Analysis of Fourier ptychographic microscopy with half of the captured images

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Abstract

Fourier ptychographic microscopy (FPM) is a new computational imaging technique that can provide gigapixel images with both a high-resolution and a wide field of view. However, the data-acquisition process is time-consuming, which is a critical issue. In this study, we analyze the FPM imaging system with the half number of captured images; i.e. if the size of the light-emitting diode (LED) array is $15 \times 15$, we use $15 \times 8$ LEDs to capture images. According to the image analysis of the conventional FPM system, we then compare the reconstructed images with different captured data. When the sample is close to amplitude-only, simulation and experimental results show that the resolution of the reconstructed image with the half number of captured data does not differ significantly from that with all the captured data.

Keywords: microscopy, computational imaging, phase retrieval

(Some figures may appear in colour only in the online journal)

1. Introduction

It is well-known that in the conventional microscope, the low numerical aperture (NA) of the objective lens produces a wide-FOV image but with a low image resolution. Fourier ptychographic microscopy (FPM) is a newly developed computational optical imaging technique, which breaks the diffraction limit of the objective lens by using an angularly varying light-emitting diode (LED) illumination and a phase-retrieval algorithm [1–3]. In the FPM system, a programmable LED array is typically used as the light source. After a stack of low-resolution images are captured at different illumination angles, an iterative phase-retrieval process is used to reconstruct the complex field of the object with an enhanced resolution, without sacrificing the wide FOV [4–6]. Because the FPM breaks the limitation of the space-bandwidth product (SBP) of the optical system and achieves gigapixel imaging, it has great potential in various applications, such as biomedical imaging [7–13] and characterizing unknown optical aberrations of lenses [14, 15].

However, the high SBP imaging capability of FPM has a drawback: the method is time-consuming, limiting its application to static objects. This limitation arises from the requirement of a large amount of low-resolution images. In addition, the low illumination intensity of the LED array induces a long exposure time during image acquisition. To improve the capture efficiency, various techniques have been proposed. In principle, these techniques can be divided into two categories. The first one is to improve the FPM illumination. For example, Kuang et al proposed using a laser instead of the LED array to enhance the intensity of the illumination, for reducing the exposure time during the capture procedure [16]. Other approaches in this category include lighting several LEDs simultaneously [12, 17–19] and non-uniform sampling of the spectrum of the object [7, 20–23] to reduce the required number of images. These techniques usually change the illumination structure. An optimal strategy that sacrifices the simplicity of the original FPM needs to be developed. Another technique involves improving the reconstruction algorithm [24–26]. For example, the Wirtinger flow optimization algorithm has been proposed to reduce the...
exposure time to approximately 80% [24]. However, this algorithm increases the calculation cost [1].

In this paper, we propose an alternative approach by analyzing the imaging process. According to the analysis, we perform simulations and experiments to demonstrate our method. In section 2, we perform a theoretical analysis of the FPM imaging process. In sections 3 and 4, simulations and experiments are conducted to validate the analysis results.

2. Analysis of the FPM imaging system

We suppose that, in an FPM system, as shown in figure 1, a complex object with the transmittance function of $O(x, y) = A(x, y) \exp[j\phi(x, y)]$ is located at the front focal plane of the microscopic objective, where $A(x, y)$ and $\phi(x, y)$ are the amplitude and phase of the object, respectively. A plane wave parallel to the optical axis illuminates the object with a wavelength of $\lambda$. Without considering the aperture size, the Fourier spectrum $H(u, v)$ located at the back focal plane of the microscopic objective is expressed as [27]

$$H(u, v) = \int_{-\infty}^{\infty} O(x, y) \exp\left[-\frac{2\pi i}{\lambda f}(ux + vy)\right] \, dx \, dy,$$  
(1)

where $f$ is the focal length of the objective, and $(u, v)$ is the spatial-frequency coordinate. If the illumination angle is $(\theta_x, \theta_y)$, the Fourier spectrum becomes

$$H_{\theta_x, \theta_y}(u, v) = \int_{-\infty}^{\infty} O(x, y) \times \exp\left[j2\pi\left(\frac{\sin \theta_x}{\lambda}x + \frac{\sin \theta_y}{\lambda}y\right)\right] \times \exp\left[-\frac{2\pi i}{\lambda f}(ux + vy)\right] \, dx \, dy,$$

$$= H(u - u_0, v - v_0),$$  
(2)

which indicates that the Fourier spectrum is shifted to $(u_0, v_0)$ owing to the illumination angle. It has been proven that $u_0 = f \sin \theta_x$ and $v_0 = f \sin \theta_y$ [28]. Because of the limited NA of the system, only a part of the Fourier spectrum transmits the tube lens. Let the radius of the transmitted sub spectrum $H_{\theta_x, \theta_y}^{\text{sub}}(u, v)$ be $r$. It can then be written as

$$H_{\theta_x, \theta_y}^{\text{sub}}(u, v) = H(u - u_0, v - v_0) \times \text{circ}\left(\frac{\sqrt{u^2 + v^2}}{r}\right),$$  
(3)

where $\text{circ}\left(\frac{\sqrt{u^2 + v^2}}{r}\right)$ is the circle function. Suppose that the tube lens has the same focal length as the objective lens. Then, the captured image at a specific illumination angle can be written as

$$I_{\theta_x, \theta_y}(x, y) = \mathcal{F}^{-1}\left\{ H(u - u_0, v - v_0) \times \text{circ}\left(\frac{\sqrt{u^2 + v^2}}{r}\right) \right\}^2 = O(x, y) \times \exp\left[j2\pi\left(\frac{\sin \theta_x}{\lambda}x + \frac{\sin \theta_y}{\lambda}y\right)\right] \otimes J(x, y)^2,$$  
(4)

where $\mathcal{F}^{-1}$ is the inverse Fourier transform, $J(x, y) = \frac{\lambda f}{\sqrt{x^2 + y^2}} \text{J}_1\left(\frac{2\pi \sqrt{x^2 + y^2}}{\lambda f}\right)$ is the inverse Fourier transform of the circle function, and $J_1(x, y)$ is the first-order Bessel function.

In the case where the phase information of the sample can be neglected, it equals an amplitude-only object, i.e. $O(x, y) = A(x, y)$, and equation (4) can be written as

$$I_{\theta_x, \theta_y}(x, y) = \left| A(x, y) \times \exp\left[j2\pi\left(\frac{\sin \theta_x}{\lambda}x + \frac{\sin \theta_y}{\lambda}y\right)\right]\right| \otimes J(x, y)^2.$$  
(5)

From equation (5), it can be seen that the captured intensity images $I_{\theta_x, \theta_y}(x, y)$ equal $L_{\theta_x, \theta_y}(x, y)$ strictly.

In the case where the phase information of the sample cannot be neglected, equation (4) should be written as

$$I_{\theta_x, \theta_y}(x, y) = \left| A(x, y) \times \exp\left[j\phi(x, y) + 2\pi\left(\frac{\sin \theta_x}{\lambda}x + \frac{\sin \theta_y}{\lambda}y\right)\right]\right| \otimes J(x, y)^2.$$  
(6)

Because of the phase factor $\phi(x, y)$, we cannot observe how the phase influences the captured intensity images directly from equation (6).

From the theoretical analysis of the FPM imaging system, we obtain a preliminary conclusion that the images captured with circular symmetrical illumination angles have no intensity difference when the object is amplitude-only. Considering that FPM uses only intensity images to reconstruct wide-FOV and high-resolution images, we speculate that the images reconstructed with the half number of low-resolution images can
attain a comparable resolution to those reconstructed with the whole images in FPM.

3. Simulation verification

We performed two simulations to verify the above analysis. In these simulations, we used a $4 \times 0.1$ NA microscopic objective and a $15 \times 15$ LED array. The wavelength of the LED array was 630 nm, and the spacing between two adjacent LEDs was 4 mm. The distance between the LED array and the sample was 110 mm. The camera sensor had $128 \times 128$ pixels, with a pixel pitch of 6.5 μm.

In the first simulation, we compared the images captured by a microscope at symmetrical illumination angles. Figure 2 shows the amplitude and phase profiles of the object used in this simulation. The original object had $512 \times 512$ pixels. The pixel number of the low-resolution pictures captured by the camera was $128 \times 128$. The object was set to be at the focal plane of the microscope.

To compare the captured images at a circular symmetrical illumination angles, we calculated the structural similarity (SSIM) index between them. The images are more identical when the SSIM index approaches 1. Figure 3(a) shows the locations of the LED pairs used in the simulation. A total of seven pairs of images were captured at different illumination angles. Figure 3(b) shows the SSIM index distribution at different illumination angles and object phase ranges. The horizontal axis is consistent with the pair numbers in figure 3(a). To observe the influence of the object phase, we set different ranges of the phase value. In figure 3(b), we observe that the images captured under circular symmetrical illumination angles exhibit no intensity difference when the sample is amplitude-only. When consider the phase factor, the SSIM is inversely proportional to the range of the phase value and directly proportional to the illumination angle. The effect of the object phase range decreases as the illumination angle increases. The simulation results agree with the analysis in section 2. Considering the numerical analysis and the simulations, we conclude that the images captured at symmetrical illumination angles are identical when the sample is amplitude-only.

The second simulation was performed to verify our speculation. The amplitude and phase profiles of the object used in the FPM simulation are the same as in the first simulation. The recovery process of the FPM follows the strategy of the phase-retrieval technique. The algorithm switches between the spatial and Fourier domains. In the spatial domain, the low-resolution intensity measurements are used as the object constraints to ensure the solution convergence. In the Fourier domain, the confined coherent transfer function of the objective lens is imposed as the support constraint. After several iterations, the high-resolution complex field of the object can be obtained. Figure 4 shows the reconstructed results for an amplitude-only object. Figure 4(a) shows the reconstructed intensity and Fourier spectra with the whole ($15 \times 15$) captured images, and figure 4(b) shows those for the half ($15 \times 8$) the captured images. Compared
with figure 4(a), we can observe contrast difference in figure 4(b). The SSIM index of the two images is 0.9177. This is because we directly apply an inverse Fourier transform to the half-spectrum of the object to reconstruct the intensity image, which can induce a ringing effect and cause a blur effect. Considering the fact that the Fourier spectrum of a real function has conjugate symmetry, we propose two methods for compensating the other half of the spectrum. Figure 4(c) shows the results of method 1. In this way, we hold back the top half of the spectrum in figure 4(b) and complement the bottom half of the spectrum by performing a conjugate symmetry translation on the above half parts. The SSIM index of the intensity images between figures 4(a) and (c) is 1.0. Figure 4(d) shows the results of method 2. We hold back all of the spectrum in figure 4(b) and complement the left spectrum in the yellow rectangle by performing a conjugate symmetry translation on the corresponding spectrum in figure 4(b). The SSIM index of the intensity images between figures 4(a) and (d) is 0.9975, which indicates that the image quality of the reconstructed intensity image in figure 4(d) is nearly the same as that of figure 4(a). Therefore, after spectrum compensation for the amplitude-only object, the intensity images reconstructed using only half of the captured images have no degradation.

To observe the influence of the phase of the object, we calculated the SSIM index between the reconstructed intensity images using all and half of the captured images with different phase ranges. Figure 5 shows the results. From the trend of the curves, we observe that the degree of image degradation for method 2 is lower than that for method 1. The reason is that method 2 retains the whole reconstructed spectrum by employing half of the captured images. Combined with the results of figure 3, we observe that the captured images exhibit less difference at large illumination angles. Because method 2 retains enough low-frequency information, we use it for reconstructing all of the experimental data.

4. Experimental verification

We built an FPM system by replacing the light source of an Olympus IX 73 inverted microscope with a programmable
LED array (32 × 32 LEDs, 4 mm spacing) controlled by an Arduino. The LEDs had a central wavelength of 629 nm and a bandwidth of 20 nm.

Two experiments were performed to verify our speculation. In one of them, the USAF-1951 resolution target was the object, which can be regarded as an amplitude-only like object. The other experiment used a microscopic object having phase information. Both samples were imaged with a 4 × 0.1-NA microscopic objective and a complementary metaloxide semiconductor (CMOS) camera (PCO, edge 4.2). The pixel size of the CMOS is 6.5 μm. In the experiments, the central 17 × 17 LEDs were turned on sequentially so that 289 low-resolution images were captured. The exposure time of the camera was 600 ms per shot. The distance between the LED array and the sample was 113.5 mm. The expected synthesized NA of the imaging system is 0.48.

The reconstructed results of the first experiment are shown in figure 6. Figure 6(b) shows one of the segments of the original captured images. Figure 6(c) shows the image reconstructed using 289 images, and figure 6(d) shows the result obtained using 153 images. Figure 6(e) shows the intensity profile along the red vertical lines in figures 6(c), (d), and (f) shows that along the yellow horizontal lines. For figures 6(e) and (f), we observe that the shapes are similar. The SSIM index between figures 6(c) and (d) is 0.8304. In both figures 6(c) and (d), we can distinguish the smallest bar that is in group 8, element 6 of the USAF resolution target, whose line width is 1.1 μm. This means that the image resolution of figures 6(c) and (d) has no significant difference.

The reconstructed results of the second experiment are shown in figure 7. Figure 7(b) shows one of the segments of the original captured images. Figure 7(c) shows the intensity image reconstructed using 289 images. Figure 7(d) shows the intensity image reconstructed using 153 images. Figure 7(e) shows the intensity profile along the red vertical lines in figures 7(c) and (d). Figure 7(f) shows the intensity profile along the yellow horizontal lines in figures 7(c) and (d). For figures 7(e) and (f), the shapes are similar. The SSIM index between figures 7(c) and (d) is 0.7507. Because the phase information of the sample is not obvious and it is close to an amplitude-only object, the similarity between figures 7(c) and (d) is still high. Compared with the amplitude-only like object in the first experiment, although the SSIM index decreases slightly, there is no significant difference in image resolution between figures 7(c) and (d).

5. Conclusion

Under the assumption of thin biological samples for FPM, both theoretical analysis and simulations of a microscopic imaging system revealed that images captured with circular symmetrical illumination angles have little intensity difference when the sample is amplitude-only; therefore, the FPM reconstructions show an insignificant difference. In FPM experiments, amplitude-only like objects were used to perform the verification. The reconstructions did not show clear
image resolution degradation between the cases where all and half of the images were used. For amplitude-only like samples, the half number of captured images can be used. Owing to the image reduction in this method, the time costs of both image capture and computational processing were reduced by half.

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Figure 7. (a) Captured image of a plant root; (b) part within the red rectangle in (a). (c), (d) Intensity images reconstructed with all and half of the captured images, respectively. (e), (f) Intensity profiles along the red vertical and yellow horizontal lines in (c) and (d), respectively.
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