Digital holographic microscopy with phase-shift-free structured illumination

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When structured illumination is used in digital holographic microscopy (DHM), each direction of the illumination fringe is required to be shifted at least three times to perform the phase-shifting reconstruction. In this paper, we propose a scheme for spatial resolution enhancement of DHM by using the structured illumination but without phase shifting. The structured illuminations of different directions, which are generated by a spatial light modulator, illuminate the sample sequentially in the object plane. The formed object waves interfere with a reference wave in an off-axis configuration, and a CCD camera records the generated hologram. After the object waves are reconstructed numerically, a synthetic aperture is performed by an iterative algorithm to enhance the spatial resolution. The resolution improvement of the proposed method is proved and demonstrated by both simulation and experiment.

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1. INTRODUCTION

Digital holographic microscopy (DHM) can provide real-time quantitative phase contrast imaging and has become one of the most extensively used tools in the life and material sciences and industries [1–5]. Usually in DHM, the specimen is illuminated by a plane wave, and the spatial resolution is limited by the wavelength (λ) and the numerical aperture (NA) of the objective lens [6]. The improvement of the spatial resolution of DHM can be done by using a short wavelength, although it presents possible photo-damage and phototoxicity to certain types of specimens (e.g., biological tissues) [7]. Another approach is to synthesize a larger hologram through moving the sensor [8], scanning the object [9], or inserting a diffraction grating between object and sensor [10–12]. Off-axis illumination can also improve the resolution by synthesizing a large effective NA. For the off-axis illumination, full spatial frequency coverage is usually achieved by illuminating the object sequentially with different directions of plane waves [13–15]. Another possibility is to use simultaneous illumination with multiple beams with polarization multiplexing [16], or by using vertical cavity surface emitting lasers (VCSEL) [17]. Speckle field illumination was also proposed to improve the resolution of DHM, and the resolution enhancement has been achieved by averaging the object waves under hundreds of different speckle illuminations [18]. Furthermore, the spatial resolution of microscopy can also be improved by projecting a periodic pattern onto the object to be investigated and shifting the illumination patterns three times [19,20]. Usually, such structured illumination is used to obtain resolution improvement for intensity-modulated objects. Recently, Mudassar and Hussain [21,22] applied a scheme for phase imaging by illuminating the object with patterns generated by interference between the beams delivered by two fibers. Ma *et al.* [23] generated the structured illumination by using a diffraction grating and applied it to DHM. Compared with the off-axis and speckle illumination [13–18], structured illumination is more compatible with the inline DHM due to the ease of phase-shifting operation, which can be realized by laterally shifting the structured illumination. Nowadays, the invention and advance of spatial light modulators (SLMs) and digital micromirror devices (DMDs) enable one to generate and shift the structured illuminations without mechanical movement, opening up the possibility for high-speed and high-resolution synthetic aperture imaging.

In our recent paper [24], we also presented the structured illumination generated by SLM for resolution enhancement and autofocusing in DHM. In order to separate the information carried by the $\pm$1st diffraction orders of structured illumination, each direction of the illumination fringes is required to be shifted laterally at least three times. The quantity shifted has to be controlled precisely in order to perform the phase shifting in the reconstruction. This requirement increases the cost of the setup and also retards the fast imaging. In this paper, we propose a scheme for structured illumination without phase shifting in DHM for resolution enhancement. An iterative algorithm is used to synthesize a larger NA among these object waves under different structured illuminations.

2. PRINCIPLE AND METHOD

The setup used for our investigation is schematically shown in Fig. 1. A laser diode with the wavelength 635 nm is used as the light source. The laser beam is expanded and directed onto a SLM to generate the structured illumination. A series of one-dimensional binary phase gratings with orientation rotated by $m \times \pi/M$ ($m = 0, 1, 2, \ldots M-1$, here M is the number of gratings) are loaded sequentially on the SLM. The light modulated by the SLM is de-magnified and projected onto the object plane by the relay optics ($L_1 - MO_1$), which allows for passing only the 0th, ±1st orders produced by each binary grating, and thus the filtered patterns projected on the object are sinusoidal. Under these illuminations, the object waves are magnified by a telescope system composed of the microscopic objective MO_2 and the tube lens L_2. The magnified object waves interfere with the off-axis reference wave to form off-axis holograms, which are recorded by the CCD camera.

For convenience of description, we denote the complex amplitude of the ith illumination field with A^i_{illum}, the off-axis reference wave with R, and the intensity distribution of the ith recorded hologram without specimen by $I^i_{\mathrm{illum}} = |R + A^i_{\mathrm{illum}}|^2$, respectively. When a specimen is inserted and illuminated by the field A^i_{illum}, we denote the wave transmitting through the specimen with A^i_O and the corresponding hologram with $I^i_o = |R + A^i_O|^2$. Provided that the sample is located in such a way that its image plane has a distance Δz from the CCD camera, the object waves can be numerically reconstructed by using the angular spectrum propagation:

$$\begin{cases} A^i_O(x, y, \Delta z) = \mathrm{FT}^{-1}\{\mathrm{FT}\{I^i_O R\} \cdot W_f \cdot H\}, \\ A^i_{\mathrm{illum}}(x, y, \Delta z) = \mathrm{FT}^{-1}\{\mathrm{FT}\{I^i_{\mathrm{illum}} R\} \cdot W_f \cdot H\} \end{cases}. \quad (1)$$

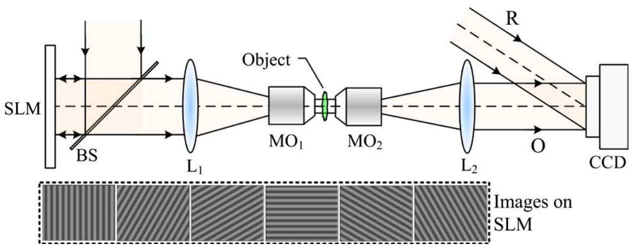

Fig. 1. Schematic of DHM with structured illumination.

Here FT{·} and $\mathrm{FT}^{-1}\{\cdot\}$ denote the Fourier transform and its inverse, respectively. $H = \exp\{ik\Delta z[1 - (\lambda\xi)^2 - (\lambda\eta)^2]^{1/2}\}$, ξ and η are the spatial coordinates in the frequency domain. The window function $W_f(\xi, \eta)$ is used to select the frequency spectrum of the primary lobe. It is worth mentioning that the defocus distance Δz can be determined numerically by searching the minimal difference between the reconstructions $O_i(\Delta z) = A^i_o/A^i_{\mathrm{illum}}$ and $O_j(\Delta z) = A^j_o/A^j_{\mathrm{illum}}$ with respect to different propagation distance Δz [24].

The schematic for the resolution improvement of DHM by the structured illumination is shown in Fig. 2. Each structured illumination can be seen as a superposition of two plane waves, which propagates along the ±1st diffraction orders of the illumination. The two plane waves shift the specimen spectrum in two different directions in the Fourier plane, as shown in Fig. 2(a), middle. Usually, some high frequency components of the object [see the four black dots in Fig. 2(a), left], which are beyond the system aperture, will be cut off by the imaging system aperture under the on-axis plane wave illumination. But under the structured illumination, these high frequency components are downshifted and pass through the system [see the three dots in Fig. 2(a), right]. After these frequency components are shifted back to their original positions, the resolution of the DHM imaging can be improved accordingly. In our experiment, we use the structured illumination that is generated by the 0th, +1st, and −1st diffraction orders of the loaded binary grating. The above analysis on the resolution improvement, as well as the following iteration-based spectrum synthesis method, is still applicable for the structured illumination with 0th, ±1st diffraction orders, and even higher diffraction orders (e.g., ±2nd, ±3rd), which will in turn provide an additional spatial resolution improvement.

The iterative reconstruction and the spectrum evolvement are sketched in Fig. 2(b). The first reconstruction with resolution improvement is obtained by averaging all the reconstructions along different directions of structured illuminations, i.e., $O = (1/M)\sum_{i=1}^{M} A^i_o/A^i_{\mathrm{illum}}$. The averaging operation is mainly used to improve the image quality. Although averaging the object waves under hundreds of different speckle illuminations [18] has been reported, it requires that each speckle illumination has an equal weight for its different frequency spectra. Within our method, the spatial resolution

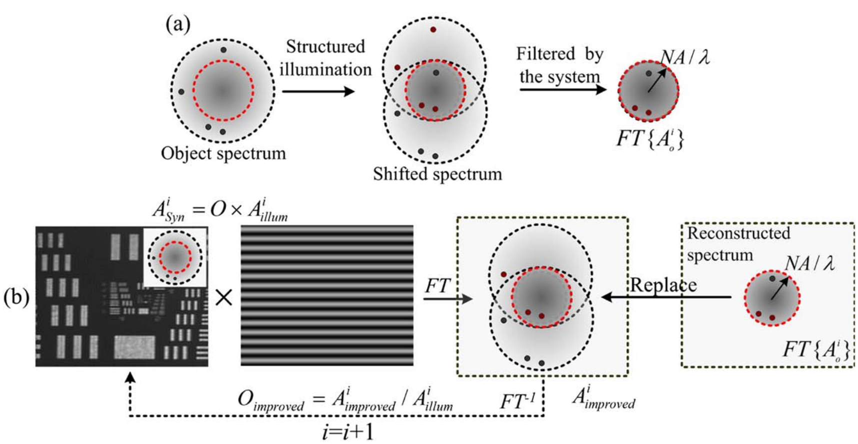

Fig. 2. Resolution enhancement of DHM by the structured illumination. (a) Spectrum evolvement of the object wave during the imaging process. (b) Flowchart of the iteration process for resolution enhancement. The black dots denote high frequency components of the object; the red-dashed circles denote the NA of the optical system.

of the reconstruction is improved by the following iterative algorithm.

Structured illumination is simulated by multiplying the object wave O by the ith structured illumination A^i_{illum}, and the object wave spectrum is duplicated and shifted into two opposite directions (carried by the ±1st order of the illumination). In this calculated spectrum $\text{FT}\{O \times A^i_{\text{illum}}\}$, the frequency components of O locate both inside and outside the NA of the system [see Fig. 2(b)]. The spectrum $\text{FT}\{O \times A^i_{\text{illum}}\}$ has less frequency component inside the NA circle compared to that of $\text{FT}\{A^i_O\}$ that is reconstructed directly from the hologram. Thus, the calculated spectrum is improved by replacing the central part (inside the NA circle) of $\text{FT}\{O \times A^i_{\text{illum}}\}$ with that of $\text{FT}\{A^i_O\}$. After an inverse Fourier transform on the obtained spectrum, the resolution enhancement of the reconstructed object wave can be achieved. In implementation, the iteration process takes the following steps:

1. Initialize the object wave by an averaging of $O = (1/M)\sum_{i=1}^{M} A^i_o / A^i_{\text{illum}}$.
2. Multiply O with the ith structured illumination A^i_{illum} to get the wave $A^i_{\text{syn}} = O \times A^i_{\text{illum}}$.
3. Make the Fourier transform on A^i_{syn}, and replace its central part with that of the spectrum of A^i_O. We denote the obtained spectrum as $\text{FT}\{\cdot\}$.
4. Make the inverse Fourier transform on the obtained spectrum. We get the improved object wave A^i_{improved}.
5. The reconstruction with resolution improved can be obtained by $O_{\text{improved}} = A^i_{\text{improved}} / A^i_{\text{illum}}$.

The iterative process is continued by replacing the initial object wave O with the newly improved object wave O_{improved}. Furthermore, the object waves reconstructed from different groups of structured-illuminated holograms are averaged in order to reduce the noise. The resolution enhancement on the reconstruction is determined by the illumination angle θ_{illum} of the ±1st diffraction orders of the structured illumination. The spatial resolution that can be obtained by using structured illumination is

$$\delta = \frac{\kappa_1 \lambda}{\text{NA} + \sin\theta_{\text{illum}}}. \tag{2}$$

The constant factor κ_1 is determined by the experimental parameters, such as coherent noise level, signal-to-noise ratio of the detector, and so on [25]. Equation (2) describes an enhancement in spatial resolution, compared with the on-axis plane wave illumination where $\theta_{\text{illum}} = 0$. The maximal resolution is $\delta = \kappa_1\lambda/(\text{NA}_{\text{MO1}} + \text{NA})$, when $\sin\theta_{\text{illum}} = \text{NA}_{\text{MO1}}$ with NA_{MO1} being the NA of the objective MO_1. Note that this method enhances the spatial resolution and maintains a large field-of-view and long working distance at the same time. Compared to [21,22], where two fibers were used to generate the structured illumination, here we use a SLM to generate and change the structured illuminations without mechanical movement; thus high speed and high repeatability of phase imaging are achieved. It is worth mentioning that the proposed iterative spectrum synthesis method works only for thin samples. For thick samples, a selective synthesis operation, which synthesizes the spectrum with small z-component, can be applied [26,27].

3. SIMULATION

Simulation has been carried out to verify the feasibility of the proposed method. A specimen is simulated with the amplitude and phase distributions shown in Figs. 3(a) and 3(b),

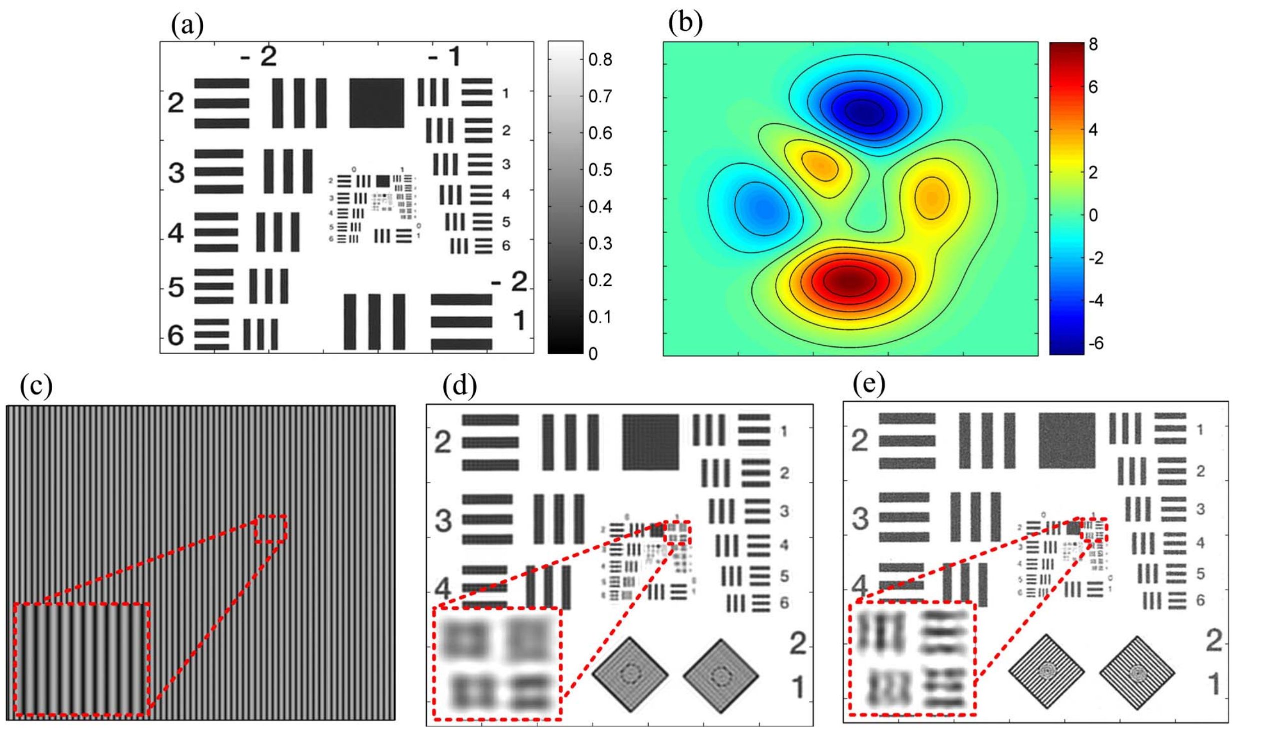

Fig. 3. Simulation results for resolution enhancement by structured illuminations. (a) Amplitude and (b) phase distributions of the simulated specimen. (c) Intensity distribution of the structured illumination along horizontal orientation. (d) and (e) Reconstructed amplitude images by using on-axis plane wave illumination and by structured illuminations with the proposed iterative method, respectively.

respectively. Six structured illuminations A^i_{illum} with the period of 6 pixels and their fringe orientations rotated by $m \times \pi/6(i = 0, 1, 2, 3, 4, 5)$ are simulated, of which one amplitude distribution is shown in Fig. 3(c). The complex amplitude of the simulated object is multiplied sequentially by the six structured illuminations. The spectrum of the obtained object wave is then low-pass filtered by a aperture that truncates the high frequency spectrum over the quantity of 1/(5pixel) in the Fourier domain in order to simulate limited aperture of the imaging system. Finally, the off-axis holograms for both the illumination waves and the object waves are simulated by $|A^i_{\text{illum}}+R|^2$ and $|A^i_O + R|^2$, respectively. Here R represents the reference wave with a linear carrier phase due to the off-axis propagation. The complex amplitudes of A^i_{illum} and A^i_O in the image plane are reconstructed by using Eq. (1). The image along the ith illumination is reconstructed by $O_i = A^i_o/A^i_{\text{illum}}$. For comparison, the imaging process of an on-axis plane wave illumination is also simulated. The reconstructed amplitude images by using the on-axis plane wave illumination and different orientations of structured illumination (reconstructed by the iterative method) are given in Figs. 3(d) and 3(e). The comparison result shows that the reconstruction in Fig. 3(e) has higher spatial resolution than that in Fig. 3(d), which verifies the feasibility of the proposed method.

4. EXPERIMENT

An experiment has been carried out based on the setup described in Fig. 1. Twelve binary phase gratings rotated by $m \times \pi/12(m = 0, 1, 2, 3, \ldots 11)$ are loaded sequentially on the SLM (Holoeye LCR-2500, 1024×768 pixels, pixel size 19 μm) to generate the structured illumination. Four of these binary gratings are schematically shown in Fig. 4(a). A transparent glass plate (1 cm × 1 cm) was used as a sample, which had different scales of phase structures on the plate. In our experiment, two lenses are used instead of the objective lenses MO_1 and MO_2 in order to image such a large sample. A telescope system $MO_2 - L_2$ with a magnification of $M = 3$ and a low $\text{NA} = 0.021$ is used to image the sample onto the CCD plane. After interfering with an off-axis reference wave, the generated holograms under different orientations of structured illumination are recorded, and one of them is shown in Fig. 4(b). We can see that the object is modulated by both the carrier fringes (along the horizontal direction) and the structured illumination fringes (along the diagonal direction). The telescope system $MO_2 - L_2$ limits the resolution of the setup to $\delta = 0.61\lambda/\text{NA} = 18$ μm for the on-axis plane wave illumination. The illumination angle of the ±1st diffraction orders of the structured illumination is $\theta_{\text{illum}} = \arcsin(M_1\lambda/\Lambda) = 0.018$ rad in the object plane. Here M_1 denotes the magnification of the telescope system $L_1 - MO_1$, and Λ denotes the period of the structured illumination in the SLM. According to Eq. (2), the theoretical resolution obtained by using the structured illumination is $\delta_{\text{str}} = 10$ μm.

The complex amplitudes of each illumination A^i_{illum} and object wave A^i_O are reconstructed by using Eq. (1). The resolution improvement was performed by using the abovementioned iterative method. For comparison, the phase images reconstructed by using the traditional on-axis plane wave illumination and by using the proposed method are compared in Figs. 4(c) and 4(d), respectively. It is seen that the resolution target in the third column (from the right side) with linewidth 12.5 μm, which is not distinguishable in Fig. 4(c), is clearly resolved in Fig. 4(d). This implies that the proposed iterative method improves the spatial resolution of the phase imaging. The number of illumination is chosen to have a compromise between the measurement time and the isotropic resolution improvement (in different directions) with low noise level. In our experiment, we used 12 structured illuminations to improve the spatial resolution of the reconstruction. Fewer structured illuminations (but no less than four) with the

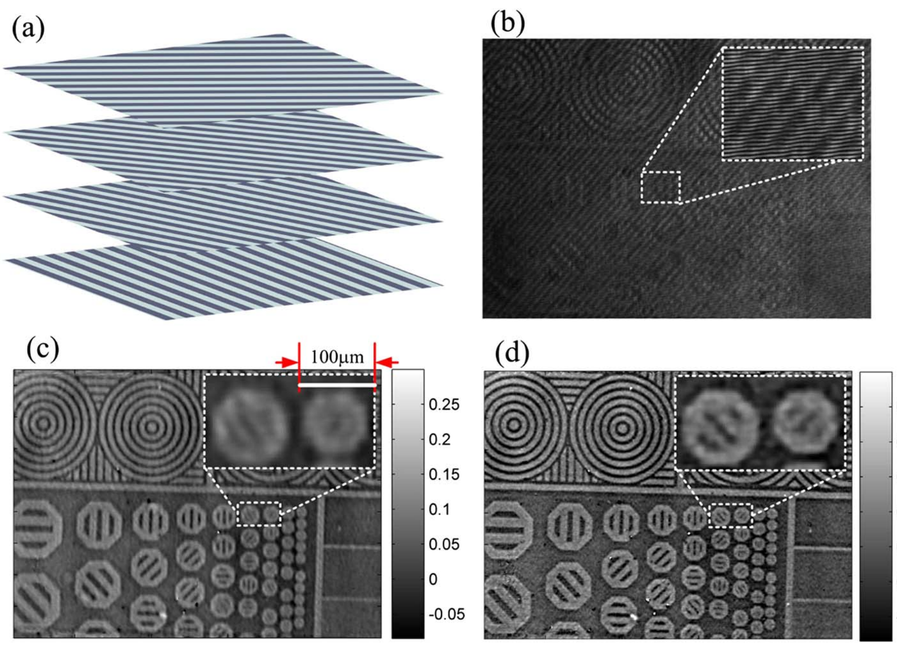

Fig. 4. Experimental results for resolution enhancement by structured illuminations. (a) Four phase gratings with different orientations loaded on SLM to generate structured illuminations. (b) Recorded hologram under one structured illumination. (c) and (d) The reconstructed phase images by using the on-axis plane wave illumination and by using the proposed iteration method, respectively.

orientations evenly distributed on a ring can be used for resolution improvement in despite of a certain degree of noise. Although a low NA of objective was used in the experiment, it can be extended to a higher NA of objective.

5. CONCLUSION

The structured illuminations with different orientations, which are generated by a SLM, are used to illuminate the specimen sequentially in DHM. An iterative method enables the reconstruction with spatial resolution enhancement for all orientations without phase shifting on the structured illuminations. The use of the SLM allows for generating and changing quickly the structured illumination without mechanical movement.

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