Optics Communications 346 (2015) 38–42

Contents lists available at ScienceDirect

Optics Communications

journal homepage: www.elsevier.com/locate/optcom

Phase unwrapping method based on multiple recording distances for digital holographic microscopy

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ARTICLE INFO

Article history:
Received 9 December 2014
Received in revised form
3 February 2015
Accepted 7 February 2015
Available online 11 February 2015

Keywords:
Phase unwrapping
Digital holography
Microscopy

ABSTRACT

We present a phase unwrapping approach based on multiple recording distances for digital holographic microscopy. It unwrappes the ambiguous phase image by synthesizing the extracted continuous components from a set of multiple reconstructed phase images obtained from a series of holograms by slightly shifting the specimen longitudinally with a step more than the longitudinal correlation length of the coherent noise field. The experimental results demonstrate that the proposed method provides a more accurate calculation and better counteraction of phase noise than the methods proposed in previous research.

1. Introduction

Digital holographic microscopy (DHM) has been applied in many areas, such as living cells investigation, microstructure topography and vibration analysis [1–3]. DHM can quantitatively measure the phase distribution of the object, which contains information about the three-dimensional (3D) shape and the inner structure of transparent samples [4]. However, for objects whose optical thickness variation is greater than the illumination wavelength, the phase images are wrapped in the range of $(-\pi, \pi]$ radians after arctangent calculation [5], which makes the phase unwrapping necessary.

Various unwrapping algorithms have been applied in DHM [6,7]. But these methods cannot unwrap invalid areas and propagate consecutive errors in neighboring regions, and they fail for step-contained structures because they unwrap the phase discontinuities by depending on the surrounding pixels. The optical unwrapping phase methods [5,8,9] have been developed to remove 2π discontinuities via two or more holograms generated by mulitple illumination wavelengths. However, a larger synthetic wavelength amplifies the noise to an unacceptable level, while an insufficient one restricts its application [10]. We have proposed an approach [11] whereby the ambiguous phase image synthesizes the extracted continuous components layer by layer from a set of multiple phase images obtained by varying the optical wavelength. However, a tunable laser is required, and the discrepancy of power between the emitted optical wavelength means that the circular variable neutral density filters should be adjusted and the intensity ratio of the object beam and reference beam should be checked after tuning the wavelength of the laser, which results in the need for more time in the data acquisition process. Khmaladze et al. proposed two phase unwrapping methods. The final unwrapped phase distribution was synthesized by extracting continuous components from multiple phase images. These images were either reconstructed at various distances of a single hologram, or alternatively obtained by adding a constant to one reconstructed phase image and re-wrapping into the modulo of 2π [12]. However, those two methods would fail, if the noisy areas in one image remain noisy in the others. Only the individual coherent noise fields within multiple phase images is mutually independent, one of the phase images is noisy in a certain area, while the others are not necessarily noisy in the same area.

In this article, the 3D spatial correlation properties of coherent noise were experimentally investigated and analyzed by using a discrete correlation algorithm. Furthermore, a phase unwrapping method for digital holographic microscopy was proposed. Instead of changing the illumination wavelength, multiple holograms were recorded by consecutively varying recording distances with each step exceeding the longitudinal correlation length of the coherent noise field. Then, the phase images were reconstructed by an appropriate algorithm in which the discontinuity occurrences were different and the phase coherent noises presented distributions in various states. Finally, the distribution of the unwrapping phase was synthesized by extracting continuous

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http://dx.doi.org/10.1016/j.optcom.2015.02.012
0030-4018/

components layer by layer using these phase images. The related experimental results were given to confirm the feasibility of the proposed method.

2. Principles

2.1. 3D spatial correlation properties

The coherent noise produced by undesired diffraction and multiple reflections is a complicated 3D field in the reconstruction plane [13]. As the coherent noise fields of the image planes are discrete values from DHM, a discrete correlation algorithm is employed to calculate the correlation coefficients of the coherent noise by using the spatial averaging instead of the ensemble averaging [13,14]. If two point positions coincide, a normalized correlation value of unity is obtained. As these points become further separated from each other, the correlation value tends to decrease. Two points are said to be totally de-correlated when this correlation function decreases to zero, which is often used to define the lateral (perpendicular to the optical axis) and longitudinal (along the optical axis) correlation length of the coherent noise field. In practice, the average correlation length is often defined as the distance at which the correlation coefficient takes the value of 0.5 in most cases [14].

2.2. Iterative algorithm

The sample under study is a one-dimensional slanted plane with several wavelength height and isotropic reflective index as shown in Fig. 1(a). When the object is illuminated by a plane wave with the wavelength λ, the phase change due to the object is $\phi = 2\pi h n_\Delta/\lambda$, where h is the height of the sample and $n_\Delta = n - n_0$ is the refractive index difference between the sample and the surrounding medium. The corresponding wrapped phase image ϕ_1 reconstructed from the hologram with the recording distance d_1 exhibits multiple phase jumps, as shown in Fig. 1(b). Assuming a set of reconstructed phase images $\phi_1, \phi_2, \ldots, \phi_m$ are obtained from the holograms with different recording distances of $d_1, d_2, \ldots, d_m$ with constant sampling interval Δd, Δd should more than longitudinal correlation length of the coherent noise field, and m is the total number of reconstructed phase images. Those phase images are wrapped in a modulo of 2π in which the distribution of discontinuity is varied with each other. The protocol of unwrapping procedures as follows:

(I) Assuming Δh is the size of unwrapping step. A selected area of ϕ_1 with values between $-\pi\Delta h n_\Delta/\lambda$ and $\pi\Delta h n_\Delta/\lambda$ and far from any discontinuities is chosen as the starting area as shown in Fig. 1(c). In practice, the reconstruction fidelity depends on the size of Δh, since a larger one would include some areas with a discontinuity, while a smaller step size may produce the correct solution and would also be more computationally demanding. Therefore, an "adaptive step" routine should be introduced to adjust the step size depending on the image topology of the actual phase.

(II) Other areas that are not adjacent to the starting area are discarded, which ensures that the chosen area is not influenced by the different phase periods. The value of this area is filled in at the same location of a blank matrix P with the same size as ϕ_1, and the first height Δh of the object is obtained.

(III) The phase image ϕ_2, shown in Fig. 1(d), is subtracted by $2\pi\Delta h n_\Delta/\lambda$ and re-wrapped in the range of $(-\pi, \pi]$ radians as a new distribution ϕ_2' whose discontinuities will appear at different places, which means that ϕ_2 is effectively moved by Δh after being converted into the actual height of object, as shown in Fig. 1(e).

(IV) The part of ϕ_2' is extracted in which the values are between $-\pi\Delta h n_\Delta/\lambda$ and $\pi\Delta h n_\Delta/\lambda$. After adding with $2\pi\Delta h n_\Delta/\lambda$, the corresponding area in matrix P is filled in to obtain the second height Δh of the object, as shown in Fig. 1(f).

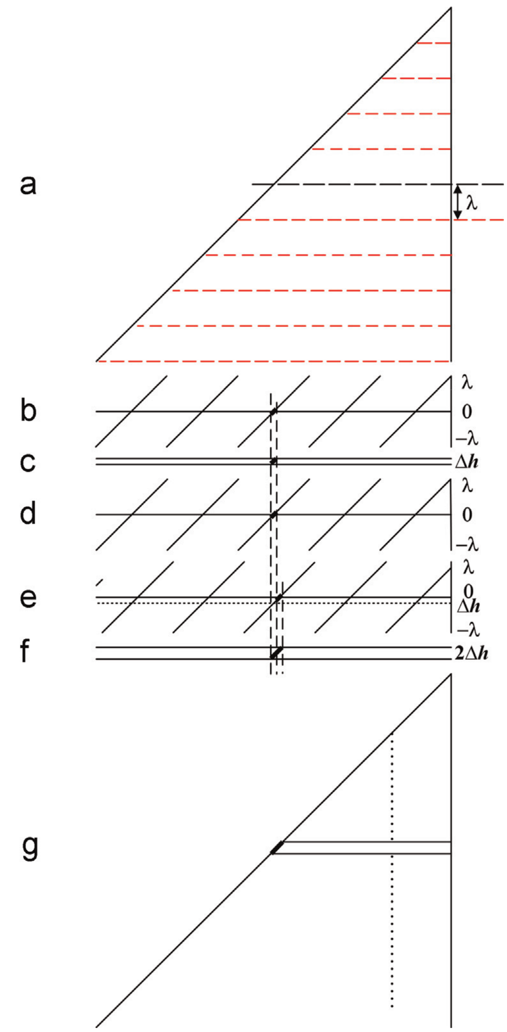

Fig. 1. Phase unwrapping by varying recording distance.

(V) The wrap-free phase distributions are obtained by repeating the steps above by moving up and down [Fig. 1(g)] until no new pixels are added to reconstruct the entire phase image layer-by-layer. In order to make sure the multiple phase images can be used repeatedly in the iteration process, when the final phase image ϕ_m is reached, the phase image sequence is then reversed; that is, the last phase image becomes the first one, the second to last one becomes the second, and so on.

3. Experimental results

3.1. Experimental setup and sample

The schematic diagram of the experimental setup is illustrated in Fig. 2. A light was emitted from a diode laser (OXXIUS S.A, LBX-638-100-CIR-PP, 638 nm, 100 mW) and divided into an illumination beam and a reference beam by using a polarized beam splitter (PBS). A half wave plate (HWP1) was conjugated with the PBS to adjust the intensity ratio between the two beams, and a neutral density filter (NF) was inserted in front of HWP1 to adjust the total intensity of the imaging system. Another half wave plate (HWP2) was used in the reference optical path to obtain the same line