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Optical diffraction tomography microscopy with transport of intensity equation using a light-emitting diode array

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ABSTRACT

Optical diffraction tomography (ODT) is an effective label-free technique for quantitatively refractive index imaging, which enables long-term monitoring of the internal three-dimensional (3D) structures and molecular composition of biological cells with minimal perturbation. However, existing optical tomographic methods generally rely on interferometric configuration for phase measurement and sophisticated mechanical systems for sample rotation or beam scanning. Thereby, the measurement is suspect to phase error coming from the coherent speckle, environmental vibrations, and mechanical error during data acquisition process. To overcome these limitations, we present a new ODT technique based on non-interferometric phase retrieval and programmable illumination emitting from a light-emitting diode (LED) array. The experimental system is built based on a traditional bright field microscope, with the light source replaced by a programmable LED array, which provides angle-variable quasi-monochromatic illumination with an angular coverage of ± 37 degrees in both x and y directions (corresponding to an illumination numerical aperture of ~0.6). Transport of intensity equation (TIE) is utilized to recover the phase at different illumination angles, and the refractive index distribution is reconstructed based on the ODT framework under first Rytov approximation. The missing-cone problem in ODT is addressed by using the iterative non-negative constraint algorithm, and the misalignment of the LED array is further numerically corrected to improve the accuracy of refractive index quantification. Experiments on polystyrene beads and thick biological specimens show that the proposed approach allows accurate refractive index reconstruction while greatly reduced the system complexity and environmental sensitivity compared to conventional interferometric ODT approaches.

1. Introduction

In microscopy imaging, the phase carries important information about the object's structure and optical properties, while this information cannot be directly visible. The typical examples of visualization methods are phase contrast microscopy [1] and differential interference contrast microscopy [2], which has been widely used in biomedical studies. But these techniques do not provide quantitative maps of phase change, making quantitative analysis and the interpretation difficult.

Interferometric method, such as digital holography microscope (DHM) [3,4], makes it possible to quantitatively measure the phase delay introduced by the heterogeneous refractive index distribution within the specimen. Nevertheless, conventional interferometry typically relies on highly coherent laser source, and the speckle noise also prevents the formation of high quality images. Over the past decades, more advanced quantitative phase microscopy techniques, that allow for self-interference under white-light illumination, have been reported to greatly improve the spatial resolution and imaging quality of the phase measurement [5–8]. On a different note, non-interferometric quantitative phase imaging can be realized with the transport of intensity equation (TIE) [9–15] only using object field intensities at multiple axially displaced planes. Furthermore, the phase can be uniquely determined by solving the TIE without complicated interferometric optical system and phase unwrapping.

Although the quantitative phase can provide reliable information about biological samples with the methods mentioned above, the measured phase only represents the optical path length along the axial direction, making the detailed volumetric information inside the sample inaccessible. Three dimensional (3D) imaging could be implemented by recording the images of specimen at various illumination angles. The 3D refractive index map of the specimen could then be reconstructed using the set of angular images. There are two ways to achieve the relative change of illumination angle. First, a sample of interest can be rotated, and the images of diffracted light field at

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different rotation angles can be used to reconstruct the 3D refractive index map of the sample [16–19]. Sample rotation enables the entire angular coverage, but the mechanical rotation inevitably makes the sample unstable, called radial run-out [17]. This method is more suitable for certain solid non-biological objects such as optical fibers. A special sample cuvette is required for the imaging of living biological cells in rotating method [20,21]. The second method is illumination scanning, the mirror mounted on a galvanometer or a piezoelectric transducer is typically used as a beam rotator, controlling the angle of the illumination beam that impinges onto a sample [22–26]. In recent years, the liquid crystals spatial light modulator (SLM) [27] and digital micro-mirror device (DMD) [28,29] have been utilized as a beam rotator for faster imaging speed and more stable beam controlling. Besides, tomographic imaging has also been implemented based on the lens-free platforms, providing both depth-resolved imaging capability as well as a very large field of view [30,31]. Nevertheless, these lens-free tomographic methods still depend on mechanical movement and laser illumination, and the resolution of 3D imaging also needs to be improved compared to existing high-NA conventional microscopy. Due to the finite numerical aperture of an objective, the lack of complete angular coverage leads to poorer axial resolution than the transverse resolution of the beam rotation method. For a specimen with prior knowledge, various algorithms have been developed to solve the problem of missing angle information [32,33].

The choice of 3D reconstruction algorithm is crucial to the spatial resolution and quantification of final reconstructed refractive index map. If the phase of transmitted field is interpreted as a line integral of the refractive index along the propagation direction for X-ray computed tomography (CT), then the filtered back-projection algorithm based on the inverse Radon transform can be applied [34]. Within the framework of diffraction tomography theory, the effect of diffraction is explicitly taken into account. Optical diffraction tomography (ODT) was first theoretically proposed by E. Wolf [35] in 1969 and the geometrical interpretation of ODT was presented by Dändliker and Weiss [36]. Lately, there have been some significant technical advances in diffraction tomography imaging for label-free 3D cell imaging [22–24,37,38]. Approximations such as the Born or Rytov approximation have been employed in ODT imaging to make the relationship between the 3D scattering potential and the two dimensional (2D) measured field straightforward [24,34,39].

However, most existing optical tomographic methods generally rely on interferometric implementation for phase measurement and sophisticated mechanical systems for sample rotation or beam scanning. Thus, the accuracy of phase measurement may be disturbed by the coherent speckle, environmental vibrations, and mechanical error during data acquisition process. To overcome these limitations, we use a light-emitting diode (LED) array as the illumination source within a conventional transmission microscope in this work. A single LED of the programmable source is turned on sequentially to generate the variable angle beam covering the angular range from −37 to 37 degrees. It should be noted that a similar experimental configuration, based on the illumination of a LED array, has been widely implemented in Fourier ptychographic microscopy for high-resolution and wide-field imaging [40–43]. Using two intensity images taken at distinct planes along the optical axis for each incident angle, the angular complex field images can be directly retrieved via the TIE. Moreover, the positional misalignment correction of the LED array is presented to correct some misplaced frequency spectrum components in 3D Fourier spectrum. The Rytov approximation, which is less restrictive than the Born approximation, will be chosen to simplify the relationship between the 3D scattering potential and the 2D measured field for biological cells imaging in this paper.

The proposed approach gets rid of coherent laser illuminations and complicated mechanical system for sample rotation and beam scanning, and bypasses the difficulties in phase unwrapping, as is common for interferometric methods. These advantages make it a competitive and powerful alternative to interference techniques for various microscopy applications in micro-optics inspection, life sciences, and biophotonics. Precise refractive index measurement of a micro polystyrene bead validates the principle and demonstrates the accuracy of this method. The experimental results on investigation of unstained Pandorina morum and human cancer cells are then presented, suggesting that the method developed can provide promising applications in the disease diagnosis and morphology study of cellular processes.

2. Experimental setup

Fig. 1 shows the schematic diagram of system illumination, the positional misalignment of LED array, and the photograph of imaging system. The illumination component of conventional microscope is replaced by a 15×15 programmable LED array beneath the specimen. It should be noted that the original LED array contains totally 64×64 LED elements, and only a fraction of them (the 15×15 LEDs on the upper right corner) are used for sample illumination, as shown in Fig. 1(c). Each LED can provide approximately spatially coherent quasi-monochromatic illuminations with narrow bandwidth (central wavelength $\lambda = 530$ *nm*, ~20 *nm* bandwidth). The light emitted from each LED can be approximately treated as a plane wave for each small image region of the specimen. The distance between every adjacent LED elements is 2.5 *mm*, and the LED array is placed 23 *mm* away from the sample stage. Thus, the maximum illumination angle of LED array is about 37 degrees in both x or y directions.

In our system, the LEDs in array are turned on sequentially and controlled by a custom-built Field Programmable Gate Array (FPGA)

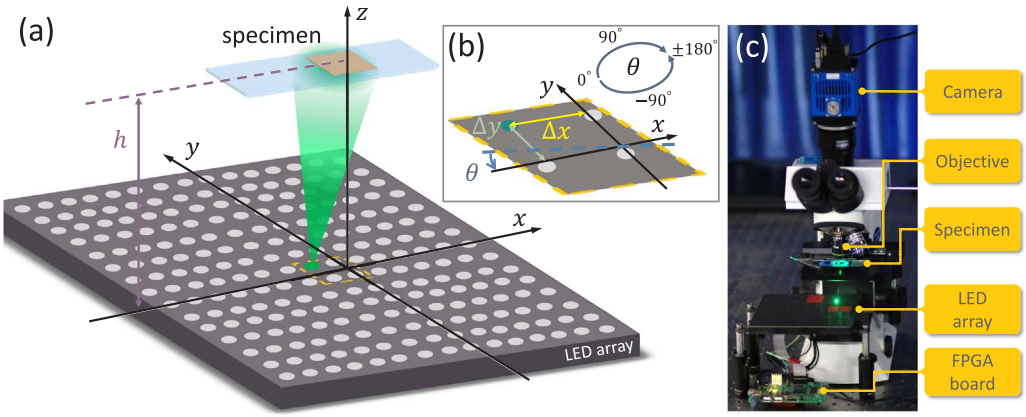

Fig. 1. (a) Schematics explaining of system illumination. A LED array is placed under the specimen and the distance from specimen to LED array is 23 mm. Spacing between adjacent LED elements is 2.5 mm. (b) The system error of translation Δx, Δy and rotation angle θ caused by the misplaced LED array. (c) Photograph of the imaging system. The crucial parts of setup in this photo are marked with the yellow boxes.