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Common-path diffraction optical tomography with a low-coherence illumination for reducing speckle noise

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ABSTRACT

Common-path diffraction optical tomography (cDOT) is a non-invasive and label-free optical holographic technique for measuring both the three-dimensional refractive index (RI) tomograms and two-dimensional dynamic phase images of a sample. Due to common-path geometry, cDOT provides quantitative phase imaging with high phase sensitivity. However, the image quality of the cDOT suffers from speckle noise; the use of a monochromatic laser inevitably results in the formation of parasitic fringe patterns in measured quantitative phase images. Here, we present a technique to reduce speckle noise in the cDOT using a low-coherence illumination source. Utilizing a Ti-sapphire pulsed laser in the cDOT, we achieved the reduction of speckle noise in both the three-dimensional RI tomograms and two-dimensional dynamic phase images.

Keywords: Optical diffraction tomography, incoherent quantitative phase imaging, speckle noise reduction

1. INTRODUCTION

Quantitative phase imaging (QPI) is one of powerful tools for imaging microscopic objects such as biological cells and tissues [1, 2]. Employing the principle of holography, the two-dimensional amplitude and phase delay maps of samples are quantitative and non-invasively measured. Three-dimensional refractive index (RI) maps can also be reconstructed from multiple QPI images obtained with various angle of illuminations using tomographic reconstruction algorithms. QPI has been exploited with various optical modalities including spectroscopic measurements [3-7] and polarization sensitive imaging [8, 9]. Recently, research has seen the potential and applicability of the QPI techniques in various applications in red blood cells [10-12], dry mass imaging [13], neuroscience [14], parasitology [15, 16], microbiology [17, 18], plankton [19], imaging through turbidity [20], and industrial fields [21-23].

Despite such advances, however, most QPI techniques suffer from speckle noise or parasitic fringe patterns. The speckle noise is caused by the use of a temporally coherence illumination which has a long coherence length. Light scattering from dust particles, multiple reflection, or other diffractive objects located far away from the sample plane contributes to the measured phase images, resulting in the low imaging qualities.

Currently, only a few sets of approaches and ideas have been explored in order to reduce unwanted speckle noise in QPI. The use of temporally incoherent sources including a Ti-sapphire pulsed laser, a LED, a white-light illumination has significantly reduced speckle noise [24-28]. The use of spatially incoherent sources by employing speckle illumination has been also employed for speckle reduction in QPI [29-32]. Recently, polarization states has been exploited for the speckle reduction [33]. However, the speckle reduction in three-dimensional RI tomographic images using QPI has not been fully explored with the notable exception in Ref. [27].

Here, we present an improved three-dimensional RI tomographic imaging technique based on common-path diffraction optical tomography (cDOT) in which speckle noise is significantly reduced utilizing a temporally low-coherence illumination source. The cDOT is a recently developed QPI technique which can measure both three-dimensional maps and two-dimensional dynamic images [34]. We demonstrate that the use of a low-coherence

illumination source can reduced the speckle noise in both 3-D RI tomograms, as well as 2-D QPI images and temporal stability.

2. METHODS

2.1 common-path diffraction optical tomography (cDOT)

The detailed experimental setup for the cDOT is described in elsewhere [34]. Briefly, in the cDOT, complex light field images of a sample, illuminated with various incident angles of plane waves, are measured using a common-path interferometry based on diffraction phase microscopy (DPM) [35, 36]. Three-dimensional RI maps of a sample are then reconstructed from these measured multiple two-dimensional field images with various illumination angles via diffraction tomography algorithm [37, 38]. Due to the limited angle of acceptance determined by the numerical aperture (NA) of the objective lenses, there exist a portion of information in Fourier spectra which cannot be optically measured, so-called the missing cone problem. To address the missing cone problem, we employed the non-negativity constraint [39].

The angle of the illumination beam is scanned by a two-axis galvanometric mirror [GM1 in Fig. 1(a)]. After passing the sample, the angle of the beam is then descanned by a two-axis galvanometric mirror, GM2, located at the conjugated plane to GM1, which ensures the optical axis is unchanged for the DPM. The DPM precisely measured the optical field images of a sample exploiting common-path interferometry. The diffracted beam from the sample is projected onto a grating. The grating splits the beam into many diffraction orders, from which the zeroth and first orders are selected to be used as a reference and sample beam, respectively. The zeroth-order diffraction beam is spatially filtered by the pinhole located in the Fourier plane, and is then converted into a plane wave. The holograms generated by the interference between the plane wave and the sample beam are recorded by a CCD camera.

2.3 cDOT with a temporally low-coherent source

Figure 1(a) shows the optical setup of the cDOT equipped with a temporally low-coherent source. A Ti:sapphire pulsed laser was used for a low-coherence illumination. The spectral density of the Ti:sapphire laser was presented in Fig. 2(b). The center wavelength and the full width half maximum of the spectrum was 794 nm and 10 nm, respectively. A HeNe laser as a coherent light source was also used for a comparison purpose.

Fig 1. (a) Experimental setup. M: mirrors; FM: flip mirror; GM1, GM2: galvanometer scanning mirror; OL1: condenser lens (NA = 0.9); OL2: objective lens (NA = 1.4); TL: tube lens; Microscopy: IX71, Olympus Inc.; L1-L5: lenses ($f = 100$ mm); L6: lens ($f = 400$ mm); G: grating; P: pinhole (diameter = 25 μ m), (b) spectral density of the Ti:sapphire laser.

3. RESULTS

In order to study the effects of temporal coherency of the illumination to the spatial speckle noise, we first compared the 2-D phase images of a clean slide glass using the Ti:sapphire laser and the HeNe laser in the cDOT. Figures 2(a) and (b) are the quantitative phase images of no sample region illuminated by the HeNe and the Ti-sapphire laser, respectively. Speckle noise caused by multiple reflection is clearly observed in the case of the HeNe laser [Fig. 2(a)], whereas the use of temporally low-coherent source significantly reduced speckle noise in QPI [Fig. 2(b)]. To quantitatively analyze the reduction of speckle noise, we calculated the probability density functions (PDFs) of optical path-length delay in the phase images in Figs. 2(a) and (b). As shown in Fig. 2(c). The PDF of the phase image obtained with the HeNe laser is significantly wider than that obtained with the Ti:sapphire laser. Standard deviations of the phase images are 31.8 and 6.7 milli-radian for the case with the HeNe and Ti:sapphire laser, respectively.

Fig 2. Speckle noise reduction in the cDOT using temporally low-coherent source. (a-b) Quantitative phase image of a clean slide glass obtained with the HeNe laser (a) and the Ti-sapphire laser (b). (c) Probability density functions of the optical path-length delay in the phase images shown in (a) and (b).

To study the effects of a low coherent source to RI tomogram quality, we measured the three-dimensional RI tomograms of a human red blood cell (RBC) using the cDOT. The cross-sectional slice images of the measured RI tomograms obtained with the HeNe laser and the Ti:Shapphire laser was shown in Figs. 3(a-b) and Figs. 3(c-d), respectively. In the RI tomograms obtained with the coherent illumination, RI values corresponding to the cell are elongated along the z-axis [Fig. 3(b)], compared to the case with the low-coherence source [Fig. 3(d)]. Furthermore, stripe-shaped artifacts are also found in the RI tomogram obtained with the coherent illumination, as indicated with the white arrows in Figs. 3(a-b). For quantitative comparison, the PDFs of RI values corresponding to the medium are calculated [Fig. 3(e)]. The PDF of the RI values obtained with the HeNe laser was wider than that obtained with the Ti:sapphire laser. Standard deviations of the RI values are calculated as 2.9 and 1.7 milli-radian for the case of the HeNe laser and Ti:sapphire laser, respectively. For a fair comparison, the iterative process based on the non-negatively constraint for solving the missing cone problem was excluded when calculating the PDFs of the RI values.

Fig 3. (a-d) The cross-sectional slice images of the measured RI tomograms obtained with the HeNe laser (a-b) and the Ti:Shapphire laser (c-d). (e) Probability density functions of RI values corresponding to the surrounding medium.

The cDOT is capable of measuring dynamic two-dimensional phase images with high stability because the cDOT employs the principle of common-path interferometry. In order to further investigate the effects of the low-coherence illumination to dynamic phase images, we measured temporal fluctuations of phase images of the same RBC, which was used in the RI tomogram measurements, with the frame rate of 125 Hz.

The result is shown in Fig. 4. The averaged phase images of the RBCs and the standard deviation map of dynamic phase images are shown in Figs. 4(a) and (b), respectively. Figure 4(c) shows the temporal fluctuation of the three representative points in the measured phase images corresponding to background, cell dimple area, and cell outer area [indicated with the letter A, B, and C in the Fig. 4(a)]. In the case of the coherence illumination, there exist elevated dynamic fluctuation signals both in the background and sample area [Fig. 4(b)]. This elevated signals are occasionally found when there is multiple reflection from moving objects such as multiply reflected light from adjacent RBCs. Even in the case, the use of temporally low-coherence source significantly reduce the speckle noise. Figures 4(d-f) are the results obtained with the Ti:sapphire laser. It is clearly shown that the temporal speckle noise is also decreased when the Ti:sapphire laser was used.

Fig 4. Dynamic two-dimensional phase images of a RBC using the cDOT with the HeNe laser (a-c) and the Ti-sapphire laser (d-f). (a) and (d): Quantitative phase images illuminated of the RBC. (b) and (e): Standard deviation map of temporal phase fluctuation. (c) and (f): Phase fluctuations of three representative points indicated as the letter A, B, and C in (a) and (d).

4. CONCLUSION

We demonstrated that the use of an incoherent illumination significantly enhance the image quality of the cDOT. The speckle noise was reduced in the two-dimensional phase images, three-dimensional RI tomograms, and dynamic membrane fluctuation images. For a fair comparison of the effects of the source coherency, we have utilized the same setup and the sample; the HeNe and Ti-sapphire laser are alternatively used as an illumination source. In the two-dimensional quantitative phase images and the three-dimensional RI tomograms, speckle noise was reduced by approximately 79% and 27%, respectively. Furthermore, the temporal phase fluctuation was also reduced by 63%. The present work clearly demonstrate that the quantitative phase imaging capability of the cDOT can be improved by the use of the temporally low-coherence illumination by significantly reducing the speckle noise. The presented method will find direct applications in measuring three-dimensional RI tomograms of biological samples because it requires the simple replacement of a coherent source to a low-coherence source. The present method can also be combined with high-speed three-dimensional RI tomographic techniques [37].

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