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A color-corrected strategy for information multiplexed Fourier ptychographic imaging

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ABSTRACT

Fourier ptychography (FP) is a novel computational imaging technique that provides both wide field of view (FoV) and high-resolution (HR) imaging capacity for biomedical imaging. Combined with information multiplexing technology, wavelength multiplexed (or color multiplexed) FP imaging can be implemented by lighting up R/G/B LED units simultaneously. Furthermore, a HR image can be recovered at each wavelength from the multiplexed dataset. This enhances the efficiency of data acquisition. However, since the same dataset of intensity measurement is used to recover the HR image at each wavelength, the mean value in each channel would converge to the same value. In this paper, a color correction strategy embedded in the multiplexing FP scheme is demonstrated, which is termed as color corrected wavelength multiplexed Fourier ptychography (CWMFP). Three images captured by turning on a LED array in R/G/B are required as priori knowledge to improve the accuracy of reconstruction in the recovery process. Using the reported technique, the redundancy requirement of information multiplexed FP is reduced. Moreover, the accuracy of reconstruction at each channel is improved with correct color reproduction of the specimen.

1. Introduction

Fourier ptychographic microscopy (FPM) is a fast-growing computational imaging technique that can overcome the space-bandwidth product limit of a low numerical aperture (NA) imaging system [1]. Both wide-field and high-resolution images of a sample can be obtained without mechanical scanning and interferometric measurements. Owing to its various applications, FPM shows its potential to be used in modern biomedical research, digital pathology, and so on [2].

With similar concept, FPM has developed rapidly with other computational imaging techniques, such as diffractive ptychography and lensfree holographic microscopy [3,4]. Compared with synthetic aperture techniques, FPM has several advantages. Rather than requiring data by mechanical scanning, a programmable LED array substitutes for the original illumination of a microscope to obtain a stack of low-resolution (LR) images of the sample under different illuminating angles. Using the phase retrieval technique, both the HR intensity and the phase information of the object can be resolved with multiple intensity measurements [5–8]. Besides, as with other computational imaging techniques, aberrations of the optical system can be corrected by implementing the wave-front correction digitally [9,10]. Meanwhile, positional misalignment of the light source could also be corrected by stochastic algorithms, such as simulated annealing method [11–13]. Furthermore, the capacity of high NA imaging has recently been reported, which achieved half-pitch resolution of 154 nm at a wavelength of 435 nm with a 10×, 0.4 NA objective lens. This shows further possibilities of label free super resolution imaging [14].

Information multiplexing theory evolved from diffractive ptychography [15–17], which has improved the efficiency of FPM data acquisition. With wavelength multiplexed FP, HR images of samples at R/G/B channels can be recovered by turning on R/G/B LED units simultaneously. Compared with capturing LR images with a monochrome camera at three different wavelengths respectively, the wavelength multiplexed FP reduces the data size by three times. However, since the same dataset is used to recover three HR images at three wavelengths, the mean values

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of HR images converge to roughly the same value. In other words, diversity of the samples at different wavelength illuminations is lost in the data acquisition process which will cause a color reproduction problem in recovered result. In digital pathology and biomedical imaging, color information can be very important, particularly in applications such as clinical diagnosis and cell classification.

In this paper, a color correction strategy for multiplexing FP imaging is introduced which requires three additional intensity measurements, while turning on a LED array at each wavelength. These three images can be considered as an incoherent summation of different coherent states. Thus, by bringing these three state multiplexed images into the recovering process and updating the recovered spectrum in Fourier space, the HR image at each channel can be recovered without any resolution (detail) loss. Both simulations and experiments are presented to support the reported scheme. This reported technique, which has been termed color corrected wavelength multiplexed Fourier ptychography (CWMFP), utilizes information multiplexing theory flexibly, which may open up new potential for information multiplexed FP.

2. Methods and simulations

2.1. Forward imaging model of Fourier ptychography

A typical FP microscope is composed of an angle-varied coherent light source (i.e. a programmable LED array or laser source), and a conventional microscope, as shown in Fig. 1(a). The parameters of the system, such as pixel size of imaging sensor and distance between the light source and the object plane affect the overlap ratio $R_{overlap}$ of the pupil in the Fourier plane. The $R_{overlap}$ will further affect the speed and reliability of convergence in reconstruction [18,19]. With the FP concept, the final NA of the system is the sum of illumination and objective NAs, $\mathrm{NA}_{eff} = \mathrm{NA}_{ill} + \mathrm{NA}_{obj}$.

For an imaging system which has a coherent impulse response $h(r)$ and a thin sample $U_o(r)$, where $r = (x, y)$ denotes the lateral coordinates at the sample plane, the imaging process can be described in its simplest form as a convolution operation, $U_i(r) = h(r) * U_o(r)$. In the Fourier plane it can be described as, $G_i(f) = G_o(f) \cdot P(f)$. Where $G_o(f) = \mathscr{F}\{U_o(r)\}$, $\mathscr{F}\{\}$ denotes the Fourier transform operation. $P(f)$ is the pupil function of the imaging system determined by the pixel size of the imaging sensor and cutoff frequency $f_0 = k_0 \cdot \mathrm{NA}_{obj}$, which can be considered as a low-pass filter in an imaging system (dashed white circle in Fig. 1(d1) and (d2)), k_0 is the wavenumber at a wavelength of λ. $f = (u, v)$ denotes the coordinates in the Fourier space [20,21].

Assuming a single LED unit illuminates the sample with an oblique plane-wave which has a wave-vector $k_n = (kx_n, ky_n)$, where $n = 1, 2, 3, \ldots, N$, N is the total number of units in LED array. The exit light wave from the illuminated sample can be written as $U_e(r) = U_o(r)e^{(ik_n r)}$ and its spectrum $G_e(f - f_n)$ in the Fourier plane has shifted, which means every LED unit corresponding to a specific region in the Fourier space. Therefore, the intensity measurement that the camera captured is

$$I_n(r) = |\mathscr{F}^{-1}\{G_e(f - f_n)P(f)\}|^2. \tag{1}$$

In the case of multiplexing, when the sample is illuminated by multiple LED units, the illumination is normally considered partially coherent. This can be considered as every LED unit at a certain wavelength corresponding to a coherent state. Hence, the multiplexed intensity can be considered as the sum of all states of illumination. For L states of illumination, for example, L monochrome units of LED were turned on every time, the mth multiplexed intensity $I_{L_m}(r)$ can be described as

$$I_{L_m}(r) = \sum_{n \in L} |\mathscr{F}^{-1}\{G_e(f - f_n)P(f)\}|^2, \tag{2}$$

where the set L is chosen from all states of illumination and the symbol $\in$ denotes that n is an element from L.

2.2. Color corrected wavelength multiplexed Fourier ptychography

It is worthwhile reviewing the recovery concept of FP, since the CWMFP is embedded in the information multiplexing FP framework. The basic tool used to recover intensity and phase information was a typical alternating projection (AP) method. That is, solving the phase retrieval problem with known constraints in an iterative manner. In the conventional case of FP, the intensity measurement was set as the constraint in spatial space and the pupil function was the constraint in Fourier space. By applying the constraints in the iterative recovery process, both HR intensity and phase distribution can be restored. Furthermore, with the embedded pupil function recovery technique, pupil function with aberrations can also be restored [22].

Fig. 2 shows a brief scheme of CWMFP. The whole recovery process starts with an initial guess of sample in spatial domain, $U^0_{\lambda,n} = A_0 e^{(i\varphi_0)}$ (the coordinates were concealed to simplify the equations) where A_0 and φ_0 could be random guesses of the sample, and an initial guess of pupil function $P^0_{\lambda,n}$ which is a binary circle determined by NA of the objective and illumination wavelength λ. In general, a selected initial guess which is close to the ideal image of the sample will further accelerate the recovery. In Fourier domain, the initial guess $O^0_{\lambda,n}$ is the Fourier transform of the initial guess $U^0_{\lambda,n}$, $O^0_{\lambda,n} = \mathscr{F}\{U^0_{\lambda,n}\}$. In the updating process, assuming the iteration indices $k = 0, 1, 2, \ldots, k_{max}$ and the initial guess is set with $k = 0$ and $n = 1$. At k_{th} iteration, a region of the spectrum corresponding to an oblique plane-wave illuminating is inverse Fourier transformed to obtain an estimate of object, $\Psi^k_{\lambda,n} = \mathscr{F}^{-1}\{O^k_{\lambda,n} \cdot P^k_{\lambda,n}\}$. For the conventional FP, the amplitude of $\Psi^k_{\lambda,n}$ is replaced by the square root of intensity measurement whilst retaining the individual phase, $\bar{\Psi}^k_{\lambda,n} = \sqrt{I_{\lambda,n}}\frac{\Psi^k_{\lambda,n}}{|\Psi^k_{\lambda,n}|}$. In the case of multiplexing, the amplitude is replaced by a decomposed amplitude which is created from the integral of each illumination function. Assuming I_{L_m} is the mth intensity measurement under the multiplexing condition, for L states of illumination, it can be described as

$$\bar{\Psi}^k_n = \frac{\sqrt{I_{L_m}}}{\sqrt{\sum_{n \in L} |\Psi^k_n|^2}} \cdot \Psi^k_n. \tag{3}$$

In the case of the wavelength multiplexing, for each wavelength $\lambda = \lambda_1, \lambda_2, \lambda_3$, which corresponds to each wavelength of LED at R/G/B, respectively, the updated estimate can be described as,

$$\bar{\Psi}^k_{\lambda,n} = \frac{\sqrt{I_{L_m}}}{\sqrt{|\Psi^k_{\lambda_1,n}|^2 + |\Psi^k_{\lambda_2,n}|^2 + |\Psi^k_{\lambda_3,n}|^2}} \cdot \Psi^k_{\lambda,n}. \tag{4}$$

Next, The updated spectrum $\bar{O}^k_{\lambda,n}$ can be obtained by transforming the updated estimate $\bar{\Psi}^k_{\lambda,n}$ to the Fourier space and applied pupil constraint, $\bar{O}^k_{\lambda,n} = \mathcal{F}\{\bar{\Psi}^k_{\lambda,n}\} \cdot P^k_{\lambda,n}$. The updated spectrum $\bar{O}^k_{\lambda,n}$ is then used to replace the corresponding region $O^k_{\lambda,n}$ in the Fourier space with the following equations:

$$O^{k+1}_{\lambda,n} = O^k_{\lambda,n} + \alpha \frac{(P^k_{\lambda,n})^*}{|P^k_{\lambda,n}|^2_{max}} [\bar{O}^k_{\lambda,n} - O^k_{\lambda,n}], \tag{5}$$

$$P^{k+1}_{\lambda,n} = P^k_{\lambda,n} + \beta \frac{(O^k_{\lambda,n})^*}{|O^k_{\lambda,n}|^2_{max}} [\bar{O}^k_{\lambda,n} - O^k_{\lambda,n}], \tag{6}$$

where $O^{k+1}_{\lambda,n}$ and $P^{k+1}_{\lambda,n}$ are new spectrum and pupil function in the $(k+1)_{th}$ iteration, $O^k_{\lambda,n}$ and $P^k_{\lambda,n}$ are spectrum and pupil function waiting for updating in the k_{th} iteration. α and β are step-size and $*$ denotes the conjugate symbol.

It should be reiterated that $n = 1, 2, 3, \ldots, N$ is corresponding to the updating sequence of reconstruction which is determined by illumination NA. That is, a spiral path from inside to outside in the Fourier space. When all regions corresponding to oblique illumination