Three-dimensional high-resolution digital inline hologram reconstruction with a volumetric deconvolution method

Subjects: Biomedical & Chemical Engineering
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The digital in-line holographic microscope (DIHM) was developed for a 2D imaging technology and has recently been adapted to 3D imaging methods, providing new approaches to obtaining volumetric images with both a high resolution and wide Field-Of-View (FOV), which allows the physical limitations to be overcome. However, during the sectioning process of 3D image generation, the out-of-focus image of the object becomes a significant impediment to obtaining evident 3D features in the 2D sectioning plane of a thick biological sample. Based on phase retrieved high-resolution holographic imaging and a 3D deconvolution technique, we demonstrate that a high-resolution 3D volumetric image, which significantly reduces wave-front reconstruction and out-of-focus artifacts, can be achieved. The results show a 3D volumetric image that is more finely focused compared to a conventional 3D stacked image from 2D reconstructed images in relation to micron-size polystyrene beads, a whole blood smear, and a kidney tissue sample. We believe that this technology can be applicable for medical-grade images of smeared whole blood or an optically cleared tissue sample for mobile phytophysical microscopy and laser sectioning microscopy.

The principle of in-line holographic microscopy was suggested by Gabor and is based on an electron holographic interference between a small object and illuminating wave, called the “electron interference microscope” [1]. Interference microscopy has been expanded into digital in-line holographic microscopy (DIHM) [2], supported by the development of digital detectors that have micron size pixels and a large image sensor, enabling the numerical calculation of diffraction and the reconstruction of a holographic image using various digital image processing techniques [3, 4]. The merit of DIHM is its capability to be adapted to highly functional and simplified microscopes without complex physical optics and an expansive light modulator [5]. For example, the high-resolution digital image reconstruction approach for a wide field-of-view (FOV) image is one of the most influential methods in biomedical applications, such as the diagnosis and pathology for various lab chip platforms [6-8]. For the wide FOV of a whole slide digital image at a single shot, its resolution and size have an inverse relationship, which affects the total measurement time and cost of the imaging. The narrow FOV of the conventional microscopic imaging method to achieve the whole slide image requires a grid scanning and digital stitching method, with a high magnification factor imaging resolution, to expand the FOV. The digitalized image and its optical calculation of the analytical wave equation, Fourier optics, have broken through the limitations of the conventional microscope, such as resolution enhancement beyond the diffraction limit [9], high-resolution [10], wide field-of-view imaging from a 2D planar hologram [11], on-chip differential interference contrast microscopy [12], and wide field-of-view lens-free fluorescent imaging [13].

Recently, the DIHM has been introduced into 3D imaging applications, providing new approaches to obtaining a volumetric image with both a high resolution and large FOV size, which allows the physical limitations and focal depth to be overcome. Based on the 2D DIHM technology and its various merits, 3D section imaging technologies relating to volumetric samples have been suggested for a thick clear tissue, widespread whole blood cells, and polystyrene beads on a sliding glass, i.e., 3D imaging of CLARITY tissue [14], three-dimensional profiling and tracking [15, 16], angled tomography [17], 3D image distortion compensation [18], and wide-field pathology slide imaging [19]. There are two significant challenges that must be overcome to achieve a wide FOV high-resolution 3D image: Phase recovery and spatial under-sampling, based on an iterative reconstruction with a single shot hologram [20, 21], multi-angle [22], multi-height [23], spatial shift [24], colorization [25] for color artifacts [26], a scattering medium [27], a diffuser [28], a scattering mask [29], and multi-wavelength [30]. In this 2D image reconstruction and phase recovery, twin image and spatial aliasing signals, along with other digital artifacts, were solved using each separate technology or an integrated propagating phaser approach based on this idea [31]. These approaches show a significant improvement in resolution and a significant elimination of reconstruction artifacts, which can aid the numerical compensation of a sub-pixel super-resolution and three-dimensional volumetric image.

However, during the sectioning process of the 3D image generation, the out-of-focus reconstructed wavefront becomes a significant impediment to obtaining clear 3D cells in the sectioning of the thick biological sample [14] or particle images.
and in its application for flow visualization [32]. Except for the off-axis interferometer approach [33], there are three approaches to solving the problem. One of these strategies uses a 2D hologram, which can be calculated from the iterative phase matching method. This wavefront reconstruction approach can generally be adopted in super-resolution image reconstruction. Another simple way to find a focused depth is to use image processing with sharp contrast detection to determine a Z-depth profile in the noisy out-of-focus image. The other approach is 3D deconvolution method, which is to remove artifacts from the out-of-focus image at the target Z-depth focusing plane [34, 35]. The 3D deconvolution method can reduce the artifacts from the out-of-focus signal using the low-resolution reconstructed wavefront, although it is necessary to improve the noise suppression and resolution enhancement in the case of a super-resolved holographic image. Since 3D deconvolution methods target a simple particle sample, i.e., a bead and low-resolution holographic image, as described in previous reports [34, 35], the 3D deconvolution method cannot reflect the real situation of a medical sample and application, such as whole blood and pathology.

Here, we present a method to improve the 3D object image quality by the volumetric deconvolution and phase recovery of a super-resolution 2D holographic image. This approach consists of two steps to obtaining fully resolved 3D volumetric images. The three-dimensional image reconstruction, from low resolution to high resolution, is based on the conventional phase retrieval super-resolution and 3D volumetric deconvolution approach to rebuilding a real object from the image plane, as shown in Figure 1. First, the super-resolution image is obtained using low-resolution subpixel movements and a phase retrieval algorithm, as shown in Figure 1b. Second, the volumetric object is reconstructed using the 3D volumetric convolution of the super-resolution hologram image, which acts as a spatial filter, as shown in Figure 1c. Based on this approach, we demonstrate that a high-resolution 3D volumetric image, which significantly reduces wavefront reconstruction and out-of-focus artifacts, can be achieved. We believe that it can produce medical-grade images of smeared whole blood or an optically cleared tissue sample for mobile phytological microscopy or laser sectioning microscopy.

References


Keywords

Digital holography; volumetric deconvolution; three-dimensional volumetric deconvolution

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