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Accuracy improvement in digital holographic microtomography by multiple numerical reconstructions

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ABSTRACT

In this paper, we describe a method to improve the accuracy in digital holographic microtomography (DHMT) for measurement of thick samples. Two key factors impairing the accuracy, the deficiency of depth of focus and the rotational error, are considered and addressed simultaneously. The hologram is propagated to a series of distances by multiple numerical reconstructions so as to extend the depth of focus. The correction of the rotational error, implemented by numerical refocusing and image realigning, is merged into the computational process. The method is validated by tomographic results of a four-core optical fiber and a large mode optical crystal fiber. A sample as thick as 258 μm is accurately reconstructed and the quantitative three-dimensional distribution of refractive index is demonstrated.

1. Introduction

As a combination of digital holographic microscopy (DHM) and optical tomography, digital holographic microtomography (DHMT) provides efficient access to quantitative three-dimensional (3D) distributions of refractive index (RI), which play a critical role in internal structure inspections of materials as well as observations of transparent samples. DHMT has been widely applied in the studies of optical fibers [1,2], plant fibers [3], biomedical specimens [4–6] and other samples [7].

In DHMT, complex wavefronts that traverse the sample under test are recorded from various directions with DHM, which provides great flexibility in phase retrieval and auto-focusing. The illumination angle can be varied with two main configurations: the illumination-scanning configuration [8–11] and the sample-rotating configuration [2–5]. A method combining both techniques was also reported [12]. The former achieves high stability and high recording speed, but the limited recording angle results in anisotropic resolutions and distorted reconstructions. The latter provides larger frequency coverage and isotropic resolutions but is a relatively slow measurement process. The high quality of reconstructed results makes the sample-rotating configuration widely employed in the measurement of solid samples, such as fibers [1–3] and lenses [13], as well as biomedical specimens in capillaries [4,5,14].

In practice, the accuracy of DHMT is likely to decline due to different factors. One of the key factors is the deficiency of depth of focus. The most basic reconstruction algorithm used in DHMT is filtered backprojection, based on the Fourier slice theorem [15], in which light is assumed to traverse the sample along straight lines. However, this assumption restricts the accurate reconstruction volume to a small region around the rotation axis. Since the algorithm does not take into account the defocusing far from the focal plane, tomograms are blurred and deformed outside of the central region when the thickness of the sample exceeds the depth of field of the imaging system. In fact, a microscope objective (MO) with a high magnification and a large numerical aperture (NA) provides a small depth of field (usually several microns). Therefore, deficiency of depth of focus is a general problem encountered in microtomography, especially at high magnification. Another key factor impairing the accuracy of sample-rotating DHMT is the rotational error during measurement. The error is mainly induced by the mechanical positioning error [16] between the axis of the sample and the rotation axis and the vibration of both the sample and the setup. The former causes a periodic deviation and the latter results in a random shift. The lateral component of the error, also called radial runout, makes the sample shift in the projection images, causing misalignment, while the axial component pulls the center of the sample away from the focus, causing defocusing.

The deficiency of depth of focus is often circumvented by an alternative algorithm called filtered backpropagation [17]. This algorithm, based on the Fourier diffraction theorem [15], takes diffraction effects into account. Numerous researches [10,13,14,18]

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have been demonstrated where the diffraction tomography algorithm is employed to obtain 3D RI maps with better accuracy and higher resolution. Kostencka et al. [19] compared filtered backprojection and filtered backpropagation and concluded that the latter achieved better accuracy of off-axis results but still suffered from a limited accurate reconstruction volume. They also proposed a modified algorithm producing high accuracy in full volume of the reconstruction. Besides, various schemes have been developed to straightforwardly extend the depth of focus for accurate tomography of thick samples. The most typical one is mechanical scanning [20,21], in which the MO moves axially so that different areas are focused successively. However, a precision and stable mechanical device is required, thereby complicating the optical setup. Numerical equivalents have also been proposed to avoid this problem. Choi et al. [9] proposed an algorithm to extend the depth of focus for illumination-scanning microtomography by heterodyne interferometry, where light fields were propagated numerically to multiple planes after reconstruction.

As for the rotational error, careful mechanical adjustment before measurement is essential for the alignment of the axis of the sample and the rotation axis. Various devices have also been applied to stabilize the sample, such as precision rotation stages, syringe needles for fibers [22], micropipettes for biomedical specimens [4,5], etc. Another method [7] was proposed where the experimental setup was rotated while the sample remained static, avoiding potential disturbance to the sample. However, the rotational error is practically impossible to eliminate completely in the setup. Therefore, numerical correction methods have been studied. The lateral component of the error, i.e. radial runout, is usually corrected by realigning the edges in the projection images [23] and the axial component can be reduced by holographically refocusing the recorded light fields [24,25].

In this paper, we achieve accuracy improvement in sample-rotating DHMT of thick samples by multiple numerical holographic reconstructions. The two problems, the deficiency of depth of focus and the rotational error, are addressed simultaneously for high efficiency. The diffraction effects are taken into account by reconstructing the hologram at a series of distances, thus extending the depth of focus to the whole range of the sample, thanks to the capability of DHM to refocus the complex field to arbitrary distances from a single hologram [26–28]. The correction of the rotational error, implemented by refocusing the light field and realigning the images, is merged into the computation process to reduce the computational burden. Quantitative 3D distribution of RI with high accuracy is obtained by the superposition of the matrices of all the recording angles.

2. Multiple numerical reconstruction method

DHM records a hologram which is generated from the off-axis interference between a reference wave and an object wave traversing a sample. The sample is magnified by an MO with the focus located at the center of the sample. The CCD camera is usually positioned behind or in front of the image plane. The preprocessing of a hologram includes frequency domain filtering [29] to eliminate zero-order and conjugate images and phase compensation [30] to correct the residual phase curvature. The light field $u_0(x,y;d=0)$ retrieved from the hologram is propagated numerically to the image plane through a reconstruction distance d with the angular spectrum method:

$$u(x,\, y;\, d) = \mathrm{F}^{-1}\left\{ U_0(k_x,\, k_y;\, d = 0)e^{id\sqrt{k_m^2-k_x^2-k_y^2}} \right\}, \tag{1}$$

where $u(x,y;d)$ is the propagated field with the focus located at distance d; $U_0(k_x,k_y;d=0)$ is the Fourier transform of $u_0(x,y;d=0)$; k_x and k_y are the wavevector projections in the x and y directions, respectively; k_m is the wavenumber of the laser in the medium and $\mathcal{F}^{-1}$ denotes the inverse Fourier transform. The quantitative phase map $\phi(x,y;d)$ is then extracted from the field:

$$\varphi(x,\, y;\, d) = \arctan\left(\frac{\mathrm{Im}\left\{ u(x,\, y;\, d) \right\}}{\mathrm{Re}\left\{ u(x,\, y;\, d) \right\}} \right), \tag{2}$$

where $\mathrm{Im}\{u(x,y;d)\}$ and $\mathrm{Re}\{u(x,y;d)\}$ denote the imaginary and real parts of the complex field, respectively.

In order to extend the depth of focus for accuracy improvement, multiple numerical propagations are required instead of a single one. The hologram is reconstructed using a series of distances and hence the focus is located at various axial positions throughout the sample. Phase distributions are combined to form a projection matrix. The superposition of the filtered matrices at multiple angles produces the tomogram, as depicted in Fig. 1.

In Fig. 1, it is seen that the out-of-focus substructures are superposed with in-focus ones at certain distances so that it is impossible to separate them properly. Through multiple reconstruction, the substructures are focused at different distances. This process, analogous to the diffraction propagation of the light field, can be regarded as a holographic implementation of the backpropagation algorithm [17], which is based on the solution of the inverse scattering problem [31,32].

As for the rotational error, the axial component, causing focus shift of the MO, is corrected by refocusing the light fields, which is also accomplished by multiple reconstructions of a hologram in DHM. Therefore, the refocusing process can be merged into the multiple reconstruction process, instead of implementing time-consuming separate processes. The lateral component of the error is corrected by realigning the edges of the sample after refocusing.

In multiple reconstruction, the interval of distances (denoted as Δd) should be coordinated with the pixel pitch Δx of the CCD in order to keep the tomograms isotropic. With the lateral magnification being M and the axial magnification being the square of it, the interval of distances is then expressed as:

$$\Delta d = \frac{\Delta x}{M}M^2 = M\Delta x. \tag{3}$$

A cubic matrix covering the whole sample thickness with the side length denoted as $2N+1$ is allocated for each recording angle before processing. The retrieved 2D phase maps are stored in the matrix as slices. The directions of the axes are indicated in Fig. 2.

The process of multiple reconstruction is divided into two stages. Refocusing to account for the MO focus shift during rotation is accomplished in the first stage. First, an initial distance d_0 between the image plane of the MO and the CCD is measured. This distance corresponds to a focus inside the sample but may not be accurately in the center. A series of distances are then chosen to be $\{d_0,\ d_0\pm\Delta d,\ d_0\pm 2\Delta d,\ldots,\ d_0\pm L\Delta d\}$ with L being an integer. L is chosen to ensure that the range $[d_0-L\Delta d,\ d_0+L\Delta d]$ covers the maximum axial shift range of the MO focus during rotation. Because the rotational error is reduced beforehand in the setup by mechanical adjustment, the axial shift of the sample is smaller than the thickness of the sample and thus L is smaller than N. Then the preprocessed hologram is numerically propagated to all the distances within the range and both the phase map and the amplitude map are retrieved. Values of a certain refocusing criterion [33] are calculated from the amplitude maps and the phase maps are stored in a matrix. The phase maps are positioned according to their distances with the d_0 map in the middle, as illustrated in Fig. 2(a). Phase unwrapping [34] may be implemented if necessary. After processing for the $2L+1$ distances, the best focusing distance, denoted as $d_0+S\Delta d$ (S is an integer and $-L\le S\le L$), is