



Quantitative polarization digital holographic microscope

Kumar, Manoj
Yoneda, Naru
Awatsuji, Yasuhiro
Matoba, Osamu

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Manoj Kumar^{1,*} , Naru Yoneda^{2,3} , Yasuhiro Awatsuji⁴ and Osamu Matoba^{2,3} ¹ Amity School of Physical Sciences, Amity University Punjab, Sector 82A, Mohali 140306, India² Graduate School of System Informatics, Department of Systems Science, Kobe University, Rokkodai 1-1, Nada, Kobe 657-8501, Japan³ Center of Optical Scattering Image Science, Kobe University, Rokkodai 1-1, Nada, Kobe 657-8501, Japan⁴ Faculty of Electrical Engineering and Electronics, Kyoto Institute of Technology, Matsugasaki, Sakyo-ku, Kyoto 606-8585, Japan

* Author to whom any correspondence should be addressed.

E-mail: manojklakra@gmail.com and matoba@kobe-u.ac.jp**Keywords:** single-shot, common-path, polarization imaging, digital holography, stokes parameters**Abstract**

Quantitative polarization digital holographic microscope (QPDHM) emerges as a powerful technique offering unprecedented capabilities in imaging and extraction of rich optical parameters, such as phase retardation, birefringence, and orientation of anisotropic structures, which are crucial for understanding biological processes and material properties at the microscale. In this work, a new configuration of single-shot common-path QPDHM with a very high temporal stability (of ~ 0.0096 rad.) is proposed and experimentally demonstrated. The system involves polarization-angular multiplexing digital holographic method in which only a single multiplexed hologram is recorded, which provides the complex amplitudes of the object under study corresponding to the orthogonal polarization components. The full polarization state of the object light field, i.e. the Stokes parameters, is quantitatively measured from the obtained complex amplitude information. The system does not require mechanical movement of any optical components or complicated devices such as phase-shifting or polarization camera. This advantage of the proposed system makes it a suitable candidate for the visualization and analysis of dynamic and fast transient polarization state measurements. The imaging performance and measurement accuracy of the proposed system are verified on several anisotropic materials, and the obtained results corroborate its relevance.

1. Introduction

Several materials, such as crystalline polymers, liquid crystals, fibrous and polymeric materials, and biological samples including collagen fibers, fibronectin, and cytoskeleton, are optically anisotropic [1, 2]. These anisotropic materials exhibit physical properties, such as optical, mechanical, and thermal behavior, that vary with direction. These directional dependencies arise from the internal structure and molecular orientation of the material, often governed by crystalline arrangements, molecular alignment, or fibrous morphology. Characterizing anisotropy is critical across a wide range of scientific and engineering disciplines, including materials science, photonics, biomedical imaging, and nanotechnology. Traditional imaging and characterization techniques, while powerful, often fall short in capturing the subtleties associated with direction-dependent properties. In this context, polarization imaging techniques have emerged as essential tools for probing anisotropic morphological and structural information with enhanced contrast, specificity, and functional insight [3–5]. Polarization, a fundamental property of light, is highly sensitive to the optical anisotropy of materials. When polarized light interacts with anisotropic media, it experiences changes in its polarization state due to phenomena such as birefringence, dichroism, and scattering anisotropy. These interactions encode structural and morphological information about the sample into the polarization state of the transmitted or reflected light. By systematically analyzing these changes, polarization imaging allows researchers to infer properties such as crystal orientation, molecular alignment, stress distribution, and sub-surface features that are otherwise invisible under conventional intensity-based imaging.

Polarization imaging techniques such as polarized light microscopy [3], polarization-sensitive optical coherence tomography [6], polarization Raman microscopy [7], fluorescence confocal polarizing microscopy [8], Fourier polarimetry [9], polarized fluorescence microscopy [10], polarization-sensitive second harmonic microscopy [11], etc, use polarized light to convey morphological, biochemical, and functional information from anisotropic objects. Therefore, these techniques have been widely used for the analysis and characterization of anisotropic samples. However, most of these techniques cannot achieve real-time measurements due to the need to rotate the polarized elements multiple times to record multiple intensity images at different polarization detection states. Therefore, there is a need to develop a single-shot polarization imaging technique. Many features make digital holography (DH) [12, 13] a compelling substitute for traditional intensity-based polarization imaging techniques. DH has the capability of simultaneously determining intensity, phase, and polarization state in a single-shot acquisition, and this feature reveals the development of varied futuristic technologies. Colomb *et al* [14] reported a Mach–Zehnder type DH system for the first time for the polarization detection, and afterwards several new advancements in this technique for polarization detection have been proposed [15–22].

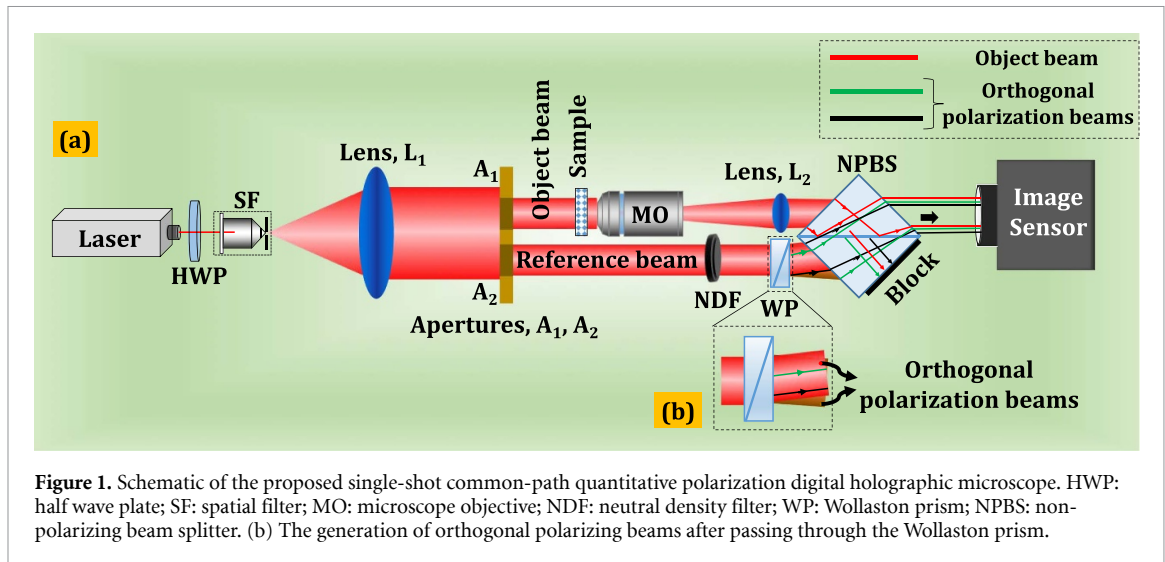
In polarization-sensitive DH, the object wavefield is allowed to interfere with two orthogonal polarization reference beams at a different off-axis angle, resulting in a polarization-angular multiplexing digital hologram. The distribution of polarization states is then obtained by numerically reconstructing the amplitude and phase of each polarization component. However, to eliminate multiple recordings and to achieve single-shot acquisition by the multiplexing method, the system requires two distinct optical pathways for creating the reference beams, and therefore, makes the optical system relatively bulky, complicated, and expensive [18, 22]. Dual-channel DH systems, typically based on Mach–Zehnder or Michelson interferometric configurations, suffer from several inherent limitations arising from the separation of object and reference beams into two independent optical arms. Because the two beams propagate through different physical paths, any environmental disturbance, such as mechanical vibrations, air turbulence, temperature fluctuations, or thermal drift, affects them differently, introducing phase noise and reducing measurement accuracy. These systems also require precise optical path length matching and careful alignment of mirrors, beam splitters, and lenses, making them sensitive to misalignment and increasing system complexity. Moreover, long or unequal optical paths can degrade coherence and fringe visibility, while maintaining stability often demands vibration-isolated optical tables or active phase stabilization, increasing cost and limiting portability.

Common-path digital holographic systems overcome these issues by allowing the object and reference waves to travel along nearly the same optical path and share most optical components. Since both waves experience identical environmental perturbations, common-mode noise is largely canceled, significantly enhancing phase stability and reducing sensitivity to external disturbances. The minimal path difference eliminates stringent path-length matching requirements and simplifies alignment. As a result, common-path systems offer superior mechanical robustness, improved temporal phase stability, and reduced phase noise, which are essential for quantitative phase measurements. Additionally, their compact architecture requires fewer components, lowering cost and enabling easier integration into microscopes or portable platforms. These advantages make common-path DH particularly well suited for live-cell imaging, dynamic biological studies, industrial metrology, and field-deployable optical systems where high stability, simplicity, and reliability are critical.

In this work, we report, for the first time, a single-shot common-path polarization-angular multiplexing quantitative polarization digital holographic microscope (QPDHM). The QPDHM is proposed for the measurement of full polarization states of a light field. In the proposed QPDHM system, the polarization-angular multiplexing is achieved by a Wollaston prism (WP) and a cube beam splitter oriented at $\sim 45^\circ$. This single multiplexed hologram enables simultaneous reconstruction of amplitude, phase, and orthogonal polarization components. The quantitative imaging and measurement capability of the QPDHM system is demonstrated on a custom-designed polarization-sensitive resolution target as well as a standard birefringent resolution target.

2. Methodology

Figure 1(a) shows the schematic of the proposed single-shot common-path QPDHM. A He-Ne laser of wavelength 632.8 nm (power 20 mW) is used as a light source. A half-wave plate (HWP) is placed in the laser path to transform the linearly polarized light into 45° linearly polarized light. The laser is spatially filtered by a spatial filter (SF) assembly that consists of a microscope objective of $40\times$ and a pinhole of $5\ \mu\text{m}$. The spatially filtered laser is collimated by a lens (L_1) of focal length 100 and diameter of 50 mm. Half of the collimated object beam of diameter $\sim 50\ \text{mm}$ is used as the object beam, and the other half



is used as a reference beam. Apertures A_1 and A_2 are used to separate the object and reference beams. In the object beam, the sample is placed on a 3D translation stage. The object beam is allowed to pass through the object under study, and the object diffracted beam is collected by a microscope objective (MO—magnification $10\times$, NA 0.3) and again collimated by a lens (L_2) of focal length 100 mm and diameter 25.4 mm. The reference beam, as depicted in figure 1(b), is allowed to pass through a WP (model—WPQ10, 1° beam separation, 400 nm–2.0 μm , uncoated quartz, Thorlabs). The WP splits the linearly polarized light into the horizontal and vertical polarizations, which serve as two reference beams. The object and the reference beams have lateral separations, and in order to create their interference on the image sensor, a non-polarizing beam splitter (NPBS) oriented at $\sim 45^\circ$ with respect to the optical axis is introduced in the optical design. The object beam is allowed to pass through the upper prism of NPBS, whereas the two reference beams pass through the lower prism. The object beam is partially reflected and partially transmitted through the NPBS and creating two replicas. The same happens with reference beams. The orientation of NPBS can be adjusted so that the reflected object beam from the base of NPBS and transmitted reference beams follow exactly the same path. Therefore, the object beam and the reference beam (which is composed of two orthogonal polarization beams) interfere to form a polarization-angular multiplexed digital hologram at the faceplate of the image sensor (Sony Pregius IMX 264 model featuring a pixel number of 2448×2048 and a pixel size of 3.45 μm). At the existence of the NPBS, two replicas of the object and reference beams are generated, and one of the replicas is recorded by the image sensor and another is blocked. The major role of NPBS is to reduce the lateral separation between the object and two reference beams and help to form a polarization-angular multiplexed digital hologram, but at the same time, it reduces the object and reference light throughputs. However, in the system with a laser power of ~ 20 mW, this problem was not observed. A neutral density filter (NDF) is used in the reference beam to adjust the contrast of the interference fringes.

The 1° separation of the WP determines the spatial carrier frequencies of the two orthogonally polarized interference terms in the multiplexed hologram. This angle was selected to generate sufficient separation of the corresponding cross-correlation terms in the Fourier domain while remaining within the Nyquist sampling limit of the camera sensor (pixel size 3.45 μm). In an off-axis digital holographic system, the angular separation between the object and reference beams determines the spatial carrier frequency of the interference fringes. For a beam tilt angle θ , the fringe spatial frequency is given by, $f_c = \frac{\sin\theta}{\lambda}$, where λ is the illumination wavelength. In the discrete Fourier domain, proper reconstruction requires that the cross-correlation terms be well separated from the zero-order term and from each other, while remaining within the Nyquist sampling limit of the image sensor, $f_c < \frac{1}{2p}$, where p is the camera pixel pitch.

If θ is too small, the spectral lobes corresponding to the orthogonal polarization components partially overlap with the zero-order term, leading to cross-talk and reconstruction artifacts. Conversely, if θ is too large, the carrier frequency approaches the Nyquist limit, increasing the risk of aliasing and reducing fringe contrast due to limited sampling. Using the camera pixel size (3.45 μm) and wavelength

(632.8 nm), the allowable angular range is calculated to ensure sufficient spectral separation with a safety margin from aliasing. The selected 1° beam separation angle of the WP places the cross-correlation terms in non-overlapping regions of the Fourier plane while maintaining high fringe visibility and efficient numerical filtering. This represents an optimal trade-off between spectral isolation and sampling constraints.

The $\sim 45^\circ$ orientation of the NPBS is required to geometrically overlap the object and reference beams while minimizing optical path difference in the common-path configuration. At this angle, the transmitted reference beams and the reflected object beam propagate collinearly toward the sensor, maximizing fringe contrast and temporal stability. Deviations from this angle reduce overlap efficiency and fringe visibility, degrading signal-to-noise ratio and reconstruction quality. The selected configuration was verified experimentally by monitoring fringe contrast and Fourier-domain separation under small angular variations.

3. Theory

The multiplexed digital hologram is composed of two groups of interference fringes in slightly different orientations and, therefore, is capable of providing simultaneously the two orthogonal polarization components of the object beam. The two orthogonal reference beams are incoherent to each other and do not contribute to forming any fringes by their interference. The intensity distribution of the multiplexed digital hologram is represented by

$$I(x, y) = |E_O(x, y) + E_{RH}(x, y) + E_{RV}(x, y)|^2 \quad (1)$$

where, $E_O(x, y) = E_{OH}(x, y) + E_{OV}(x, y)$, $E_{RH}(x, y)$, and $E_{RV}(x, y)$ are the object wavefield, horizontal reference wavefield, and vertical reference wavefield, respectively. The complex amplitude of the object wavefield for the two orthogonal polarization components can be numerically reconstructed by using the angular spectrum method [12] and these are represented by the following expressions by following the method reported by Dou *et al* [18, 22], as

$$E_H = \beta |E_{OH}| |E_{RH}| e^{i(\theta_H)} \quad (2)$$

$$E_V = \beta |E_{OV}| |E_{RV}| e^{i(\theta_V)} \quad (3)$$

where, β is the constant related to exposure time and response of the image sensor, θ_H , and θ_V are the dynamic phases with horizontal and vertical polarization states, respectively. Further, the azimuthal and polar angles can be calculated from the obtained complex amplitudes corresponding to the two orthogonal polarization states, as

$$2\psi = \arg\left(\frac{E_H}{E_V}\right) - \Delta\theta \quad (4)$$

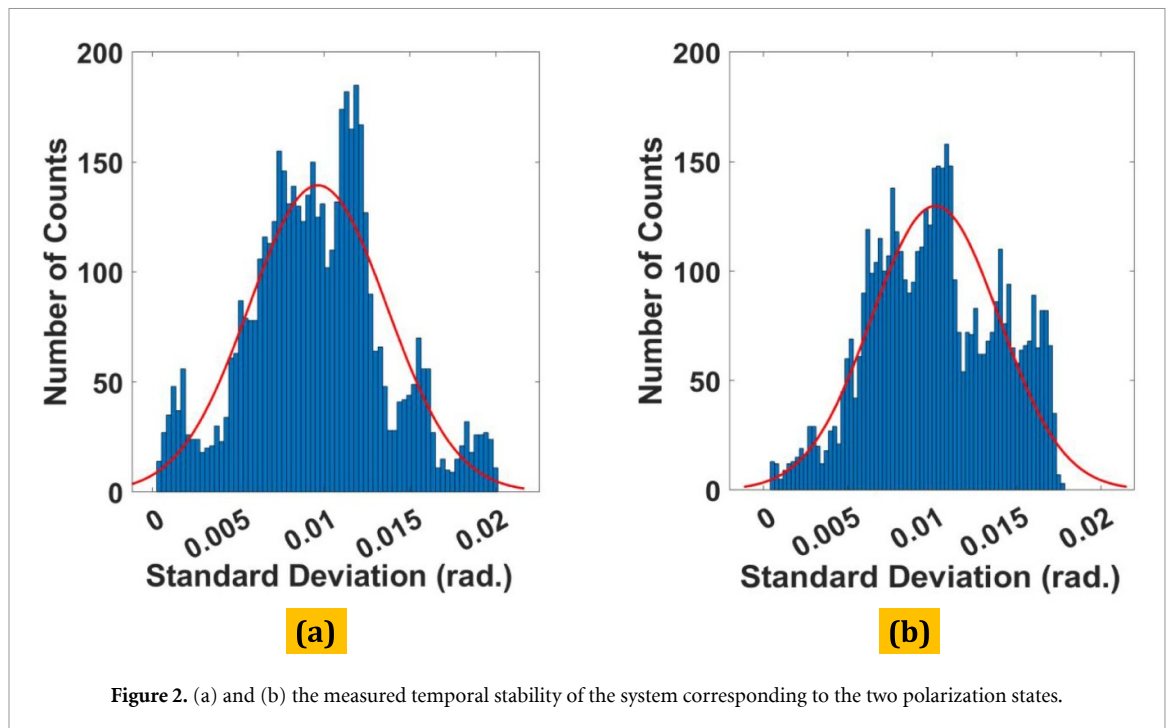
$$2\chi = \frac{\pi}{2} - 2\arctan\left|\frac{E_V}{E_H}\right|. \quad (5)$$

Finally, the Stokes parameters can be calculated by the measured azimuthal and polar angles and represented by

$$\left. \begin{aligned} S_1 &= \sin(2\chi) \\ S_2 &= \cos(2\chi) \cos(2\psi) \\ S_3 &= \cos(2\chi) \sin(2\psi) \end{aligned} \right\}. \quad (6)$$

4. Results and discussion

Owing to the common-path optical configuration, the QPDHM system is expected to provide higher temporal stability. Therefore, first, the temporal stability of the proposed QPDHM system is measured in order to assess its performance. A time series of multiplexed holograms (5000 holograms) is recorded



at the rate of 40 frames per second. The phase distribution for a small region ($200 \text{ pixels} \times 200 \text{ pixels}$) in each recorded hologram is numerically reconstructed, followed by calculating the standard deviation (SD) at each spatial location within the region of interest. Figures 2(a) and (b) show the histograms of the SD corresponding to two polarization states, indicating the mean fluctuation is 0.0096 radian. This measured temporal phase stability is more than 20 times higher compared to a traditional double channel DH system [23]. The spatial resolution of the proposed system was evaluated experimentally using a USAF 1951 1X resolution target (Edmund Optics). The smallest resolvable feature corresponds to element 4 of group 6, yielding a measured spatial resolution of approximately $5.52 \mu\text{m}$. In addition, the space-bandwidth utilization (SBU) and field of view (FOV) of the system were characterized. The measured SBU is 19.63%, and the FOV is $8.44 \text{ mm} \times 7.06 \text{ mm}$ along the x and y directions, respectively.

Further, the accuracy of the measurement is verified by conducting an experiment on a standard theoretical object, a combination of a polarizer and an HWP. The multiplexed holograms, at different orientations angles of HWP in steps of 10° , are recorded and then processed, as discussed in equations (1)–(6), to obtain the Stokes parameters. Figure 3 shows the theoretical and experimental results of the measured Stokes parameters (S_1 and S_2). To evaluate the accuracy and stability of the proposed system, ten repeated measurements were performed using a linearly polarized beam with an angle of $\pi/6$. The corresponding results are summarized in table 1, including the theoretical values, the mean of the ten measured values, the SD, and the root mean square error (RMSE). The close correspondence between the experimentally obtained and theoretical values of the Stokes parameters confirms the accuracy and reliability of the proposed system.

Next, an experiment is performed with a USAF resolution target (Edmund optics USAF 1951, 1X) as an object. The two pieces of cellophane tape are attached to the USAF target. The locations of these tapes are indicated by two rectangular boxes, as schematically shown in figure 4. The dotted area of the target, as shown in figure 4, corresponding to the ‘group 0’ element of the resolution target, is imaged on the image sensor, and a multiplexed digital hologram is recorded. The cellophane tape behaves as a half-wave retarder in a broad wavelength band [20, 24]. The polarization state for the area with double tape should be the same as the area without the tape. Similarly, the polarization state for the areas with single tape should be the same. Figure 5(a) shows the recorded multiplexed digital hologram, and its Fourier transform is depicted in figure 5(b). The DC term and the ± 1 order terms are clearly shown in figure 5(b). In $+1$ order term, the spatial spectra for the horizontal (E_H) and vertical (E_V) polarization components are marked with red and yellow circles, respectively. The retrieved amplitudes corresponding to the orthogonal polarization states are shown in figures 5(c) and (d), respectively. The retrieved

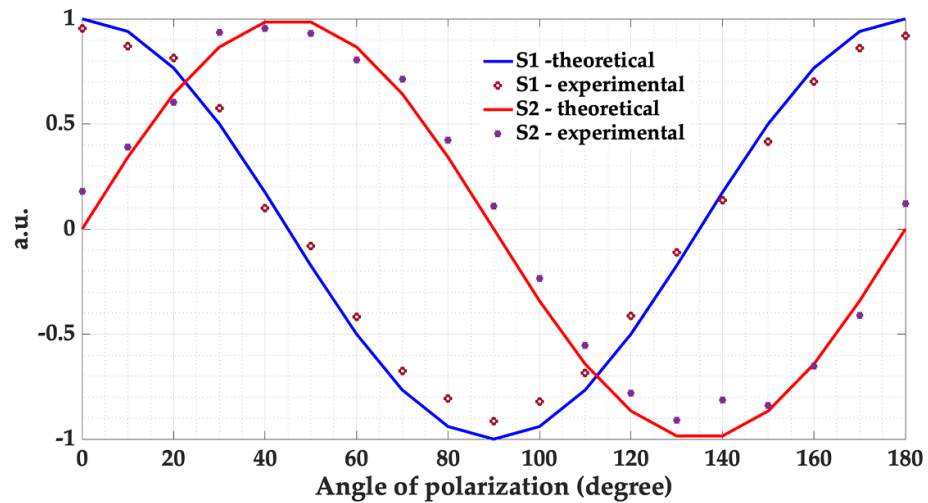


Figure 3. Comparison of the measured and theoretical values of Stokes parameters.

Table 1. Measurement results of the polarization beam with an angle of $\pi/6$.

Stokes parameters	True value	Measured value	Average measured value	SD	RMSE
S1	0.50	0.512	0.5023	0.008 22	0.008 13
S2	0.866	0.858	0.8595	0.008 20	0.010 13

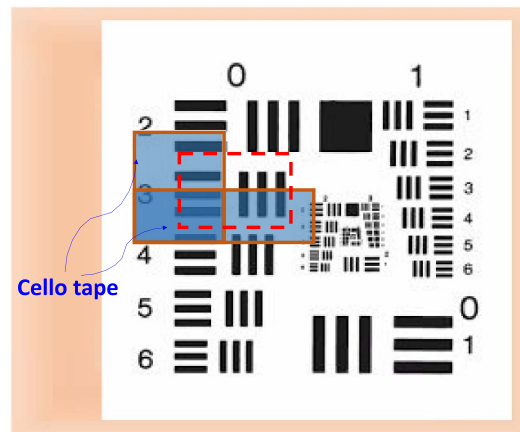


Figure 4. Photograph of the USAF resolution target with two cellophane tape strips attached onto it shown as light blue color. The dotted rectangular depicts the imaged area by the proposed system.

phase distributions for the orthogonal polarization states are shown in figures 5(e) and (f), respectively. The two complex amplitude images (amplitude and phase) that were retrieved match exactly point-to-point. As a result, image matching is not required, and the measurement error brought about by image processing is completely eliminated. The Stokes parameters are calculated by using the equations (4)–(6) and the obtained results for these parameters are shown in figure 6. The obtained phase, amplitude, and state of polarization results validate the QPDHM system's imaging and measurement capabilities.

Furthermore, another experiment is conducted on a birefringent USAF 1951 resolution target (Thorlabs, R3L1S1B). The obtained Stokes parameters S_0 , S_1 , S_2 , and S_3 for the birefringent USAF target are shown respectively in figures 7(a)–(d).

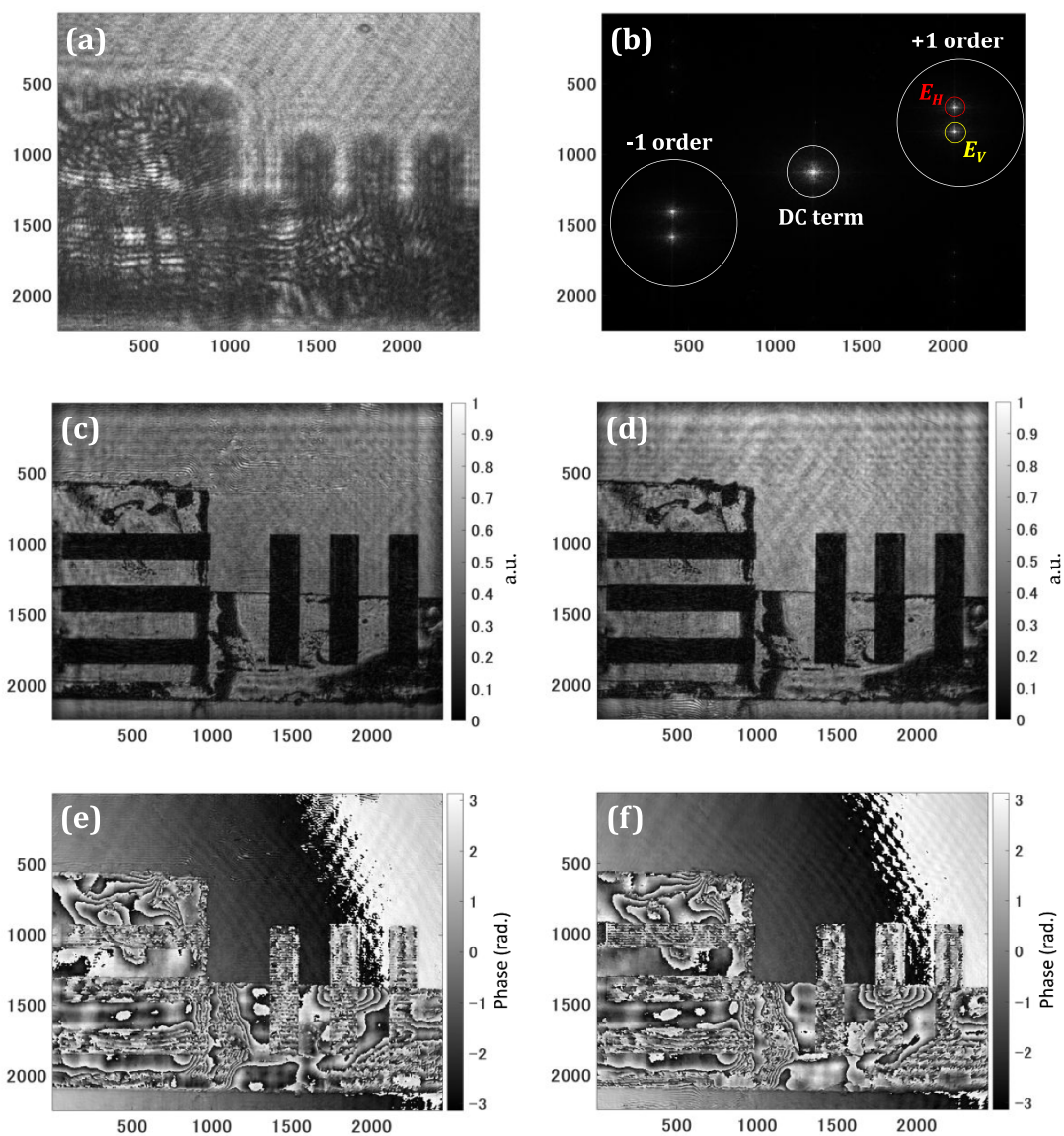


Figure 5. Experimental results: (a) recorded multiplexed digital hologram; (b) Fourier spectrum of (a); (c) and (d) the amplitude images for two orthogonal components; and (e) and (f) their phase distributions.

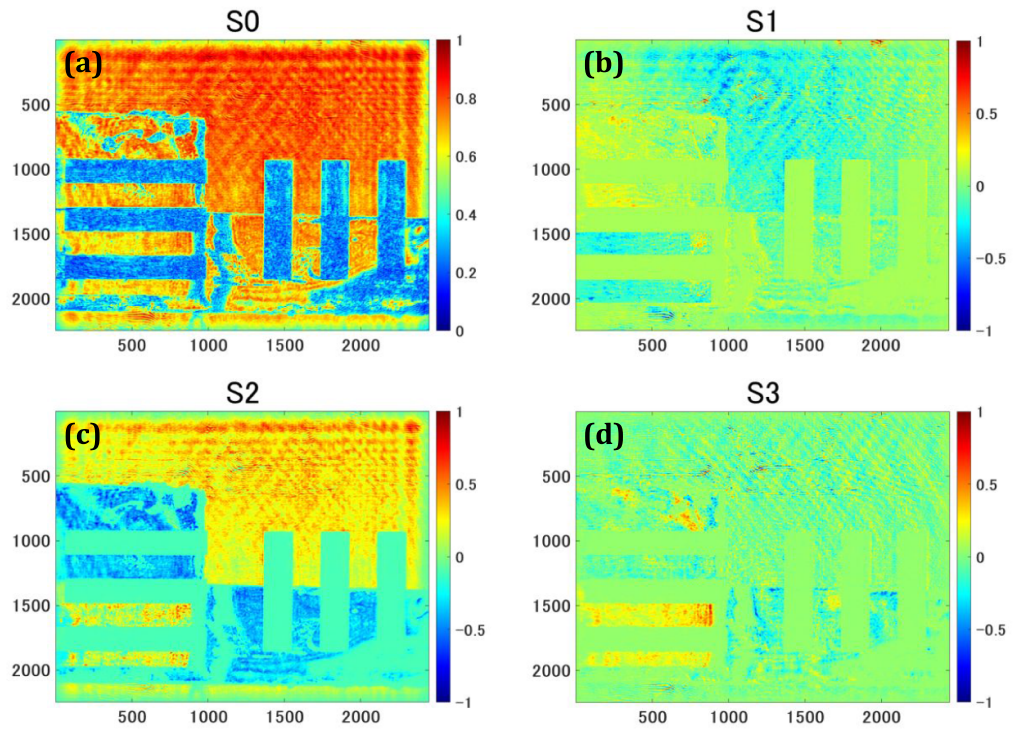


Figure 6. Experimental results on the resolution target with cellophane tape. Measured values of Stokes parameters (a) S_0 , (b) S_1 , (c) S_2 , and (d) S_3 .

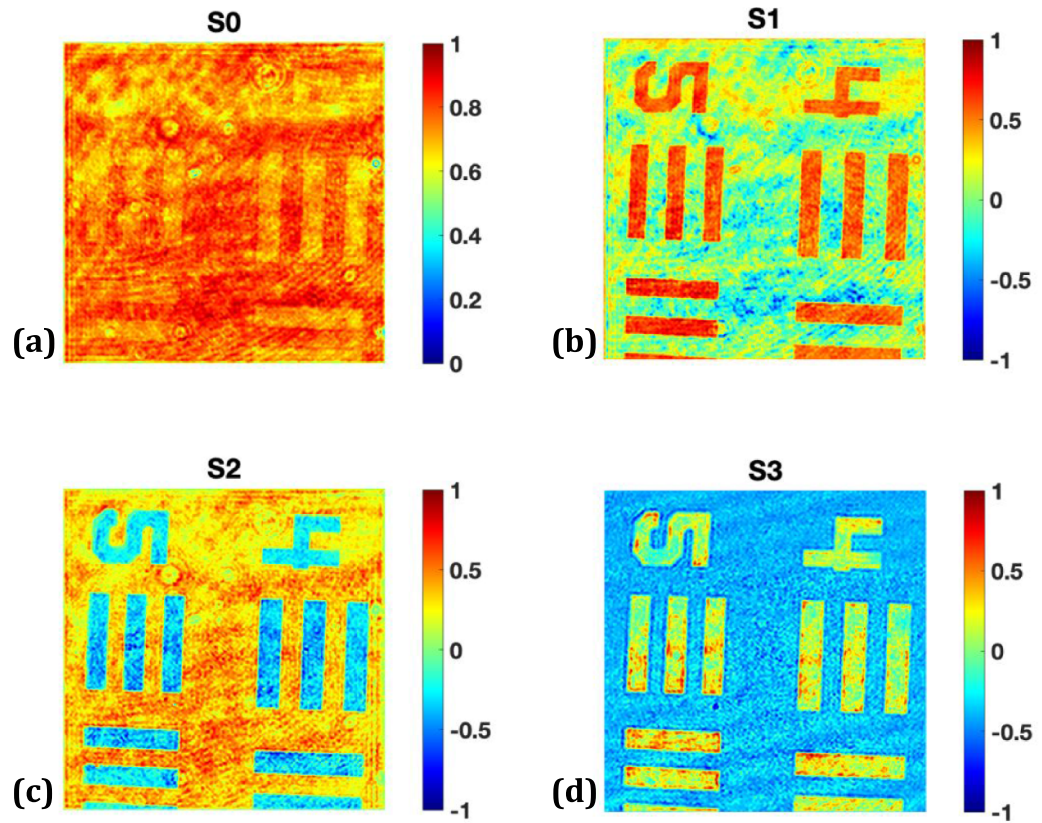


Figure 7. Experimental results on birefringent resolution target. The measured values of Stokes parameters (a) S_0 , (b) S_1 , (c) S_2 , and (d) S_3 .

5. Conclusion

In summary, in this work, a new configuration of single-shot and common-path QPDHM is proposed and experimentally demonstrated. The novelty of the system lies in its compact optical design and single-shot acquisition. The common-path design offers several significant advantages, particularly in terms of stability, simplicity, and robustness. Since the reference and object beams share the same optical path, they experience similar environmental fluctuations (e.g. vibrations, air turbulence), which minimizes relative phase shifts. Further, the common-path configuration eliminates the need for beam splitters and mirrors used in traditional Mach–Zehnder or Michelson-type setups, making the system compact and cost-effective at the same time. The single-shot imaging capability enables to achieve dynamic and fast transient polarization measurements in almost real-time, limited by the sensor frame rate. Several experiments are performed to verify the stability, measurement accuracy, and amplitude, phase, and state of polarization measurement capability of different samples. The obtained results corroborate the proposed QPDHM system's applicability, which can further be extended to a wide range of research disciplines. For example, in materials science, QPDHM can contribute to characterizing birefringent materials, detecting defects, and assessing mechanical properties based on polarization-sensitive measurements, therefore, providing critical insights for quality control and structural integrity assessments. In biomedical imaging, QPDHM may play a crucial role in studying birefringent biological cells and tissues. It enhances contrast and reveals microstructural organization, aiding in early disease diagnosis, including cancer detection. In conclusion, QPDHM represents a paradigm shift in microscopy, empowering researchers with enhanced capabilities to explore and manipulate the intricate world of biological and material sciences through precise, quantitative polarization imaging.

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Data availability statement

The data that support the findings of this study are available upon reasonable request from the authors.

Conflict of interest

The authors declare no conflicts of interest.

Author contributions

Manoj Kumar  0000-0003-2870-0677

Conceptualization (lead), Data curation (lead), Formal analysis (lead), Investigation (lead), Methodology (lead), Resources (equal), Software (lead), Supervision (lead), Validation (lead), Visualization (lead), Writing – original draft (lead)

Naru Yoneda  0000-0001-9964-5420

Formal analysis (equal), Investigation (supporting), Methodology (supporting), Validation (supporting), Writing – review & editing (equal)

Yasuhiro Awatsuji  0000-0001-9967-1178

Formal analysis (equal), Investigation (equal), Methodology (equal), Validation (equal), Writing – review & editing (supporting)

Osamu Matoba  0000-0001-9767-0059

Formal analysis (equal), Funding acquisition (equal), Investigation (equal), Methodology (equal), Project administration (equal), Resources (equal), Supervision (equal), Validation (equal), Writing – review & editing (equal)

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