Tomographic microscope for low-coherent quantitative phase imaging

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ABSTRACT

A novel tomographic microscope setup utilizing the principle of Holographic Incoherent-light-source Quantitative Phase Imaging is introduced. This setup combines the advantages of achromatic off-axis holography with the ultrafast operation of a digital micromirror device-based tomographic illuminator. The imaging theory is explained, and the optical design of the microscope is described. The functionality of the microscope modules is demonstrated experimentally using a light source of limited spatial coherence.

Keywords: Quantitative phase imaging, Tomography, Digital holographic microscopy

1. INTRODUCTION

Quantitative Phase Imaging (QPI) has become an indispensable tool in live cell research due to its ability to provide label-free imaging of phase samples. This technique is particularly useful for studying cellular responses to specific stimuli, such as drug treatments, while minimizing disturbances to natural cell behavior.[1,2] Typically, two-dimensional phase information is retrieved from the hologram, which is the complete record of the electromagnetic wave propagated through the sample. The hologram is recorded using a quantitative phase microscope that incorporates an interferometer. In QPI, phase contrast represents a projection of the sample's three-dimensional refractive index distribution, which may lead to misinterpretation of the sample's bulk morphology.[3]

Phase tomography enables three-dimensional reconstructions of the refractive index, providing more accurate insights into the internal structures and metabolic processes of cells.[4] This technique combines quantitative phase microscopy with tomographic illumination. Many quantitative phase microscopes implement different tomographic illumination approaches, such as sample rotation,[5,6] beam scanning approach,[7,8] and others. Each approach presents specific advantages and challenges regarding system stability, resolution, sample manipulation, and limited spatial frequency coverage.[4] Most systems employ lasers for sample illumination due to their high temporal and spatial coherence, which facilitates microscope alignment and allows for off-axis interferometer configuration with single-shot capability. However, the highly coherent light source introduces significant disadvantages, such as speckle noise that corrupts the sample information in the image.[3]

The Holographic Incoherent-light-source Quantitative Phase Imaging (hiQPI) technique[9] employs the concept of achromatic off-axis Mach Zehnder interferometry with a spatially broad low-coherent light source. Compared to traditional off-axis interferometric systems that utilize lasers, hiQPI microscopy achieves a resolution similar to the wide-field microscopy standard while reducing noise and interference artifacts.[10,11] Additionally, it offers an enhanced accuracy of up to 1 nm in optical path difference,[11] making it a powerful tool for live cell imaging. Its ability to perform real-time two-dimensional imaging of unlabeled living cells while minimizing speckle noise has been successfully demonstrated in several studies.[12–14] However, despite its advantages, the hiQPI system has yet to be fully exploited for tomographic imaging.

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In this work, we introduce a novel tomographic microscope setup that utilizes the hiQPI principle, integrating its benefits into a three-dimensional imaging system. The system employs a Digital Micromirror Device (DMD) for illumination beam scanning, allowing stable, high-speed operation without any mechanical movement.[8,15] The implementation of tomographic hiQPI promises improved spatial resolution, speckle-free imaging, and compatibility with living cell applications.

2. METHODS

The proposed tomographic system comprises a DMD illumination unit, a Mach-Zehnder interferometer, and a detection unit. The optical design of the system is illustrated in Fig. 1. Light from every point of a broad polychromatic light source (SC) is focused by primary collector lenses (PC) onto the front focal plane of the primary condenser lens (PCD). The primary condenser lens (PCD) directs light towards the TIR prism (TIR), which reflects it onto the chip of the digital micromirror device (DMD), providing homogeneous illumination based on the principle of Köhler illumination.

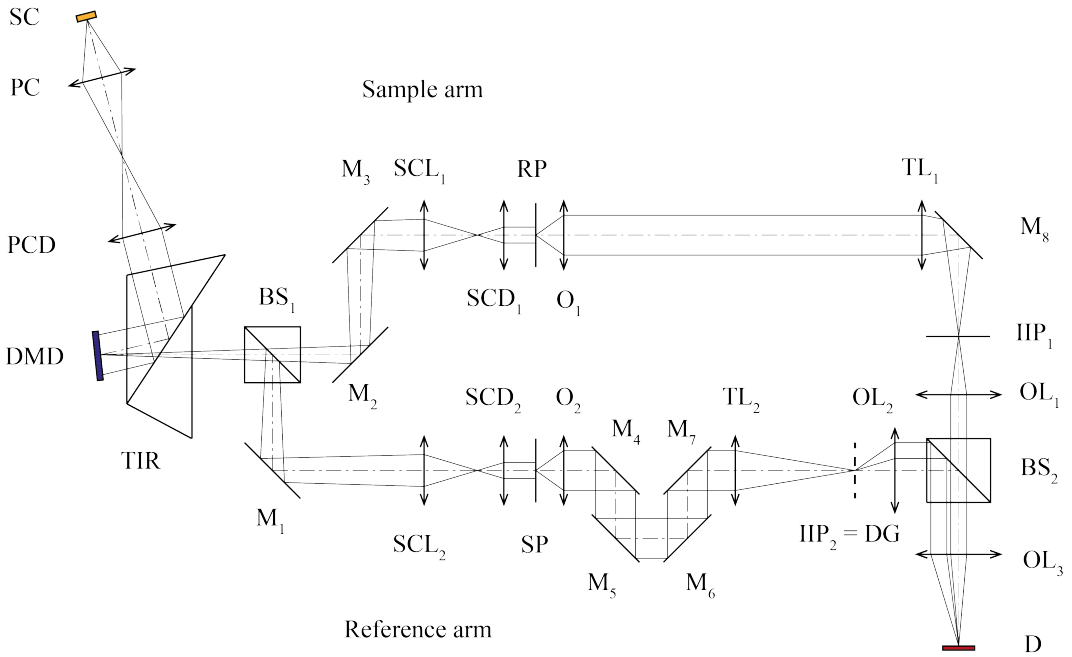

Figure 1. Optical scheme of the hiQPI tomographic microscope. Light source (SC), primary collector (PC), primary condenser (PCD), digital micromirror device (DMD), TIR prism (TIR), beamsplitter (BS), mirrors (M), secondary collector (SCL), secondary condenser (SCD), sample plane (SP), reference plane (RP), microscope objective (O), tube lenses (TL), intermediate image plane (IIP), diffraction grating (DG), output lens (OL), detector (D).

The DMD chip (DMD) serves as the digitally driven secondary light source for the standard hiQPI microscope, utilizing the Köhler principle for the second time. The DMD chip (DMD) reflects the incoming beam, which is not, in this case, reflected but passes through the TIR prism (TIR) towards 50:50 beamsplitter (BS_1), which divides the light between the sample and reference arms. Secondary collector lenses (SCL) form images of the DMD chip in the back focal planes of the secondary condenser lenses (SCD), thereby illuminating the sample plane (SP) and reference plane (RP) with homogeneous light.

The second illuminator is followed by microscope imaging systems in both arms. The microscope objectives (O) and tube lenses (TL) propagate the light from the sample plane (SP) and reference plane (RP), respectively, to form the intermediate images. In the reference arm, the intermediate image plane (IIP_2) is coincident with the diffraction grating (DG) and back focal plane of the output lens (OL_2). The blazed diffraction grating (DG) diffracts the light to the +1st diffraction order, which is then propagated by the output lens (OL_2).

The other diffraction orders are blocked. In the sample arm, a diffraction grating is not used, as the 0th diffraction order is necessary for interference. Therefore, the light is transmitted directly through the output lens (OL_1). Both beams pass through the beamsplitter (BS_2) and the output lens (OL_3), recombining at the chip of the detector (D). The interference between the +1st diffraction order of the reference beam and the 0th diffraction order of the object beam equalises the optical paths for different wavelengths[16] and the achromatic interference pattern is formed at the detector plane (D) with the carrier frequency of the fringes f_c directly proportional to the diffraction grating frequency f_{DG}

$$f_c = \frac{f_{DG}}{m_O}, \tag{1}$$

where m_O represents the lateral magnification between the intermediate image plane (IIP_2) and plane of the detector (D).

The tomographic illumination beam scanning approach is realized by employing the DMD chip (DMD) illuminated with homogeneous light. As the DMD image is formed in the back focal plane of the secondary condenser lenses (SCD), the pattern projected on the DMD chip (DMD) corresponds to the direction of the illumination beam in the sample plane (SP) and reference plane (RP). For successful tomographic reconstruction, several holograms of the sample illuminated from different angles are needed. High illumination angles are necessary for sufficient frequency coverage. Therefore, microscope objectives with high numerical aperture must be used as secondary condenser lenses (SCD). The high resolution of a DMD chip, along with its operational frequency reaching several kHz, offers great speed, versatility and user-friendly control over the illumination beam direction. Due to the grating structure of the DMD chip, the spatial filter must be used after the DMD to block unwanted diffraction orders. The functionality of the proposed illumination technique was demonstrated by recording the illumination spot on the detector located close to the sample plane (SP) while changing the illumination mask on the DMD, as illustrated in Fig. 2.

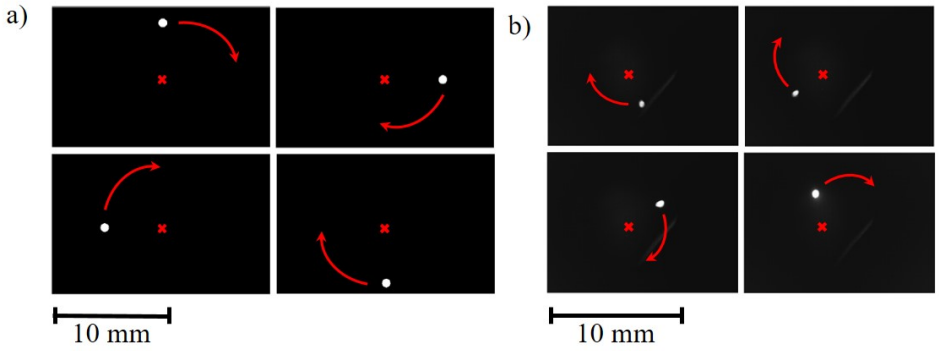

Figure 2. a) The mask projected onto the DMD chip and b) The spot recorded by the detector placed close to the sample plane demonstrates the concept's functionality. The rotation direction is indicated with red arrows.

3. RESULTS

The functionality of the tomographic illuminator was successfully demonstrated. However, before its full integration, the functionality of the microscope holographic part must be tested. A preliminary experiment was conducted without using the tomographic illumination module. In this configuration, the setup operates as a conventional hiQPI microscope. A quasi-coherent source, consisting of a HeNe laser and a rotating diffuser, was employed in the microscope assembly instead of a DMD chip to illuminate both the sample and reference planes. The rotating diffuser reduces spatial coherence while preserving the high temporal coherence of the HeNe laser. This trade-off allows for a relatively simple alignment of the setup while also suppressing unwanted speckle artefacts in the image. Thus, it is suitable for a preliminary proof of concept.

The sample used in the experiment was a PMMA phase step structure deposited on a glass substrate, fabricated through a nanolithographic process. This structure comprises five rows of steps. The first four rows feature a uniform step depth of approximately 300 nm, while the fifth row exhibits a gradually decreasing depth ranging from 1600 nm to 90 nm.

The sample hologram of the structure and reference hologram of the background were acquired using 10x/0.25 objectives (O) for imaging and 20x/0.50 objectives as secondary condensers (SCD). The acquired holograms were further processed numerically using Fourier techniques.[17] The Fourier spectra allowed filtering of the sidelobe spectrum of the interference fringes, followed by demodulation and application of a Hanning window function in Fourier space. By performing an inverse Fourier transform, both amplitude and phase images of the sample were obtained. To improve the image background, the empty phase image was subtracted from the sample's phase images. In addition, background enhancement was manually performed in the region of interest (ROI) of the phase image by fitting it with a third-order polynomial, which was subsequently subtracted from the original image. The resulting image is shown in Fig. 3 a).

For reference, an image of the phase structure was also acquired using a commercial phase microscope, the Telight Q-Phase, and processed with the Telight SophiQ software, which is shown in Fig. 3 b). The 10x/0.30 objectives were used for imaging along with condensers with a numerical aperture of 0.30. The Q-Phase microscope uses an LED with a 10 nm bandwidth centred at 660 nm for illumination. Except for one step structure, which shows a bigger phase step with Q-Phase, the imaging quality is comparable. The Q-Phase image appears sharper, likely due to better alignment, higher stability, and the superior quality of the camera. The difference in illumination wavelength between the two microscopes is relatively small compared to the background noise and phase value fluctuations. No significant differences in phase values were observed. The structure's line profiles acquired with both microscopes are compared in Fig. 3 c). The profile obtained with Q-Phase appears slightly curved, likely due to the background enhancement algorithms utilized for image reconstruction in SophiQ software, which may introduce minor inaccuracies compared to manual background correction. Despite this, the profiles show significant similarities.

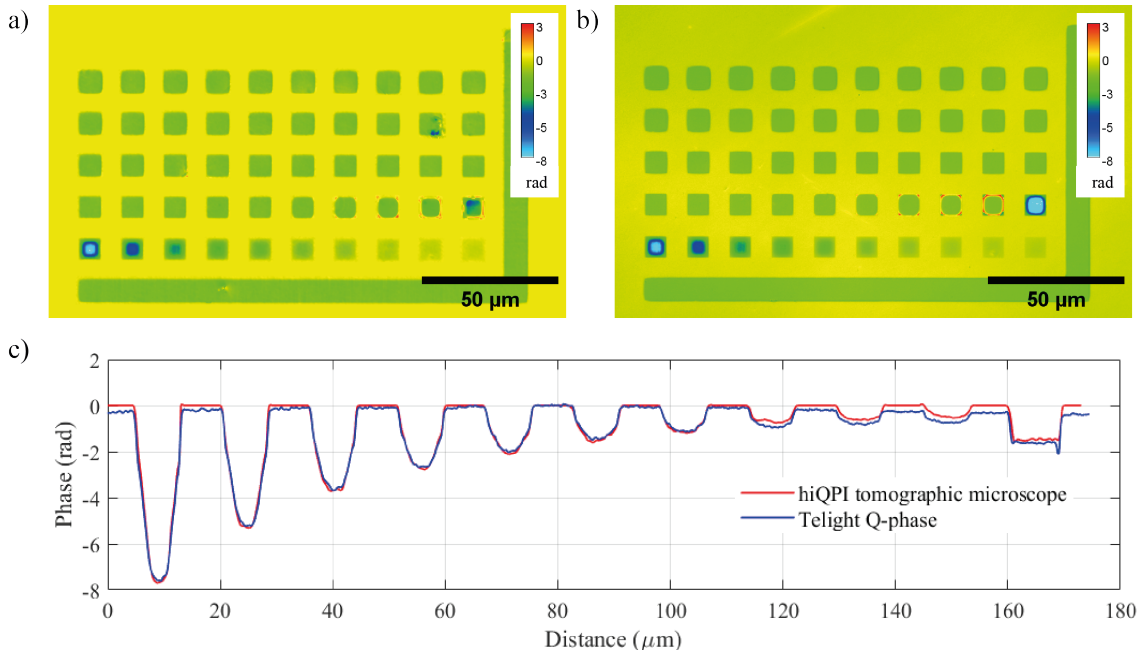

Figure 3. The phase step PMMA structure imaged with a) hiQPI tomographic microscope and b) Telight Q-Phase. c) The plot of a structure line profile marked in a) and b) with the solid lines.

4. CONCLUSION

The concept of a novel tomographic microscope utilizing the principles of hiQPI was introduced. Its working principle was explained, and the optical design was thoroughly described. The functionality of the concept was experimentally validated for both components of the microscope, the tomographic illuminator and the holographic microscope, using a quasi-coherent light source. The imaging capability of the microscope was compared to that of a commercial device, achieving similar results. Following this successful preliminary experiment, the tomographic microscope can be further developed into a fully functional, complex device with the potential for real-time, label-free high-resolution volumetric imaging of live cells.

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