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Resolution enhancement phase-contrast imaging by microsphere digital holography

Yunxin Wang [a,b], Sha Guo [a], Dayong Wang [a,b,*], Qiaowen Lin [a], Lu Rong [a,b], Jie Zhao [c]

[a] College of Applied Sciences, Beijing University of Technology, Beijing 100124, China
[b] Beijing Engineering Research Center of Precision Measurement Technology and Instruments, Beijing University of Technology, Beijing 100124, China
[c] The Pilot College, Beijing University of Technology, Beijing 101101, China

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ABSTRACT

Microsphere has shown the superiority of super-resolution imaging in the traditional 2D intensity microscope. Here a microsphere digital holography approach is presented to realize the resolution enhancement phase-contrast imaging. The system is designed by combining the microsphere with the image-plane digital holography. A microsphere very close to the object can increase the resolution by transforming the object wave from the higher frequency to the lower one. The resolution enhancement amplitude and phase images can be retrieved from a single hologram. The experiments are carried on the 1D and 2D gratings, and the results demonstrate that the observed resolution has been improved, meanwhile, the phase-contrast image is obtained. The proposed method can improve the transverse resolution in all directions based on a single exposure. Furthermore, this system has extended the application of the microsphere from the conventional 2D microscopic imaging to 3D phase-contrast microscopic imaging.

1. Introduction

Digital holography, as a powerful interference technique, has the great capability to retrieve both quantitative amplitude and phase information of an object through a flexible digital processing. It shows remarkable success in the measurement of 3D microstructure, and has been applied in many fields including the morphological monitoring of biology cells, shape inspection, particle analysis, *etc.* [1–4]. However, the limited numerical aperture (NA) constrains the resolution of digital holography seriously, which has prevented it from the practical applications of high resolution imaging [5,6].

Many attempts have been made to achieve super-resolution imaging to overcome the resolution limit by moving CCD [5], scanning the object [6], and angular multiplexing with a structured illumination [7–12]. Osten et al. developed a deep ultraviolet off-axis digital holographic microscope in combination with the oblique illumination [10], and the detected resolution is enhanced to 250 nm using a laser with the center wavelength 193 nm. However, most of these methods need to record a set of sub-holograms in time sequence by adjusting the optical elements. The detection of the rapid and dynamics information is obstructed by its time multiplexing, meanwhile, the synthesize reconstruction of the sub-holograms needs complex image processing additionally. It is worth mentioning that tomographic phase microscopy can be achieved to supply inner structure of object based on digital holography and angular scanning [13–15], which usually needs dense angular multiplexing in a large angular range. To realize fast and automatic resolution enhancement imaging, the spatial light modulator (SLM) has been successfully applied to boost the transverse resolution in digital holography [16,17]. Nevertheless, the usage of SLM brings difficulty in terms of the accurate measurement of phase information due to the discrete pixel and phase error of SLM [18,19]. Furthermore, angular multiplexing was combined with polarization, incoherence or wavelength multiplexing to achieve single-exposure super-resolution digital holography [20–23]. Two pairs of incoherent object waves and reference waves with orthogonal polarization states were designed to record a super-resolution hologram [20]. This method can only record two subholograms in a single hologram. Zhai et al. adjusted the time delay of the pulsed laser source to ensure the incoherence overlapping of the three subholograms [21]. The light source with three wavelengths and three illumination angles was utilized by Micó et al. to achieve the single-exposure super-resolution imaging using a color CCD. Besides, a 1D or 2D grating can also be inserted between the object and CCD to raise the resolution based on a single hologram [24,25]. However, these approaches can only

* Corresponding author.
E-mail addresses: guosha0725@163.com (S. Guo), wdyong@bjut.edu.cn (D. Wang).

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enhance the transverse resolution in several separate directions, and the improvement of the resolution in all continuous directions is not possible.

The microspheres or nanoparticles in combination with the white-light microscope have been used to achieve the super-resolution imaging by transforming the near-field evanescent wave into far-field propagating wave [26,27]. These methods are characterized by high performance, simple operation, and low cost [28]. Nevertheless, only the 2D intensity information of the object can be detected and the phase distribution is lost. Considering digital holography can preserve the phase-contrast image, a random and time varying flow of metallic nanoparticles was recently introduced to digital holography to improve the resolution and field of view through theoretical analysis and numerical simulation [29], which had not been verified by the experiments yet.

In this study, we report on a microsphere digital holography approach to realize resolution enhancement imaging. To our knowledge, this is the first time to get the resolution enhancement phase-contrast image by combining a static microsphere with digital holography. This setup can increase the resolution in all directions based on a single hologram. The resolution enhancement amplitude information of the 1D and 2D objects has been obtained experimentally, meanwhile, the phase images also have been detected.

2. Principle of the resolution enhancement imaging

The principle of the resolution enhancement imaging is demonstrated as shown in Fig. 1(a). For a conventional microscope without the microsphere, the numerical aperture *NA* ($NA = n_0 \sin\theta_0$) of the microscopic objective (MO) determines the ideal resolution $\delta = 0.82\lambda/NA$ of the imaging system [30], where n_0 is the refractive index of the surrounding medium, θ_0 is half of the aperture angle of the MO, and λ is the wavelength of the incidence light. The θ_0 also represents the largest acceptable diffraction angle of the object wave.

If a microsphere with radius *R* is put on the proximal surface of the object, the object wave with larger diffraction angle in the shadow areas can be coupled by refraction [31,32], and converted to a wave below the cutoff frequency. Then the object wave with larger diffraction angle can enter the MO and be detected by CCD camera. Therefore, the largest diffraction angle is magnified from the original angle of θ_0 to the present angle of θ_1, and the resolution of imaging system can be increased beyond the resolution limit correspondingly.

Next, the amplified factor of the imaging system is discussed. The object is firstly imaged by the microsphere, and a virtual and magnified image is located at the image plane of microsphere as shown in Fig. 1(b). Then the image is magnified twice by the MO. The distance *L* between the MO and image plane of microsphere is set to the work distance of MO. If the object distance and image distance of microsphere are *s* and *s*' respectively, the amplified factor of the microsphere should be [33]

$$M_s = \frac{f}{f - s} \quad (1)$$

where *f* is the focal length of the microsphere.

The total amplified factor *M* of imaging system will become $M_s M_{MO}$, where M_{MO} is the amplified factor of MO.

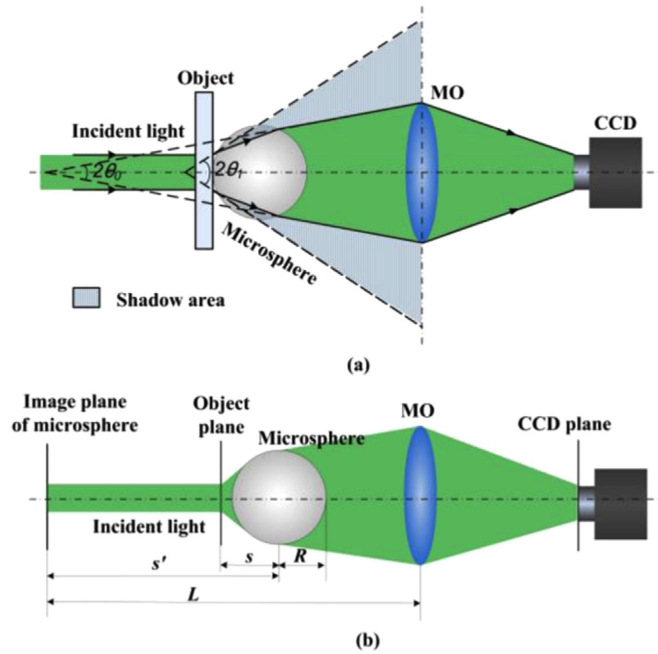

Fig. 1. Imaging based on the microsphere and MO. (a) Schematic of the resolution enhancement imaging. (b) Amplification of imaging system.

3. Experimental results and analysis

3.1. Microsphere digital holography system

In order to explore the resolution enhancement imaging of the microsphere in digital holographic microscope, the setup of microsphere digital holography is assembled in combination with the image-plane digital holography. The optical setup is described in Fig. 2. The polystyrene microspheres are prepared and set on the surface of the object as close as possible. A single-longitudinal-mode laser with the center wavelength of 532 nm is used as the light source. The laser beam is coupled into a fiber by a laser-to-fiber coupler (LFC), and then divided into two beams by a 1×2 fiber coupler (FC). One beam collimated by a fiber collimator (FCL) is employed to illuminate the object and is called the object beam. Another beam exiting from the end of the fiber is applied as the reference beam. The diffraction wave including the high frequency of the object is transformed by the microsphere, and then the MO ($20\times$, NA=0.4) produces a magnified image of the object. The hologram is produced by the interference of the object and reference beams. The CCD is located at the image-plane of the object to record the hologram. Two fiber attenuators (FA) are used to adjust the contrast of the interference fringes. The CCD camera has a resolution of 1280×1024 pixels and the pixel pitch of $4.65\ \mu m \times 4.65\ \mu m$.

3.2. Resolution of digital holographic system without microsphere

Before analyzing the resolution enhancement by microsphere,

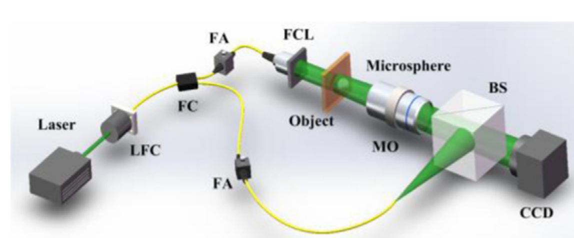

Fig. 2. Optical setup of microsphere digital holography.