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Relation between substructure position of phase objects in optical axial direction and phase information in quantitative phase imaging

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ABSTRACT

Substructure position of biological cells and tissues owns much more information about the state and evolution of these cells and tissues. Comparing with the substructure position of these biological objects at the plane perpendicular to the optical axial, the substructure position of these objects in optical axial direction is hard to be measured by the general quantitative phase imaging (QPI). Here, based on the split-step beam propagation theory, we numerically investigate the relation between substructure position of phase objects in optical axial direction and phase information in QPI. Five groups of two-sphere model with the internal sphere at different positions in optical axial direction are studied. The results show that the position of the internal sphere in optical axial direction does not change the general morphology of the phase map, but it leads to some differences near the boundary of the internal sphere. Further, we get the relation between the maximum positive phase deviation and the position of the internal sphere in optical axial direction. Such a relation will provide the theoretical basis for the analysis of experimental results in phase imaging.

1. Introduction

Biological cells and tissues play the fundamental role in biology and medicine and have attracted much more attention. The biological cells and tissues are rich in inner substructure, whose information is a reasonable indicator of the cells' or tissues' life and evolution [1–5]. This information includes the composition of the substructure, the shape of the substructure and the position of the substructure. Compared with both the composition (evaluated through the refractive index) and the 2D shape of the substructure which have been investigated broadly [6–11], the position of the substructure is rarely studied.

The composition and shape of the biological cells and tissues are usually investigated by Quantitative phase imaging (QPI) [8–20]. In this technique, some strategies [9,10,16–18] have been used to separate both the shape information and the refraction index distribution from the original images. Most of these strategies are based on the fact that the phase information is modulated by the biological cells or tissues as well as their substructure. And thus by these strategies the position of the substructure in the plane perpendicular to the optical axial direction (SPPO) can be clearly demonstrated. Compared with SPPO, the substructure position in the optical axial direction (SPO) is hard to be determined from a single image from QPI method [21,22] due to that little change in phase information occurs when varying SPO. It is worth noting that SPO is necessary in evaluating the variation of 3D substructure position [21]. Therefore, how to determine SPO becomes a critical work to recover all the information of the biological cells or tissues.

One way to determine SPO is to extract SPO from the 3D distributions of RI reconstructed by optical tomographic methods in QPI [8,23–25]. The common idea of the optical tomographic methods is to reconstruct 3-D information from multiple 2-D images with various illumination angles by the projection [8,23,24] or the diffraction tomography algorithms [25–28]. In the projection algorithm, diffraction of light in a sample is assumed to be negligible, while in diffraction tomography algorithms, the effect of diffraction is considered. Both algorithms have been successfully used in reconstructing a 3D RI distribution of the biological cells [8,23–25]. However, due to the finite numerical aperture of an imaging system, these methods are in the lack of complete angular coverage. To fill the missing information, the iterative constraint algorithm [27,29] can be executed to the tomograms reconstructed from both the projection and diffraction algorithms, but it is time consuming. In addition, to obtain multiple 2-D images in tomographic methods, the sample rotation [24]

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or the illumination beam rotation [8,23,27,28] is necessary, making the experimental study of the 3-D maps of refractive index distribution in biological cells generally more complicated. Therefore, to obtain SPO of biological cells and tissues, a reasonable way is extracting information from a single image of the biological cells obtained from QPI, which may offer real-time imaging.

It is noteworthy that the image of biological cells obtained from QPI may implicate the information of SPO due to the diffraction effect. In the existing results of QPI [14,19], we note that the phase distribution around a phase object is affected by the phase object. This phenomenon is induced by the diffraction effect, which may be modulated by the position of the small structure in the optical axial direction. Similarly, such a diffraction effect may exist near the substructure inside biological cells or tissues and can be used to evaluate SPO. Therefore, in this paper, we will investigate the relation between SPO and the diffraction effect.

Since it is very difficult to investigate the influence of SPO on QPI by experimental method, computer simulation can be the best choice. Simulation optical wave propagation through many types of materials such as inhomogeneous, anisotropic and nonlinear is often accomplished via the split-step beam propagation method (BPM) [30]. Although this method does not work for highly diffractive samples, it is applied appropriately for the biological cells and tissues in which refractive index generally does not change significantly. Therefore, BPM is introduced in this paper to analysis the light propagation through the biological cells and tissues in order to investigate the diffraction effect induced by the biological cells and their substructure. The reliability of this method will be demonstrated with the phase imaging simulation of a one-sphere model. Then five groups of two-sphere model with the internal sphere at different positions in optical axial direction will be used for the phase imaging by this method. And in these cases the influence of the internal sphere position on quantitative phase imaging will be studied.

2. Method

The simulation method for QPI, which is based on four-step phase-shifting digital holography, has been detailed in elsewhere [31]. According to this simulation method, the crucial issue for studying the influence of SPO on the information of QPI is to obtain the more accurate object wave on the object plane. In the present case, the optical axial thickness of the object can not be ignored again in the simulation of the object wave on the object plane. Therefore, BPM, which has been confirmed to be useful for simulating propagation through many types of materials, is introduced to simulate the object wave through the object with substructure in this paper.

According to BPM, the beam evolution can be calculated numerically by the following expression for each increment of propagation distance Δz [30]:

$$U_e(x,y,z+\Delta z) = \exp(\hat{D}\Delta z)\exp(\hat{S}\Delta z)U_e(x,y,z) \quad (1)$$

where $U_e(x,y,z)$ is the slowly varying envelope of the field, $\hat{D} = \nabla_t^2/2jk_0$ and $\hat{S} = -j\Delta n k_0$ are the linear operator and nonlinear operator, in which ∇_t^2 denotes the transverse Laplacian operator, $k_0 = 2\pi n_0/\lambda$, λ is the free-space wavelength, n_0 is the ambient refractive index of the medium, and Δn is the change in the refractive index over the ambient refractive index n_0. The first operator on the right hands of Eq. (1) is the propagation operator that takes into account the effect of diffraction between position z and $z+\Delta z$. Under paraxial approximation, the diffraction propagation calculation can be expressed as [32]

$$\exp(\hat{D}\Delta z)U_e(x,y,z) = \mathcal{F}^{-1}\{\mathcal{F}\{U_e(x,y,z)\}H_{\mathcal{F}}(f_x,f_y)\} \quad (2)$$

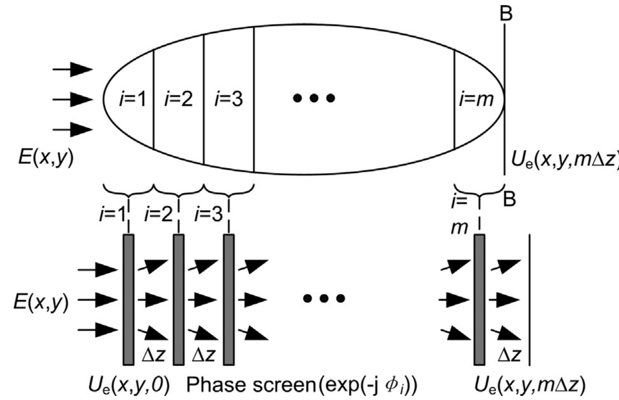

Fig. 1. Physical interpretation of BPM in an ellipsoid phase object.

where $H_{\mathcal{F}}(f_x,f_y) = \exp\{jk\Delta z[1-(\lambda^2(f_x^2+f_y^2)/2]\}$ is Fresnel transfer function, f_x and f_y are the spatial frequency in x direction and y direction, respectively, $\mathcal{F}$ and $\mathcal{F}^{-1}$ express Fourier transform and inverse Fourier transform, respectively. The second operator on the right hands of Eq. (1) describes the propagation in the presence of medium inhomogeneities without considering the effect of diffraction, and it can be incorporated in the spatial domain. Therefore, the algorithm for a single step Δz can be gotten by

$$U_e(x,y,z+\Delta z) = \exp(\hat{S}\Delta z)\mathcal{F}^{-1}\{\mathcal{F}\{U_e(x,y,z)\}H_{\mathcal{F}}(f_x,f_y)\} \quad (3)$$

By repeating the above process, the field in desired plane can be obtained. In our case, it is assumed that a plane wave radiates upon an ellipsoid phase object, as shown in Fig. 1. Then the transmitted light in B-B plane can be calculated by using BPM. For the calculation, the phase object model is evenly split into m parts along the optical axis, and the phase modulation of each part to its incident wave is considered as a phase screen in corresponding position in Fig. 1. The process of the plane wave transmitting through the object can be broken into a series of finite steps, which includes the transmitting process of the wave through a phase screen and the diffraction of wave for a distance of Δz. It is noteworthy that in each part, the phase delay is less than $\pi/2$ due to the following reason. First, there is no major change in the refractive index of the object ($\Delta n \leq 0.03$) and only very small difference between the refractive index of the object and that of surrounding ($\Delta n \leq 0.04$). Second, each part has only a thickness of $\sim 1\ \mu$m. Therefore, the BMP, similar to the diffraction method with Born approximation (the scattered field is much weaker than the incident field) [27], can be used in our cases. From the lower part of Fig. 1, one can see that the plane wave $E(x,y)$ irradiates upon the first phase screen, which acts as a phase modulation induced by the first part of the object shown in the upper part of the Fig. 1. And then the resulting field $U_e(x,y,0)$ is diffracted a distance of Δz. After this diffraction, the diffraction field is phase-modulated by the second phase screen before being diffracted another distance of Δz. The transmitting process mentioned above is repeated until getting the final output $U_e(x,y,m\Delta z)$, which expresses the object wave on the B-B plane in the upper part of the Fig. 1. However, the object wave on the B-B plane is not the desired information in our phase imaging simulation. The desired information is the object wave on the object plane. Therefore, if we choose the center plane in the optical axial direction as the object plane, the desired object wave $O_0(x,y)$ can be obtained from $U_e(x,y,m\Delta z)$ by invert diffraction calculation and expresses as following:

$$O_0(x,y) = \mathcal{F}^{-1}\{\mathcal{F}[U_e(x,y,m\Delta z)]\exp\{jkd[1-(\lambda^2(f_x^2+f_y^2)/2)]\}\} \quad (4)$$

where $d = -m\Delta z/2$ in this case.