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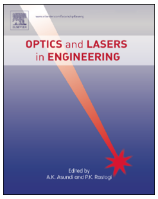

Disturbance-free digital holographic microscopy via a micro-phase-step approach

Chi-Ching Chang [a,*], Ming-Tzung Shiu [b], Je-Chung Wang [c], Chia-Hao Wu [d], Yang-Kun Chew [a]

[a] Department of Electro-optical and Energy Engineering, Ming Dao University, Changhua 52345, Taiwan, ROC
[b] School of Defense Science, National Defense University, Tashi, Taoyuan 33509, Taiwan, ROC
[c] Department of Applied Chemistry & Material Science, National Defense University, Taiwan, ROC
[d] Department of Material Science and Engineering, Ming Dao University, Changhua 52345, Taiwan, ROC

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ABSTRACT

This work proposes a cost-effective, simple, micro-phase-step (MPS) method for suppressing the zero-order diffraction and conjugate-image interferences that are caused during digital holographic microscopic image reconstruction. The proposed MPS method replaces the conventional phase modulation approach; it uses a rotatable cover glass that enables smooth modification of the incidence angle and the optical path of the reference beam. This setup allows the phase step to be accurately estimated by shifting the reference wave phase more freely close to $\pi/2$, at which the background noise can be suppressed more effectively. In the proposed MPS method, the optimal conditions for suppressing conjugate-image interference are identified using a relatively moderate intensity distribution and suppression of noise in the numerically reconstructed object wave-field. In addition, the proposed method mitigates the effect of disturbances that are caused by environmental factors, such as minor vibrations and small changes in temperature and humidity. Importantly, only two holograms are required to satisfy the objective of image reconstruction. The results in this work reveal that even with intentional interference caused by minor vibrations, conjugate-image interference can still be suppressed by determining the phase deviation between the two original holograms.

1. Introduction

The concept of holography was first proposed by British scientist Gabor in 1948. [1] Due to the limited coherence of the light sources to which Gabor had access, his experiment was performed in an in-line configuration. Such an in-line configuration for holography causes interference among the reconstructed object's image, the reconstructed conjugate image of the object, and the zero-order diffraction caused by penetration of the reference beam. Following improvements in light coherence that were achieved by the first laser device in the 1960s, Leith and Upatnieks [2,3] proposed an off-axis configuration for holography in which the signals and the noise of the reconstructed images are filtered and processed separately to provide better-quality outcomes.

Following rapid progress in digital camera and digital image processing technologies, digital recording and the reconstruction of numerical holograms, known as digital holography (DH), have developed rapidly. Goodman pioneered the work on numerical hologram reconstruction in 1967 [4]; this work was followed by the work of Kronrod [5] and Demetrakopoulos and Mittra [6]. Schnars and Juptner [7] successfully captured holographic interference fringes using a charge-coupled device (CCD); they achieved numerical object wavefront restoration and phase information retrieval. Their achievements laid the foundation for the development of DH. Due to the limitation on CCD resolution, the development of DH is limited to in-line or small-angle off-axis holography. When a CCD is used as a recording medium in an off-axis configuration, the angle between the reference and the object beams is limited to within a few degrees [8]. Consider a CCD with a 4.67-μm pitch and a laser with a 0.6328-μm wavelength; the maximum angle $\theta_{\max} \approx \lambda/(2\Delta x) = 3.9^{\circ}$. Therefore, using simple filtration to eliminate the aforementioned interference noise is difficult.

Kreis and Juptner [9] proposed the elimination of the zero-order diffraction by subtracting the mean intensity from the full hologram frame. Takaki et al. [10] utilised a phase-shifting method that efficiently eliminates the twin image and suppresses zero-order diffraction. Demoli [11] developed a procedure based on the stochastic change of the speckles in the primary-fringe patterns and on the subtraction of two such patterns to suppress the zero-order diffraction disturbance. Chen et al. [12] developed an approach in which a numerical operation is performed to suppress

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[*] Corresponding author.
E-mail address: chichang@mdu.edu.tw (C.-C. Chang).

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zero-order diffraction in DH. This numerical suppression involves subtracting the numerically generated intensities of the object and reference beams from the digital hologram. Cho et al. [13] developed a method for suppressing the zero-order diffraction in in-line digital holographic microscopy (DHM); they utilised the mean intensity of a variable number rather than a fixed number of pixels. The zero-order suppression ratio was similar to that associated with the averaging method that was proposed by Kreis et al. [9], but the image quality is better. Dong and Wu [14] described a numerical space-shifting reconstruction approach that exploits the periodicity and space-shifting property of the inverse discrete Fourier transform for use in DH. This method requires no additional holograms and imposes no special requirements on the optical recording. When combined with the intensity-averaging approach, this numerical space-shifting reconstruction method can be used effectively to remove the zero-order diffraction in the reconstruction and so constitutes a practical means of removing the zero-order diffraction from an in-line or off-axis DH. Loïc Denis et al. [15] developed inverse filtration, inspired by Fresnel diffraction and numerical approximation, using the iterative propagation method in which a Gerchberg–Saxton-derived method is used to suppress the twin-image interference noise.

Yamaguchi and Zhang [16] proposed a method of phase-shifting digital holography (PSDH) for forming 3-D images, which yields images of higher quality that can be viewed from a wider range of viewing angles than images obtained using conventional DH with an off-axis configuration. Zhang and Yamaguchi [17] subsequently applied PSDH to DHM by deriving the complex amplitude of the light that was scattered from microscopic 3-D objects through a microscope objective lens via video recording, phase-shifting analysis, and numerical reconstruction. Cai et al. [18] proposed a method for reconstructing object wavefronts in phase-shifting interferometry with arbitrary, unknown phase steps. Their method allows for the retrieval of the original object field, including its amplitude and phase distributions with arbitrary and unequal phase steps, using three or four holograms. Cai et al. subsequently [19] developed a method for extracting arbitrary unknown and unequal phase steps in phase-shift interferometry using interferograms that were recorded on the diffraction wave-field of an object. Similar approaches with various formulae have been used in many experiments. [20–26]

A phase-shifting DHM (PSDHM) uses a computer-controlled piezoelectric transducer (PZT) to modulate the reference beam phase. Zhang and Yamaguchi [17] demonstrated that the early stage of phase modulation of a reference beam is representative of PSDH; their scheme uses a CCD to separately record four objective-lens-magnifying holograms of the object and the interference with the reference wave to eliminate the interference of the zero-order and conjugate-image diffractions. Subsequently, related techniques were extensively used to detect semiconductor microstructures, micro-lenses, living cells and microorganisms, and flows [27–32]. However, precision equipment is required to enable a PSDHM to accurately control the phase difference between the object and reference beams, thereby increasing cost and requiring a low-interference environment.

Holger et al. [33] proposed a modified phase-shifting algorithm for use in DH that eliminates the zero-order and conjugate-image diffractions. Hong and Kim [34] retrieved the unknown arbitrary phase shift in self-interference incoherent DH. Both of these proposed algorithms were applied iteratively until a convergent result was obtained. Liu and Poon [35] developed a phase-shifting method that requires only two recordings, while Tahara et al. [36] proposed a parallel phase-shifting method that requires only one recording, but at the cost of a halving of the resolution.

Chang et al. [24] developed an arbitrary phase-step DH (APSDH) in which two holograms are used to suppress distortion. This APSDH uses a microscope cover glass as a phase-shifting component to adjust the phase difference. This approach has successfully resolved the issues of both cost and precision. However, adjusting the phase of the reference beam tends to increase both the measuring difficulty and the estimation errors. These errors and disturbances, which are caused by environmental factors (such as humidity, vibration, temperature, and so on), can blur the reconstructed image.

Because a minimal change in any of the above factors can cause a disturbance and thereby degrade the quality of the outcome of holographic image reconstruction, this work proposes a new in-line DHM scheme that can effectively reduce the effect of such disturbances. In this work, an accurate, simple, and cost-effective micro-phase-step method is used to effectively suppress the zero-order diffraction and the conjugate-image interferences in DHM.

2. Theoretical background

The diffraction field of a digitally acquired hologram can be obtained through numerical calculation. The image of the object wave can then be reconstructed on the image plane using free space transformation. However, the image can interfere with the conjugate of the object wave and the non-diffractive reference wave. The in-line structure method offers a better solution when a higher-resolution object wave image and phase information are desired, up to the CCD resolution limit. Therefore, effectively suppressing the zero-order and conjugate-image interferences in the in-line setup and the small angle off-axis setups is important. The zero-order blurring can be eliminated by recording the intensities of the object wave and the reference wave separately and subtracting the intensities of the object wave $\psi_O = a_O \exp(i\varphi_O)$ and the reference wave $\psi_R = a_R \exp(i\varphi_R)$ from the digital hologram:

$$I'_H = I_H - |\psi_R|^2 - |\psi_O|^2 = \psi_O \psi_R^* + \psi_O^* \psi_R \tag{1}$$

A problem that is encountered in DH is conjugate-image blurring during numerical reconstruction. A method proposed by Yamaguchi and Zhang [16] for suppressing such blurring has led to substantial advances in PSDH. However, this method is effective only for suppressing the formation of twin images in numerical reconstruction and is not ideal for precise phase shifting in a serial, special, and stable shifting procedure. The arbitrary phase-step (APS) approach has been widely investigated. Chen et al. [21] discussed such an approach for numerical wavefront reconstruction; their experiments established that only two digital holograms and a simple estimation procedure are required for conjugate-image blurring suppression and numerical reconstruction.

Let I_{H1} and I_{H2} be the digital holograms that record the interference of the object wave before and after the reference wave phase delay of $\Delta\varphi$. The intensity distribution of these holograms can be expanded as:

$$I_{H1} = |\psi_O + \psi_R|^2 = a_O{}^2 + a_R{}^2 + \psi_O \psi_R^* + \psi_O^* \psi_R \tag{2}$$

$$\begin{aligned} I_{H2} &= |\psi_O + \psi_R \exp(i\Delta\varphi)|^2 \\ &= a_O{}^2 + a_R{}^2 + \psi_O \psi_R^* \exp(-i\Delta\varphi) + \psi_O^* \psi_R \exp(i\Delta\varphi) \end{aligned} \tag{3}$$

The zero-order term noise cancellation technique is used to calculate I'_{H1} and I'_{H2} :

$$I'_{H1} = I_{H1} - |\psi_R|^2 - |\psi_O|^2 = \psi_O \psi_R^* + \psi_O^* \psi_R \tag{4}$$

$$\begin{aligned} I'_{H2} &= I_{H2} - |\psi_R|^2 - |\psi_O|^2 \\ &= \psi_O \psi_R^* \exp(-i\Delta\varphi) + \psi_O^* \psi_R \exp(i\Delta\varphi) \end{aligned} \tag{5}$$