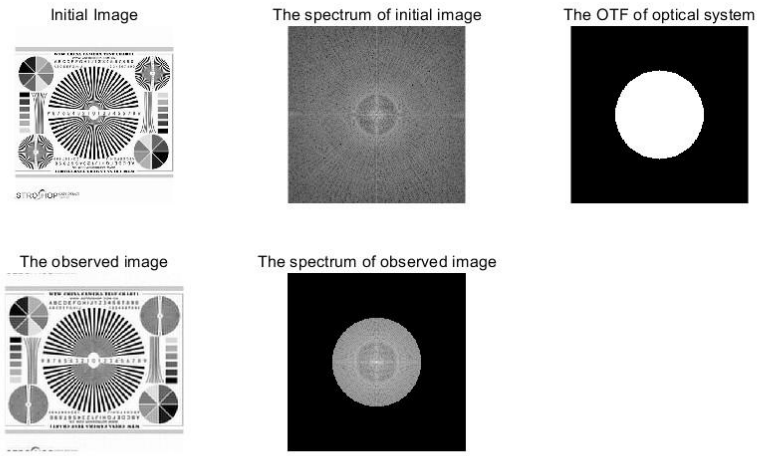

Fig. 2. The effect of OTF

3. Generate images illuminated by structured illumination

Because of only passing low-frequency information and filtering out high-frequency information, the object will lose the high-frequency information of the image after imaging by the optical system, and this is the explanation of the resolution limit of the optical system in Fourier optics theory. In order to let the high frequency information of the image enter the OTF of the optical system, we adopt the method of structured light illumination to modulate the image information. The mathematical principle is as follows:

$$\widetilde{D_{\theta,\phi}}(\{k)\} = \left[\widetilde{I_{\theta,\phi}}(k) * \tilde{S}(k)\right] \cdot \widetilde{H}(k)$$

$$= \frac{I_0}{2}\left[\tilde{S}(k) - \frac{m}{2}\tilde{S}(k - p_\theta)e^{-i\theta} - \frac{m}{2}\tilde{S}(k + p_\theta)e^{i\theta}\right] \cdot \widetilde{H}(k)$$

The equation corresponding to the image of objects illuinated by structured illumination of three different phases and the same direction is expressed in the form of a matrix as follows:

$$\begin{bmatrix} \tilde{D}_{\theta,\phi_1}(\mathbf{k}) \\ \tilde{D}_{\theta,\phi_2}(\mathbf{k}) \\ \tilde{D}_{\theta,\phi_3}(\mathbf{k}) \end{bmatrix} = \frac{I_o}{2} \begin{bmatrix} 1 & -\frac{m}{2} & -\frac{m}{2} \\ 1 & -\frac{m}{2}e^{-i\phi_2} & -\frac{m}{2}e^{+i\phi_2} \\ 1 & -\frac{m}{2}e^{-i\phi_3} & -\frac{m}{2}e^{+i\phi_3} \end{bmatrix} \begin{bmatrix} \tilde{S}(\mathbf{k})\tilde{H}(\mathbf{k}) \\ \tilde{S}(\mathbf{k}-\mathbf{p}_\theta)\tilde{H}(\mathbf{k}) \\ \tilde{S}(\mathbf{k}+\mathbf{p}_\theta)\tilde{H}(\mathbf{k}) \end{bmatrix}$$

$$\text{make} \quad \mathbf{M} = \begin{bmatrix} 1 & -\frac{m}{2} & -\frac{m}{2} \\ 1 & -\frac{m}{2}e^{-i\phi_2'} & -\frac{m}{2}e^{i\phi_2'} \\ 1 & -\frac{m}{2}e^{-i\phi_3'} & -\frac{m}{2}e^{i\phi_3'} \end{bmatrix}$$

The corresponding image and its spectrum diagram are as follows:

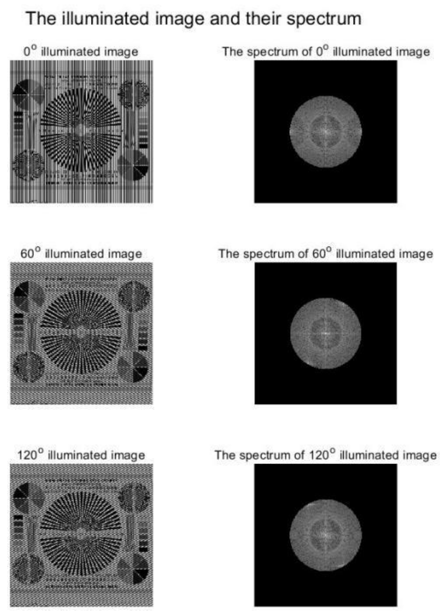

Fig. 3. The illuminated image and their spectrum

4. Decomposition and merger the spectrum

From the above equations, we can solve the shifted frequency components in the spectrum of the image illuminated by structured illumination by the following equation:

$$\begin{bmatrix} \tilde{S}(\mathbf{k})\tilde{H}(\mathbf{k}) \\ \tilde{S}(\mathbf{k}-\mathbf{p}_\theta)\tilde{H}(\mathbf{k}) \\ \tilde{S}(\mathbf{k}+\mathbf{p}_\theta)\tilde{H}(\mathbf{k}) \end{bmatrix} = \frac{2}{I_0}\mathbf{M}^{-1} \begin{bmatrix} \tilde{D}_{\theta,\phi_1}(\mathbf{k}) \\ \tilde{D}_{\theta,\phi_2}(\mathbf{k}) \\ \tilde{D}_{\theta,\phi_3}(\mathbf{k}) \end{bmatrix}$$

To show that the three components on the left side of the equal sign are three independent variables in the simulation code, we let:

$$\begin{bmatrix} \tilde{S}_M(\mathbf{k}) \\ \tilde{S}_1(\mathbf{k}) \\ \tilde{S}_2(\mathbf{k}) \end{bmatrix} = \begin{bmatrix} \tilde{S}(\mathbf{k})\tilde{H}(\mathbf{k}) \\ \tilde{S}(\mathbf{k}-\mathbf{p}_\theta)\tilde{H}(\mathbf{k}) \\ \tilde{S}(\mathbf{k}+\mathbf{p}_\theta)\tilde{H}(\mathbf{k}) \end{bmatrix}$$

Where: $\widetilde{S_M}(k)$: spectrum components that have not been moved; $\widetilde{S_1}(k)$: Spectrum components that are shifted to the left; $\widetilde{S_1}(k)$: Spectrum components that are shifted to the right.

And then we apply the following calculation method to shift each shifted frequency components back to their original position.

$$\widetilde{S_{S2}}(\boldsymbol{k}) = \widetilde{S_2}(\boldsymbol{k} + \boldsymbol{p}_\theta)$$

$$\widetilde{S_{S1}}(\boldsymbol{k}) = \widetilde{S_1}(\boldsymbol{k} - \boldsymbol{p}_\theta)$$

Then we combine the various frequency components that are decomposed from the spectrum diagram to form a complete spectrum diagram that extends approximately twice in each direction.

$$\widetilde{S_{SIM,\theta}}(\boldsymbol{k}) = \widetilde{S_{M,\theta}}(\boldsymbol{k}) \cup \widetilde{S_{SL,\theta}}(\boldsymbol{k}) \cup \widetilde{S_{SR,\theta}}(\boldsymbol{k})$$

$$\widetilde{S_{SIM}}(\boldsymbol{k}) = \widetilde{S_{SIM,\theta_1}}(\boldsymbol{k}) \cup \widetilde{S_{SIM,\theta_2}}(\boldsymbol{k}) \cup \widetilde{S_{SIM,\theta_3}}(\boldsymbol{k})$$

The whole process of decomposition, translation, and merging is shown in the figure below:

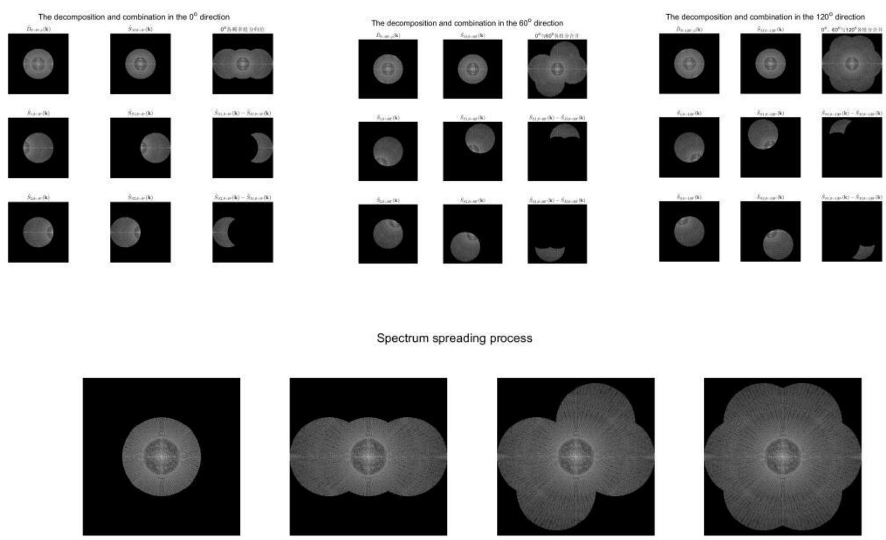

Fig. 4. The process of decomposition, translation and merging

5. Decomposition and merger the spectrum

Finally, I performed inverse Fourier transform on the combined spectrum and reconstructed the image with approximately twice the resolution in all directions.

At this point, we have completed the entire simulation process of the structured light illumination microscope imaging reconstruction algorithm. However, it should be noted that the algorithm I presented ignores the uncertainty of structured light phase, direction, and spatial frequency in actual experiments, as well as the noise that may be introduced during the actual image acquisition process.

The effect of the final SIM image reconstruction algorithm is shown in the following figure:

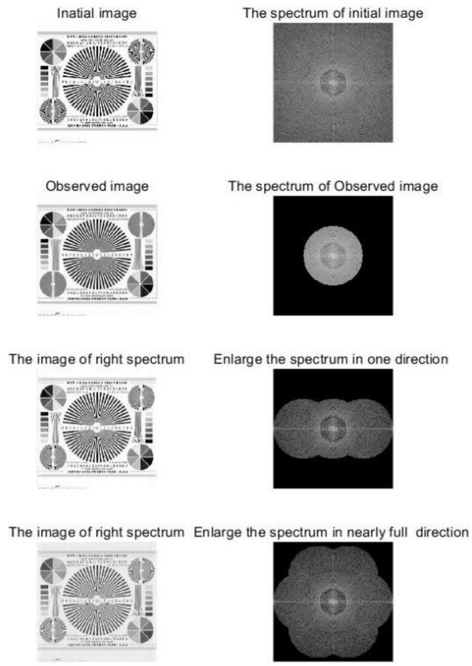

Fig. 2. The effect of SIM

Discussion

The comparison between the image processed by the SIM reconstruction algorithm and the image formed by the wide-field microscope shows that the SIM imaging technology can indeed double the resolution of the microscope. I have demonstrated every detail of the SIM reconstruction algorithm in detail through simulation. Although many undesirable factors in actual experiments are ignored, this does not prevent readers from deeply understanding the SIM reconstruction algorithm through this article.

In addition, since the SIM reconstruction algorithm uses Fourier transform and requires a large amount of calculation (because it processes image data), before the fast Fourier transform algorithm (FFT) was invented and the computing power of computers was improved to the required requirements, the implementation of SIM technology was impractical and uneconomical. Therefore, this technology was not proposed by the academic community until 1999. It can be seen that the development of various technologies is mutual promotion. The problems we can currently study depend not only on our own capabilities, but also on the technical capabilities of the current era.

Compared with other technologies, the improvement of resolution by SIM imaging technology is negligible, only two times. However, the success of SIM imaging technology has shown us a completely different way to improve the resolution of optical systems, that is, by modulating the object, so the information that could not enter the optical system can enter the optical system, and then using a specific algorithm to extract that information to improve the resolution of the image. Is there a better method than Fourier optics ? This is a question worth exploring. Currently, many teams have begun to explore the use of deep learning methods to reconstruct images and have made impressive progress.

References

1. A. Lal, C. Shan and P. Xi, "Structured Illumination Microscopy Image Reconstruction Algorithm," in IEEE Journal of Selected Topics in Quantum Electronics, vol. 22, no. 4, pp. 50-63, July-Aug. 2016, Art no. 6803414, doi: 10.1109/JSTQE.2016.2521542.