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Weighted adaptive spatial filtering in digital holographic microscopy

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ABSTRACT

Spatial filtering, a key point to realize real-time measurement, is used commonly in digital off-axis holography to extract desired terms. In this paper, we propose a weighted adaptive spatial filtering method by weighting the adaptive filtering window (obtained from image segmentation) based on signal to noise ratio. The advantages of this method are evaluated by simulations and further verified by recorded digital image plane holograms. The results demonstrate that our method is effective in suppressing noise and retaining the sharp edges in the reconstructed 3D profiles.

1. Introduction

Different from traditional optical microscopy, digital holographic microscopy is able to obtain the phase information of the wavefront, i.e. the 3D profile of the specimen [1]. It is widely used in living cell image measurement [2], particle image velocimetry [3], and micro-electromechanical systems surface analysis [4–6] due to its significant advantages such as real-time [5,7–9], non-contact, and high resolution [10,11]. In the phase retrieval process, the zero order image and conjugate image have to be eliminated by phase-shifting or spatial filtering. The phase-shifting technique is a most common way to eliminate the undesired orders for in-line holography. It requires recording at least two holograms and additional devices to implement [12,13], which will restrict the real-time dynamic measurement. Through the spatial filtering technique, the interested diffraction order in the spectrum of off-axis holography is selected and the real-time characteristic is obtained.

Researchers performed the spatial filtering by manual selection of a regular shape filtering window [14,15] without considering the distribution of the desired order or the background noise in spectrum. In practice, the distribution of the desired order spectrum is irregular and varies with different specimen. Therefore, a more accurate automatic spatial filter is needed to get a more accurate automatic measurement. Rincon et al. [16] used a distance criterion between the maximum values in the amplitude spectrum as a clustering parameter to design the filter. Weng et al. [17] employed the histogram analysis of the power spectrum of the hologram to extract the required order. Li et al. [18] proposed an adaptive spatial filtering method based on region growing and the characteristic of the spectrum separation. They all handled the filtering as a problem of image segmentation in digital image processing and acquired better measuring results than the regular shape filters. But their filters were binary adaptive filters without attenuation as observed in conventional filters (e.g. Gaussian filter). Matrecano et al. [19] used a Butterworth filter that has smoothing effect to design the filter window in consideration of the background noise in the spectrum of the hologram. But the regular shape of the Butterworth filter was still retained, hence undesired spectral components were included.

In this paper, we demonstrate a weighted adaptive spatial filter for automatic analysis in digital off-axis holographic microscopy by combining the advantages of adaptive filter and smoothing filter, where the significant distribution of the desired order and the noise smoothing function are considered simultaneously. Both the simulations and experimental results demonstrate that our method has a better performance.

2. Angular spectrum distribution and spatial filtering in digital holography

The hologram in off-axis geometry is generated by the interference of object wave O and reference wave R with a certain angle. The hologram recorded by the camera can be expressed as: $I = |O|^2 + |R|^2 + OR^* + O^*R$, where * denotes the conjugate. The first and second terms compose the zero order of diffraction. The third and fourth terms are the +1 order (virtual image) and –1 order (real image), respectively. The spectrum of the desired term (+1 order) can be obtained by employing a proper filter H in Fourier domain:

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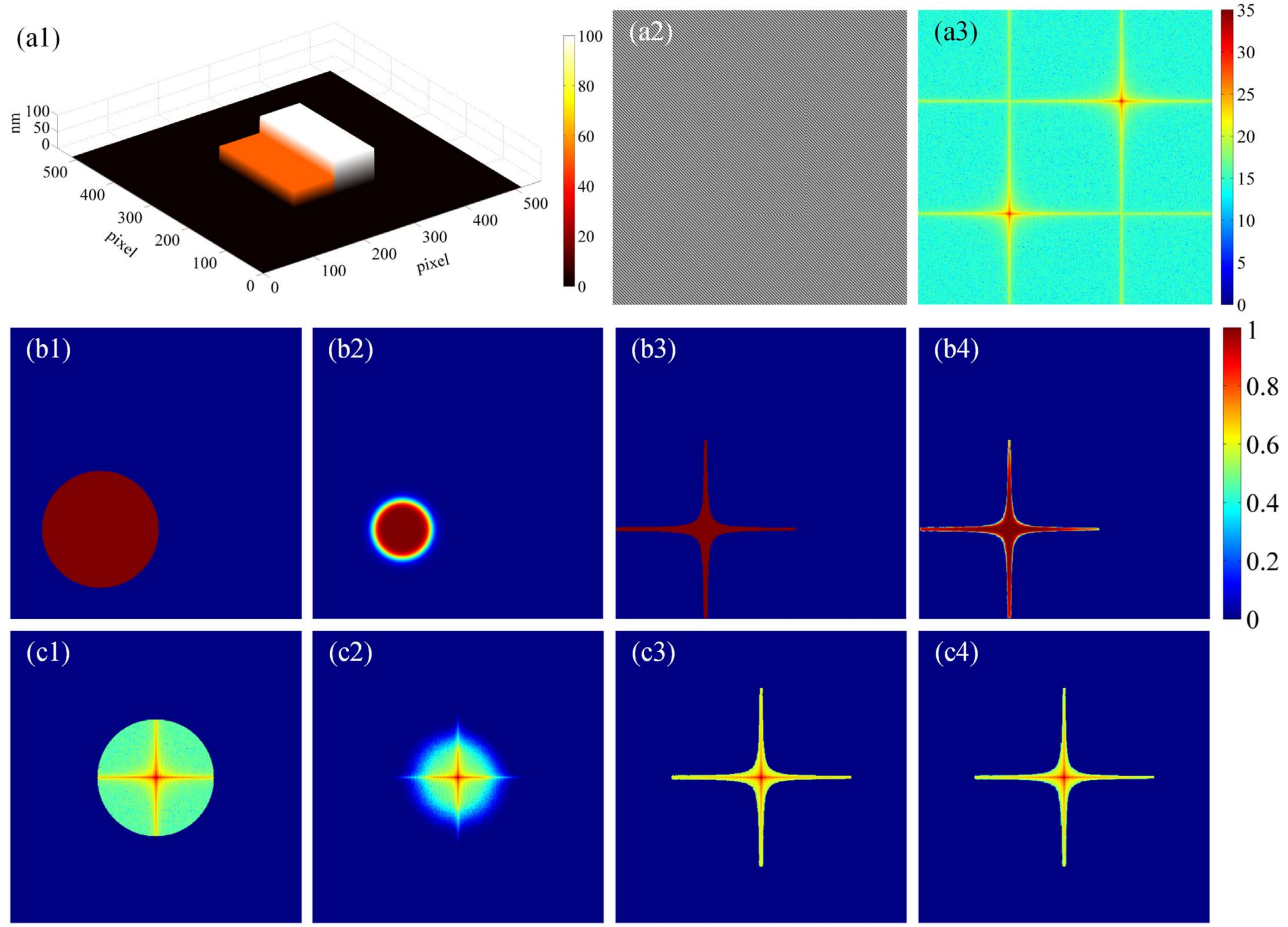

Fig. 1. Simulation of a pure phase sample: (a1) 3D topography; (a2) simulated hologram; (a3) logarithm of the power spectrum of (a2); (b1) regular shape manual filter; (b2) Butterworth filter; (b3) binary adaptive filter; (b4) weighted adaptive filter; (c1) filtered and centered spectrum by H_M; (c2) filtered and centered spectrum by H_{BTW}; (c3) filtered and centered spectrum by H_{BA}; (c4) filtered and centered spectrum by H_{WA}.

$$A_{+1}\left(f_X, f_Y\right) = \mathrm{FT}\left\{OR^*\right\} = H \times \mathrm{FT}\left\{I(x, y)\right\} = H \times \iint I(x, y)\exp\left[-j2\pi\left(f_X x + f_Y y\right)\right]dxdy. \tag{1}$$

where FT{} denotes the Fourier transform, (x, y) are the coordinates of the hologram plane, and f_X and f_Y are spatial frequencies.

As described in angular spectrum theory [20], we can consider the light waves entering the camera as a sum of many simple plane waves. Supposing the direction cosines of the propagation direction of a plane wave are $\cos\alpha$ and $\cos\beta$ respectively, we rewrite Eq. (1) as:

$$A_{+1}\left(\frac{\cos\alpha}{\lambda}, \frac{\cos\beta}{\lambda}\right) = H \times \iint I(x, y)\exp\left[-j2\pi\left(\frac{\cos\alpha}{\lambda}x + \frac{\cos\beta}{\lambda}y\right)\right]dxdy, \tag{2}$$

where λ is the wavelength, $\cos\alpha = \lambda f_X$, and $\cos\beta = \lambda f_Y$. By analyzing the physical meaning of Eq. (2), we find that the value at point $(\cos\alpha/\lambda,\ \cos\beta/\lambda)$ in angular spectrum is determined by the quantity of the entered light waves with the direction cosines $(\cos\alpha, \cos\beta)$. Generally, the reference wave is considered to be a beam of inclined plane wave with a fixed angle and plays the role of carrier in a communication theory viewpoint [21]. That is to say, the spectrum location of the desired term is determined by the tilt angle of reference wave, and the spectrum distribution is determined by the direction distribution of the object waves. If the object waves reflected or transmitted from the sample are uniform at all directions, we will acquire a regular shape distribution of the desired term. This condition only occurs when the sample is perfectly smooth and homogeneous, and hence the filter can be designed into regular shape. However, the sample cannot be so idealization in practice. The uneven surface and inconsistent reflectivity (or transmittivity) lead to non-uniform distribution of the object waves and make the distribution of the desired term irregular. It is important to determine a filter H with an adaptive filter window. The extraction of +1 order spectrum is an issue of image segmentation as described in Refs. [16–18]. The key point is to find a threshold for picking out the irregular filter window. Weng et al. [17] and Li et al. [18] made some interesting efforts on this field. Both of them designed effective methods to carry out adaptive filters which were proved more precise than manual filter. However, their filters are very similar to the ideal filter for their binary property. The frequencies within the filter window pass without attenuation and the outside frequencies are all cut off. This will result in blurring and ringing in filtered 2D images [22]. Our simulations and experiments indicate the same influence in 3D profile. To deal with these issues, Matrecano et al. [19] used a Butterworth filter with smooth edge. The Butterworth filter showed better results than Gaussian filter and increased the image quality with less noise. But they ignored the irregular shape of the