

Dual-shot polarization digital holographic microscopy with phase bias equalization for full-field Jones matrix imaging

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Abstract: Polarization digital holographic microscopy (PDHM) enables noninvasive, quantitative mapping of birefringence via full-field Jones matrix analysis. Conventional implementations rely on multiple sequential acquisitions and are vulnerable to phase drift, whereas single-shot variants are optically complex and alignment sensitive. We introduce a simple dual-shot PDHM that reconstructs the full-field Jones matrix from two holograms acquired with orthogonal incident polarizations. A motorized half-wave plate sets the input states, and a polarization-sensitive CMOS camera records off-axis holograms that embed four analyzer channels per exposure. To suppress inter-shot phase drift, we propose a phase bias equalization that leverages isotropic reference regions to align complex phases across Jones elements. Experiments on biaxially oriented polypropylene (BOPP) films yield retardance and fast axis maps in agreement. Imaging of mouse brain sections and human cornea further demonstrates robust visualization of anisotropic microstructures. The method balances speed, hardware simplicity, and quantitative performance, offering an accessible route to polarization-sensitive biomedical imaging and full-field Jones matrix microscopy.

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1. Introduction

Digital holographic microscopy (DHM) recovers quantitative complex optical fields from interferometric measurements [1–3]. When combined with polarization analysis, DHM enables full field mapping of anisotropy via the Jones matrix formalism. While intensity-based Stokes - Mueller approaches describe partially polarized and depolarizing samples [4–7], they do not encode the optical phase. Jones formalism, on the other hand, captures polarization dependent amplitude and phase for fully polarized fields, making it a more comprehensive approach for the analysis of birefringent samples [8,9].

A customized DHM setup was proposed by Colomb et al. [10], utilizing a Mach - Zehnder interferometer comprising two linearly polarized reference waves at orthogonal polarization axes and an object wave to image the polarization state of a bent fiber. This approach only provided partial polarization characterization. In a subsequent study, Wang et al. [11] demonstrated the extraction of the whole-field Jones matrix using an off-axis DHM configuration requiring four-step acquisitions for a single fully resolved Jones matrix. For high-speed measurements, Kim et al. [12] employed a dual step method using a common-path interferometer with a grating and a custom-made optical chopper to alternate between two orthogonal polarization states. Park et al. [13] measured the full, spatially resolved Jones matrix by recording four interferograms under distinct polarization states. The requirement of four sequential acquisitions potentially renders the system sensitive to temporal phase fluctuations and mechanical instabilities. Chaubey et al.

[14] demonstrated a dual-shot Jones-matrix DHM using a polarization-sensitive configuration with a triangular Sagnac reference geometry and angular multiplexing, retrieving the full Jones matrix from sequential $+45^\circ$ and -45° illuminations with a reported inter-shot delay of ~ 30 s. They applied the method to human glioblastoma cells to quantify cellular anisotropy, and extended the analysis beyond Jones-element visualization by extracting decomposition-based maps such as retardance, diattenuation, and inhomogeneity.

Liu et al. [15] employed single shot Jones matrix measurement using two laser sources in an interferometric setup and multiplexing four distinct interference patterns. Another DHM based approach employs an image-splitting device with polarization filters to capture four complex field images simultaneously [16]. This method achieves single shot imaging at the expense of added complexity of two laser sources, two reference arms, a custom image splitting device, and precise alignment of these. In a follow up work, Park et al. [17] demonstrated single-shot Jones matrix microscopy for living eukaryotic cells using a spatially multiplexed off-axis interferometric scheme combined with synthetic aperture imaging to boost polarization sensitivity. The sample image was duplicated into a 2×2 array using a 2-D grating, and the two rows were analyzed using linear polarizers at $+45^\circ$ and -45° , respectively. Two independent He-Ne lasers provided orthogonally polarized reference waves that were spatially separated at the sensor, such that the first column interfered with a 0° polarized reference and the second column with a 90° polarized reference. By synthesizing Jones matrices acquired over many illumination angles, they reported substantial suppression of intrinsic phase noise and a strong SNR improvement, enabling visualization of weak retardance in living cells. Using the resulting phase-retardation metrics, they further showed significantly higher retardation-related values in pulmonary lung cancer cell lines than in pulmonary alveolar cells.

Another single shot polarization DHM (PDHM) approach combines constrained sample window with two reference windows at orthogonal polarization states in common path scheme together with a cross grating in a 4-f imaging system [18]. In this case, spatial multiplexing limits the type and the extend of the input. Additionally, sample window size and reference beam diffraction angle are required to be optimized to preserve resolution [19]. Li et al. [20] proposed a slightly off-axis, wavelength division multiplexed digital holographic system that leverages Kramers-Kronig (KK) relations to enable single shot Jones matrix measurement with substantially improved spatial bandwidth utilization (reported increase from 39.27% to 78.54%) and real-time operation without iterative reconstruction. The approach relies on dual wavelength illumination recorded on a color camera and requires channel crosstalk correction; it can be limited for wavelength sensitive specimens whose refractive index or birefringence varies with wavelength. Another real-time full field Jones imaging microscope employed a fast switching polarization state generator (based on a liquid crystal variable retarder) for orthogonal incident polarizations and a polarization-sensitive camera [21]. Time resolved four channel Jones matrix measurement using ultrafast pulses with angular multiplexing has also been demonstrated [22]. On top of DHM based methods, vectorial Fourier ptychography (vFP) has also been explored for Jones matrix imaging [23]. By synthesizing a larger numerical aperture from multi-angle intensity measurements, vFP can improve resolution and space-bandwidth product with relatively simple optics; however, it typically requires tens to hundreds of sequential acquisitions and iterative reconstruction, which limits temporal resolution and increases computational burden (e.g., 324 acquisitions in [23]). Recent pupil-plane multiplexing vFP further reduces polarization-channel overhead by encoding orthogonal analyzer states across two halves of the pupil (with circularly polarized illumination), but still relies on multi-angle illumination and iterative ptychographic synthesis (e.g., 169 acquisitions in Chae et al.) [24]. Liu et al. [25] realized single-shot and inline holographic Jones matrix synthesis via spatial polarization state generation and polarization-based beam displacement. Compact common path polarization holography based on polarization angular multiplexing has been proposed for full field Jones matrix imaging with two acquisitions

using orthogonal polarization states [26]. Unlike the earlier example [16], this method doesn't suffer from the field of view or resolution limitations.

In this work, we present practical dual shot polarization digital holographic microscopy (PDHM) methods for full-field Jones matrix reconstruction based on a minimally modified off-axis system. Methods reconstruct the full field Jones matrix from two holograms acquired with orthogonal incident linear polarizations ($0^\circ/90^\circ$ or $45^\circ/135^\circ$). A motorized half wave plate sets the input states, and a polarization-sensitive CMOS camera with a $0^\circ/45^\circ/90^\circ/135^\circ$ micro polarizer mosaic records off-axis holograms containing four analyzer channels per exposure. This configuration removes analyzer rotation and avoids specialized multiplexing hardware, while retaining a simple, replicable setup.

Despite the progress in single shot and few shot designs, a practical gap remains between single shot & multiplexed architectures that provide temporal simultaneity but often require specialized optical components, additional multiplexing channels, or tighter calibration and alignment, and simpler few shot approaches that are easier to replicate but remain sensitive to inter frame phase drift. The dual shot PDHM presented here targets this gap by retaining the simplicity and alignment tolerance of a standard off-axis DHM layout while reducing the acquisition burden to two exposures.

Because the two frames are acquired sequentially, the measurement remains vulnerable to a time varying inter shot phase offset. We therefore introduce a phase bias equalization that leverages non birefringent (isotropic) regions to estimate and remove this offset, restoring phase consistency and amplitude accuracy across Jones matrix elements. This dual shot strategy explicitly trades temporal simultaneity for optical simplicity and is therefore best suited to static or quasi static specimens in its current implementation. We discuss the method's scope and limitations (e.g., fully anisotropic fields lacking isotropic patches) and show that equalization improves reconstruction robustness under realistic drift. We validate the system on uniform birefringent samples, biaxially oriented polypropylene (BOPP) films [27], and then apply it to biological samples (mouse brain sections and human cornea), demonstrating utility for quantitative polarization microscopy in biomedical contexts.

2. Methods

2.1. Optical setup

A modified off-axis Mach - Zehnder interferometer (Fig. 1(a)) was built around a 632.8 nm He-Ne laser. A fixed wire grid polarizer (Thorlabs WP25M-VIS) followed by a half wave plate (Thorlabs AHWP10M-600) in a motorized rotation mount (Thorlabs K10CR2/M) sets the incident linear polarization to 0° , 45° , 90° , or 135° . A non-polarizing beam splitter (Thorlabs CCM1-BS013/M) divides the polarized beam into object and reference arms with 50:50 splitting ratio. The object arm uses a Nikon $4\times/0.13$ NA objective and a 500 mm focal length tube lens (Thorlabs AC254-500-A-ML), yielding an effective magnification of $10\times$. The reference arm includes a Keplerian telescope ($f_1 = 50$ mm, Thorlabs LB1471-A; $f_2 = 500$ mm, Thorlabs LA1908-A) for beam expansion and wavefront curvature matching. A second non-polarizing 50:50 beam splitter (Thorlabs BS016) recombines the object and reference beams at a small angle. This beam splitter was mounted on a kinematic cage cube platform (Thorlabs B4C/M and Thorlabs C6WR) allowing the fine adjustment of the angle between the two beams. Besides, the beam folding mirrors (Thorlabs BB1-E02) on both arms of the interferometer were mounted on kinematic mirror mounts (Thorlabs KCB1C/M and Thorlabs KM100) for further alignment of the angle and the overlap of the reference and the object beams on camera plane. This configuration allows flexible adjustments of the off-axis carrier spatial frequency to avoid aliasing of the DC and twin-image terms.

Holograms are recorded with a polarization sensitive CMOS camera (The Imaging Source DZK-33UX250, Sony IMX250MZR, $3.45\ \mu\text{m}$ pixels) featuring a 2×2 micro polarizer mosaic

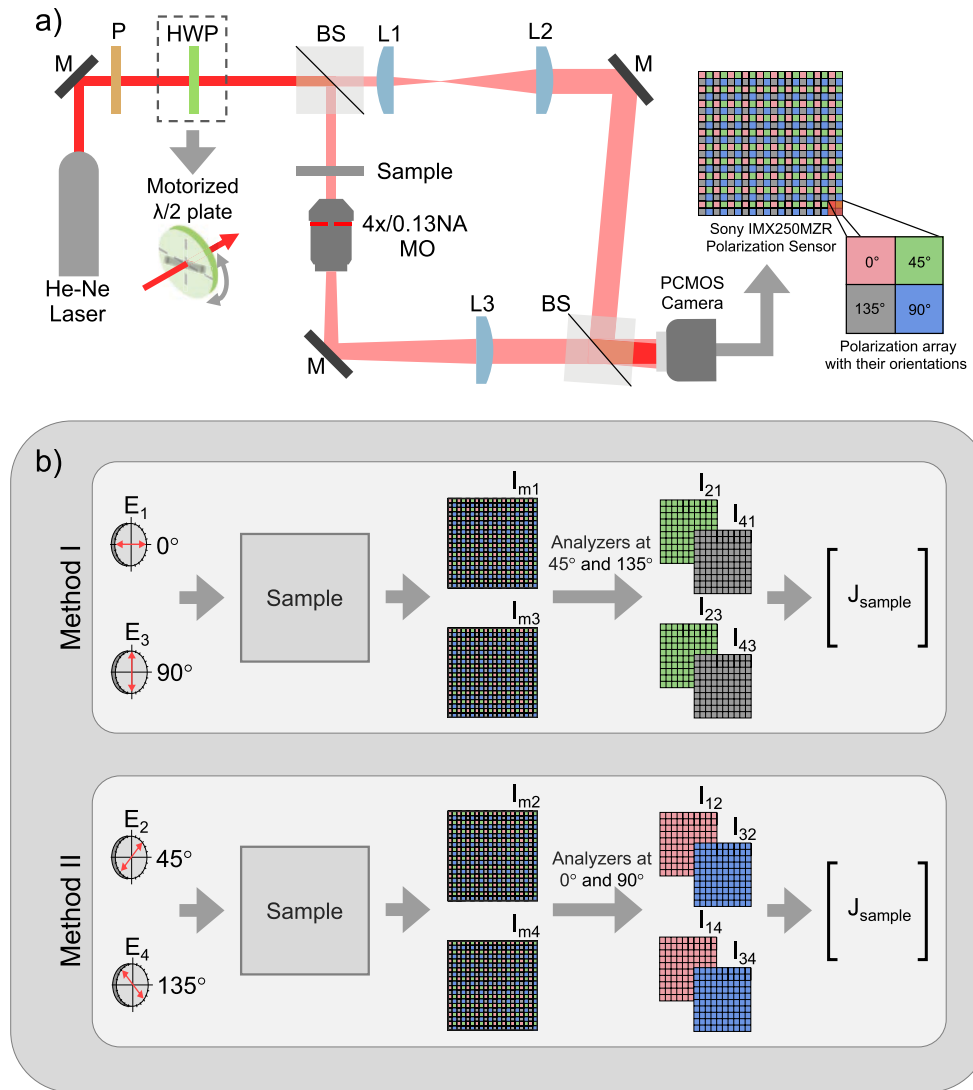


Fig. 1. Dual-shot polarization digital holographic microscopy (PDHM). **(a)** Optical layout. M: mirrors, P: wire grid polarizer, HWP: motorized half wave plate, BS: non-polarizing beam splitters, L1 ($f = 50$ mm) and L2 ($f = 500$ mm) form a Keplerian telescope for reference beam expansion, MO: microscope objective (Nikon 4x/0.13 NA), L3: 500 mm focal length tube lens, ND: neutral-density filter, PCMO: polarization-sensitive CMOS camera with 2×2 micro-polarizers at 0°, 45°, 90°, 135°. **(b)** Acquisition schematic. Two incident states (illustrated here as E_1 – E_4 for 0°, 45°, 90°, 135°; either E_1 and E_3 or E_2 and E_4) are set by the HWP. For each incident state, the polarization camera records one exposure that is demosaiced into four analyzer resolved holograms I_{1n} , I_{2n} , I_{3n} , and I_{4n} . Two of these four holograms are used to reconstruct complex fields. Two complex fields from the two exposures are then used for full field Jones matrix reconstruction.

(0°, 45°, 90°, 135°). This provides four analyzer resolved channels per exposure. The incident polarization state angles are calibrated with respect to the camera's analyzer axes by angle of linear polarization (AoLP) analysis from Stokes measurements without a sample, and the half wave plate was indexed to these axes (down to motor accuracy $\pm 0.14^\circ$).

2.2. Jones matrix reconstruction

For each input linear polarization, we record one exposure on the polarization camera, which provides four analyzer orientations. We index analyzers by $n \in \{1, 2, 3, 4\} \equiv \{0^\circ, 45^\circ, 90^\circ, 135^\circ\}$ and input states by $n \in \{1, 2, 3, 4\} \equiv \{0^\circ, 45^\circ, 90^\circ, 135^\circ\}$. The Jones vectors for the input states are defined as:

$$E_1 = \begin{bmatrix} 1 \\ 0 \end{bmatrix}, \quad E_2 = \frac{1}{\sqrt{2}} \begin{bmatrix} 1 \\ 1 \end{bmatrix}, \quad E_3 = \begin{bmatrix} 0 \\ 1 \end{bmatrix}, \quad E_4 = \frac{1}{\sqrt{2}} \begin{bmatrix} 1 \\ -1 \end{bmatrix}. \quad (1)$$

The sample Jones matrix at each pixel is:

$$J_s = \begin{bmatrix} J_{xx} & J_{xy} \\ J_{yx} & J_{yy} \end{bmatrix} \quad (2)$$

Each analyzer on the polarization camera can be represented using its Jones matrix:

$$P_1 = \begin{bmatrix} 1 & 0 \\ 0 & 0 \end{bmatrix}, \quad P_2 = \frac{1}{2} \begin{bmatrix} 1 & 1 \\ 1 & 1 \end{bmatrix}, \quad P_3 = \begin{bmatrix} 0 & 0 \\ 0 & 1 \end{bmatrix}, \quad P_4 = \frac{1}{2} \begin{bmatrix} 1 & -1 \\ -1 & 1 \end{bmatrix}. \quad (3)$$

After analyzer m , object and reference fields at the sensor are defined by:

$$O_{mn} = P_m \times J_s \times E_n, \quad R_{mn} = A_{ref} e^{i\varphi_{ref}} \times P_m \times E_n \quad (4)$$

where $A_{ref} e^{i\varphi_{ref}}$ is a scalar reference amplitude and phase. Eventually a recorded hologram intensity is given by:

$$I_{mn} = |O_{mn} + R_{mn}|^2 = |O_{mn}|^2 + |R_{mn}|^2 + O_{mn} R_{mn}^* + O_{mn}^* R_{mn} \quad (5)$$

Here the complex field $O_{m,n}$ is reconstructed via numerical filtering:

$$O_{mn} = \frac{\mathcal{F}^{-1}\{\mathcal{F}\{I_{mn}\} \cdot M\}}{\mathcal{F}^{-1}\{\mathcal{F}\{I_{mn}^r\} \cdot M\}} \quad \text{where} \quad O_{mn} = |O_{mn}| e^{i\varphi_{mn}} \quad (6)$$

where M is the band pass filter mask, and I_{mn}^r refers to reference holograms without any sample. From these fields, the Jones matrix elements are calculated using one of the following two methods.

Method I: Using linearly polarized incidence at 0° and 90° with analyzers at 45° and 135°:

$$\begin{aligned} J_{xx} &= |O_{21}| e^{i\varphi_{21}} + |O_{41}| e^{i\varphi_{41}} \\ J_{xy} &= |O_{23}| e^{i\varphi_{23}} - |O_{43}| e^{i\varphi_{43}} \\ J_{yx} &= |O_{21}| e^{i\varphi_{21}} - |O_{41}| e^{i\varphi_{41}} \\ J_{yy} &= |O_{23}| e^{i\varphi_{23}} + |O_{43}| e^{i\varphi_{43}} \end{aligned} \quad (7)$$

Method II: Using linearly polarized incidence at 45° and 135° with analyzers at 0° and 90° [11]:

$$\begin{aligned} J_{xx} &= |O_{12}|e^{i\varphi_{12}} + |O_{14}|e^{i\varphi_{14}} \\ J_{xy} &= |O_{12}|e^{i\varphi_{12}} - |O_{14}|e^{i\varphi_{14}} \\ J_{yx} &= |O_{32}|e^{i\varphi_{32}} - |O_{34}|e^{i\varphi_{34}} \\ J_{yy} &= |O_{32}|e^{i\varphi_{32}} + |O_{34}|e^{i\varphi_{34}} \end{aligned} \quad (8)$$

Ideally, Methods I and II produce identical Jones matrices. In practice, because the two frames are acquired sequentially, each method is affected by a frame-to-frame phase offset. This time varying phase offset is due to the relative phase drift between the object and reference fields, which appears as an additional global phase term between the two acquisitions. This offset is mainly driven by optical path length fluctuations caused by mechanical vibrations, air turbulence, slow thermo-mechanical drift of opto-mechanics, and intrinsic laser phase noise. To compensate for the phase offset, we introduce a phase bias equalization strategy that enforces a common phase reference between the two sequential acquisitions by exploiting a non-birefringent (isotropic) region in the field of view. The impact of the inter shot phase and the equalization procedure differ for Methods I and II. For Method I (inputs 0° and 90° ; analyzers 45° and 135°), the Jones elements formed from the 0° exposure carry one phase offset b_1 , while those from the 90° exposure carry b_3 :

$$\begin{aligned} J_{xx}^{(1)} &= e^{ib_1}(|O_{21}|e^{i\varphi_{21}} + |O_{41}|e^{i\varphi_{41}}) \\ J_{xy}^{(1)} &= e^{ib_3}(|O_{23}|e^{i\varphi_{23}} - |O_{43}|e^{i\varphi_{43}}) \\ J_{yx}^{(1)} &= e^{ib_1}(|O_{21}|e^{i\varphi_{21}} - |O_{41}|e^{i\varphi_{41}}) \\ J_{yy}^{(1)} &= e^{ib_3}(|O_{23}|e^{i\varphi_{23}} + |O_{43}|e^{i\varphi_{43}}) \end{aligned} \quad (9)$$

Here b_1 and b_3 denote the inter shot phase offset of the 0° and 90° inputs, respectively. Each pair of analyzer channels from the same physical exposure is used to form a given Jones element. Hence, the bias appears as a common phase factor, and the amplitudes are not degraded. However, the relative phase between the diagonals and off diagonals is inconsistent when $b_1 \neq b_3$. To equalize the phase, we estimate the offset by spatial averaging on a non-birefringent region and re-phase the elements derived from the 90° exposure. This approach effectively sets $b_3 \rightarrow b_1$, restoring a common phase reference across all Jones elements while preserving their amplitudes.

$$\begin{aligned} J_{xx}^{(2)} &= e^{ib_2}|O_{12}|e^{i\varphi_{12}} + e^{ib_4}|O_{14}|e^{i\varphi_{14}} \\ J_{xy}^{(2)} &= e^{ib_2}|O_{12}|e^{i\varphi_{12}} - e^{ib_4}|O_{14}|e^{i\varphi_{14}} \\ J_{yx}^{(2)} &= e^{ib_2}|O_{32}|e^{i\varphi_{32}} - e^{ib_4}|O_{34}|e^{i\varphi_{34}} \\ J_{yy}^{(2)} &= e^{ib_2}|O_{32}|e^{i\varphi_{32}} + e^{ib_4}|O_{34}|e^{i\varphi_{34}} \end{aligned} \quad (10)$$

For Method II (inputs 45° and 135° ; analyzers 0° and 90°), every Jones element combines fields from different exposures, so the inter shot offsets b_2 and b_4 appear inside the sums and differences and can corrupt both amplitude and phase. To avoid biasing the sums and differences in the calculation, we pre align the 45° and 135° fields before forming (10). On a non-birefringent region, we estimate the offset by spatial averaging, and we re-phase the 135° fields. We then compute $J^{(2)}$ via (10). This cancels the relative offset between b_2 and b_4 and prevents amplitude and phase degradation. Accordingly, Method I is advantageous in biomedical measurements where preserving amplitude contrast under inter-shot phase drift is critical. Method II remains sensitive to inter-shot phase offsets in both amplitude and phase prior to correction, but after

phase bias equalization it provides consistent Jones matrix reconstruction with phase consistent element maps.

After phase bias equalization, we factor out the isotropic (non-birefringent) phase (i.e., the common phase shared by the two linear basis states) so that the remaining Jones matrix maps emphasize birefringence. We compute the isotropic phase as the average of the diagonal phases:

$$\varphi_{iso} = \frac{\arg(J_{xx}) + \arg(J_{yy})}{2} \quad (11)$$

For visualization and downstream analysis, we report φ_{iso} as a separate scalar image and form phase-normalized Jones elements so the complex phase in Jones matrix images reflects anisotropic retardation rather than path length phase.

To complement the isotropic phase map, we also estimate isotropic amplitude images (A_{iso}). For each pixel, we identify the largest amplitude among the four Jones matrix elements and use this value as a local isotropic reference. The amplitudes of all elements at that pixel are then divided by this reference to produce a normalized amplitude map. This normalization suppresses the contrast in the areas where the sample behaves isotropically, where all amplitudes are similar, and enhances contrast where anisotropy is present, improving the visibility of birefringent structure.

2.3. Sample preparation

Biaxially oriented polypropylene (BOPP) film samples:

Commercial BOPP packing sheets were cut to size and mounted between glass slides using Norland Optical Adhesive 65 (NOA65). To determine the principal axis orientations prior to imaging, each sheet was inspected between crossed polarizer sheets and rotated to locate extinction positions; representative specimens were then oriented and fixed to present fast axes nominally at 0° and 45° for validation.

Biological tissues:

Mouse brain sections at 20 μm thickness were prepared by cryosectioning. Imaging focused on the corpus callosum, whose densely packed, myelinated axons exhibit organized anisotropy suitable for Jones matrix analysis [28].

Ex vivo human cornea was mounted using double-sided adhesive tape placed between two microscope slides; the tape had a rectangular cutout that served as a shallow well. The cornea was placed in phosphate buffered saline (PBS) within the cutout and gently compressed between the slides to flatten the native shape while maintaining hydration. This configuration stabilized the tissue, minimized curvature induced defocus, and provided a uniform optical path for polarization measurements.

3. Results and discussion

3.1. Jones matrix reconstruction on BOPP film samples

3.1.1. BOPP sample with vertical principal axis orientation

Figure 2 reports Jones matrix reconstructions using Method I for a BOPP film whose principal axis is aligned vertically. The field of view contains the film on the left and an empty region on the right to enable side by side comparison of birefringent and isotropic areas. Panel (a) illustrate the structure of BOPP sample. It shows the orientation of the BOPP film sandwiched between two microscope slides, mounted using Norland Optical Adhesive. Panels (b) and (c) show conventional incoherent intensity images between parallel and crossed sheet polarizers, respectively; because the first polarizer is coaxial with the sample's principal axis, no polarization rotation is produced, and the crossed polar image remains dark over both regions.

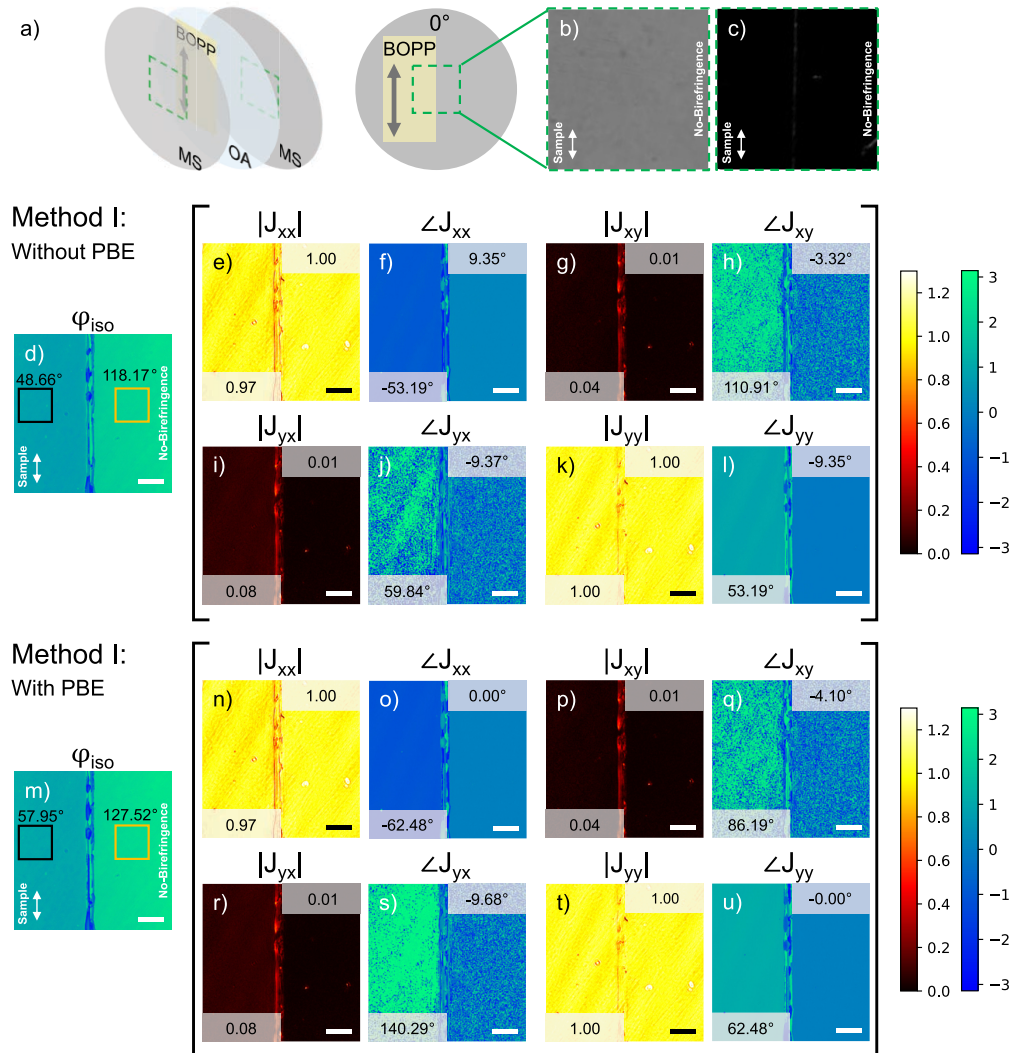


Fig. 2. BOPP film with vertical principal axis – Method I. (a) Schematic showing the orientation of the BOPP film sandwiched between two microscope slides (MS), mounted using Norland Optical Adhesive (OA). (b) Parallel polarizer intensity image. (c) Crossed polarizer intensity image. (d, m) Isotropic phase φ_{iso} from Method I without/with phase bias equalization (PBE), respectively; black and orange boxes denote ROIs (200 × 200 pixels each) used for the reported averages. (e–l) Amplitude and phase maps of the Jones matrix elements from Method I without equalization. (n–u) Corresponding maps with phase bias equalization. Scale bar: 100 μ m

Panels (d–l) present results from Method I without phase bias equalization; panels (m–u) repeat the analysis with equalization. The isotropic phase image is shown outside the brackets in (d) and (m); inside each bracket, the amplitude maps (e, g, i, k, n, p, r, t) and the corresponding phase maps (f, h, j, l, o, q, s, u) are displayed side by side for the four Jones matrix elements. The black and orange squares in (d) and (m) mark the ROIs used to compute the average values annotated on the panels.

As expected for axis alignment with the camera basis, the diagonal terms J_{xx} and J_{yy} exhibit near unity amplitudes across the film (e, k, n, t), while the cross terms J_{xy} and J_{yx} are strongly suppressed over both film and background (g, i, p, r). Amplitude averages therefore carry limited diagnostic value in this configuration; the relevant contrast resides in the phase. Extracting the isotropic phase (d) isolates non-birefringent contributions and leaves birefringence induced phase variations inside the brackets. Consistent with the Method I model, amplitudes are insensitive to the inter shot phase offset, whereas phases are biased. After applying phase bias equalization, the diagonal phase difference ($\angle J_{yy} - \angle J_{xx}$, retardance) increases from 106.38° to 124.96° , which is approximately a $\lambda/3$ plate at 632.8 nm.

We repeated the reconstruction with Method II (inputs $45^\circ/135^\circ$, analyzers $0^\circ/90^\circ$); results are arranged in Fig. 3 analogously to Fig. 2. A key difference from Method I is that Method II combines fields from different exposures, so an uncorrected inter shot phase offset affects both phase and amplitude. Consistent with this model, the unequalized amplitude maps (e, g, i, k) overestimate the cross terms J_{xy} and J_{yx} (values rising to ~ 0.3 in the isotropic region) and underestimate the diagonal terms J_{xx} and J_{yy} (reduced from near-unity), relative to Method I. After phase bias equalization, the amplitudes (n, p, r, t) closely match their Method I counterparts: cross terms are strongly suppressed over both film and background, and diagonals approach unity. The retardance derived from the diagonal phase difference is 125.16° , in agreement with $\sim \lambda/3$ behavior in Method I.

3.1.2. BOPP sample with diagonal principal axis orientation

Figure 4 shows Jones matrix reconstructions for a BOPP film whose principal axis is oriented at approximately 45° to the camera basis. Panel (a) shows the samples structure, and panels (b) and (c) provide conventional intensity images between parallel and crossed polarizers; unlike the vertical axis case, the crossed polarizer image is bright within the film, consistent with polarization conversion by a retarder oriented at 45° . Panels (d–l) present Method I results without phase bias equalization; panels (m–u) repeat the analysis with equalization. The isotropic phase map is displayed outside the brackets in (d) and (m), while the bracketed panels show, side by side, the amplitude and phase for each Jones element. ROIs used for the panel annotations are marked by the black and orange squares.

As expected for a $\sim 45^\circ$ axis, the off diagonal elements J_{xy} and J_{yx} now carry the signal: their amplitudes are high over the film (panels g, i, p, r; 0.85 and 0.88 in the ROIs), whereas the diagonals J_{xx} and J_{yy} are reduced (panels e, k, n, t; 0.46 in the ROIs). The diagonal phase difference within the film is small (panels f, l, o, u), yielding a small retardance when estimated from $\angle J_{yy} - \angle J_{xx}$ from the camera basis. Using the ROI values from the Fig. 4 as $|J^{s45^\circ}| = [0.46 \ 0.88; 0.85 \ 0.46]$ and $\angle J^{s45^\circ} = [1.86^\circ \ 76.65^\circ; 98.99^\circ - 1.86^\circ]$ along with the approximate rotation of 45° to the sample in Fig. 2, we calculate the expected Jones matrix elements of vertically aligned sample $|J^{s0^\circ}| = [0.95 \ 0.17; 0.17 \ 0.98]$ and $\angle J^{s0^\circ} = [-63.4^\circ \ 7.88^\circ; 177.75^\circ \ 59.73^\circ]$ through the Jones matrix rotation. Back calculated retardance ($\angle J_{yy} - \angle J_{xx}$) of 123.13° has less than 2° deviation from the results presented in Fig. 2. Phase bias equalization leaves Method I amplitudes essentially unchanged (as in the vertical-axis case) and restores consistent phase across the elements.

Figure 5 reports the Method II reconstruction for the same sample using the same panel layout. Before equalization, the inter shot phase offset mixes fields from different exposures and degrades both phase and amplitude, causing the off diagonal amplitudes to be overestimated and the diagonals underestimated relative to Method I. After equalization, the amplitude and phase patterns closely match Method I: off diagonals remain dominant in the film, diagonals are suppressed, and the diagonal phase difference stays near zero across the BOPP region. The retardance is calculated to be 119.11° between $\angle J_{yy}$ and $\angle J_{xx}$ for the results presented in Fig. 5 after a similar treatment explained to relate Fig. 4 to Fig. 2 for the BOPP retardance. Together,

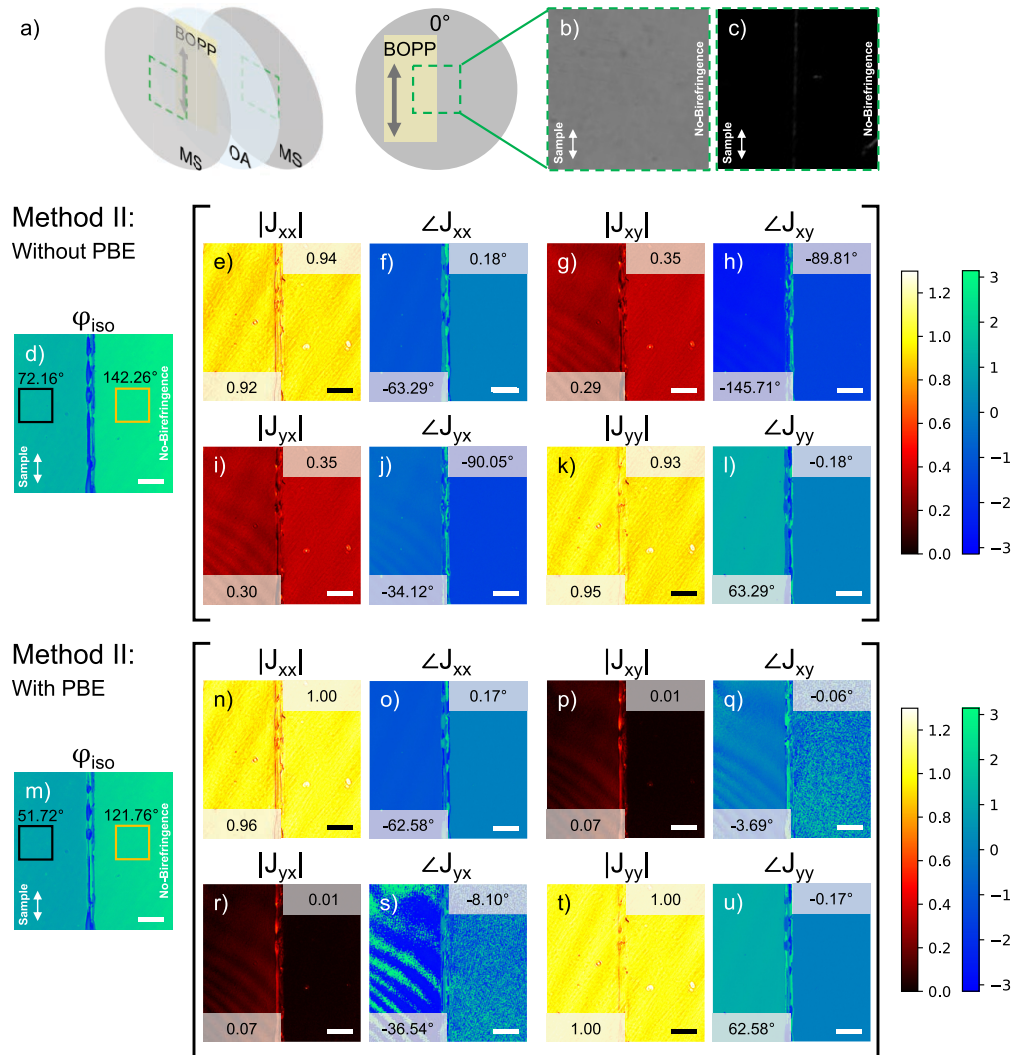


Fig. 3. BOPP film with vertical principal axis – Method II. (a) Schematic showing the orientation of the BOPP film sandwiched between two microscope slides (MS), mounted using Norland Optical Adhesive (OA). (b) Parallel polarizer intensity image. (c) Crossed polarizer intensity image. (d, m) Isotropic phase φ_{iso} from Method I without/with phase bias equalization (PBE), respectively; black and orange boxes denote ROIs (200 $\times$ 200 pixels each) used for the reported averages. (e–l) Amplitude and phase maps of the Jones matrix elements from Method I without equalization. (n–u) Corresponding maps with phase bias equalization. Scale bar: 100 μm

Figs. 4,5 confirm the expected basis-dependent behavior of a 45° retarder and the consistency of the two dual shot reconstructions once phase-bias equalization is applied.

3.2. Jones matrix reconstruction of biological samples

3.2.1. Mouse brain section

Results from a coronal mouse brain section are shown in Fig. 6. For anatomical context, we first acquired a whole section bright field mosaic on a commercial microscope (Zeiss Axio

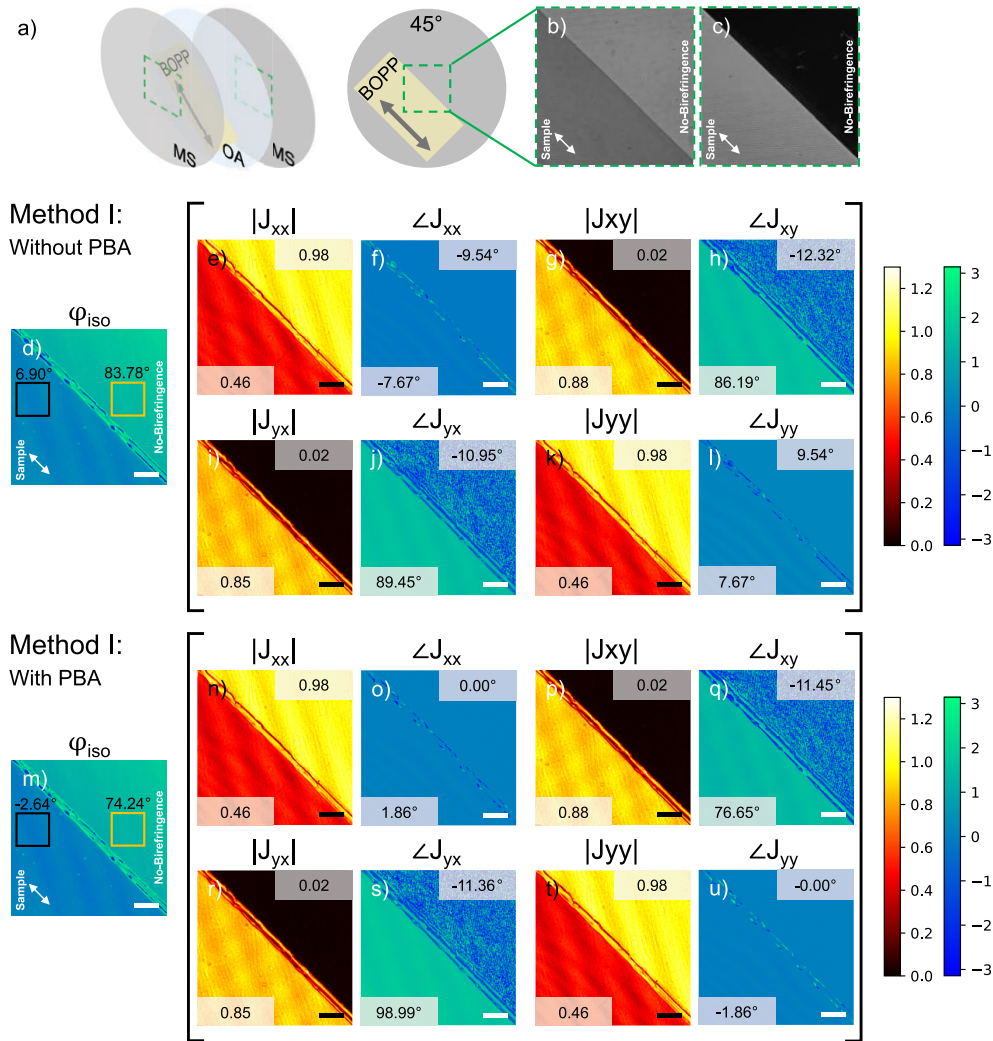


Fig. 4. BOPP film with diagonal principal axis – Method I. (a) Schematic showing the orientation of the BOPP film sandwiched between two microscope slides (MS), mounted using Norland Optical Adhesive (OA). (b) Parallel polarizer intensity image. (c) Crossed polarizer intensity image. (d, m) Isotropic phase φ_{iso} from Method I without/with phase bias equalization (PBE), respectively; black and orange boxes denote ROIs (200×200 pixels each) used for the reported averages. (e–l) Amplitude and phase maps of the Jones matrix elements from Method I without equalization. (n–u) Corresponding maps with phase bias equalization. Scale bar: 100 μm

Observer 7; Fig. 6(a)). The region highlighted in Fig. 6(b) was selected as the ROI because it contains well characterized birefringent structures. Our PDHM provides a per tile field of view of approximately $700 \times 700 \mu\text{m}$, which is smaller than this area; therefore, we used a 10×10 manual tile acquisition with 50% overlap and stitched the complex fields. This strategy enables millimeter scale coverage while retaining the polarization information per tile, and it demonstrates the robustness of our reconstruction (minimal seams and no visible tiling artifacts). We display two derived scalar maps to guide interpretation: the isotropic amplitude A_{iso} and the isotropic

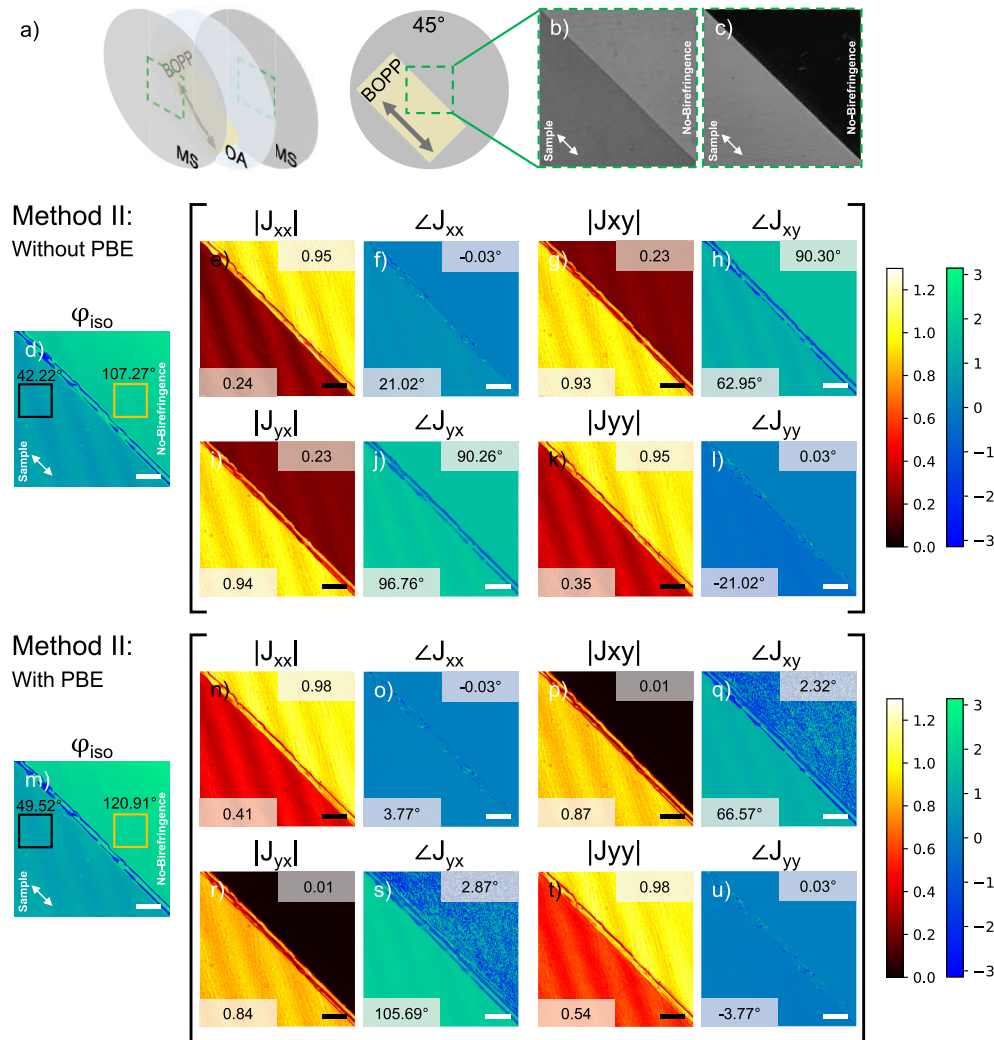


Fig. 5. BOPP film with diagonal principal axis – Method II. (a) Schematic showing the orientation of the BOPP film sandwiched between two microscope slides (MS), mounted using Norland Optical Adhesive (OA). (b) Parallel polarizer intensity image. (c) Crossed polarizer intensity image. (d, m) Isotropic phase φ_{iso} from Method I without/with phase bias equalization (PBE), respectively; black and orange boxes denote ROIs (200 × 200 pixels each) used for the reported averages. (e–l) Amplitude and phase maps of the Jones matrix elements from Method I without equalization. (n–u) Corresponding maps with phase bias equalization. Scale bar: 100 μm

phase φ_{iso} . These isolate background (isotropic) contributions and unveil anisotropic structure, making the Jones element maps easier to read across heterogeneous tissue.

For mosaicking, we equalized tile wise phase offsets by assuming that areas with near zero phase difference between the diagonal elements behave isotropically; the correction was applied conservatively to avoid bias when local fast axes approach $\pm 45^\circ$ to the instrument basis. This procedure preserved the continuity of retardance across tile boundaries without altering the qualitative contrast between white and gray matter.

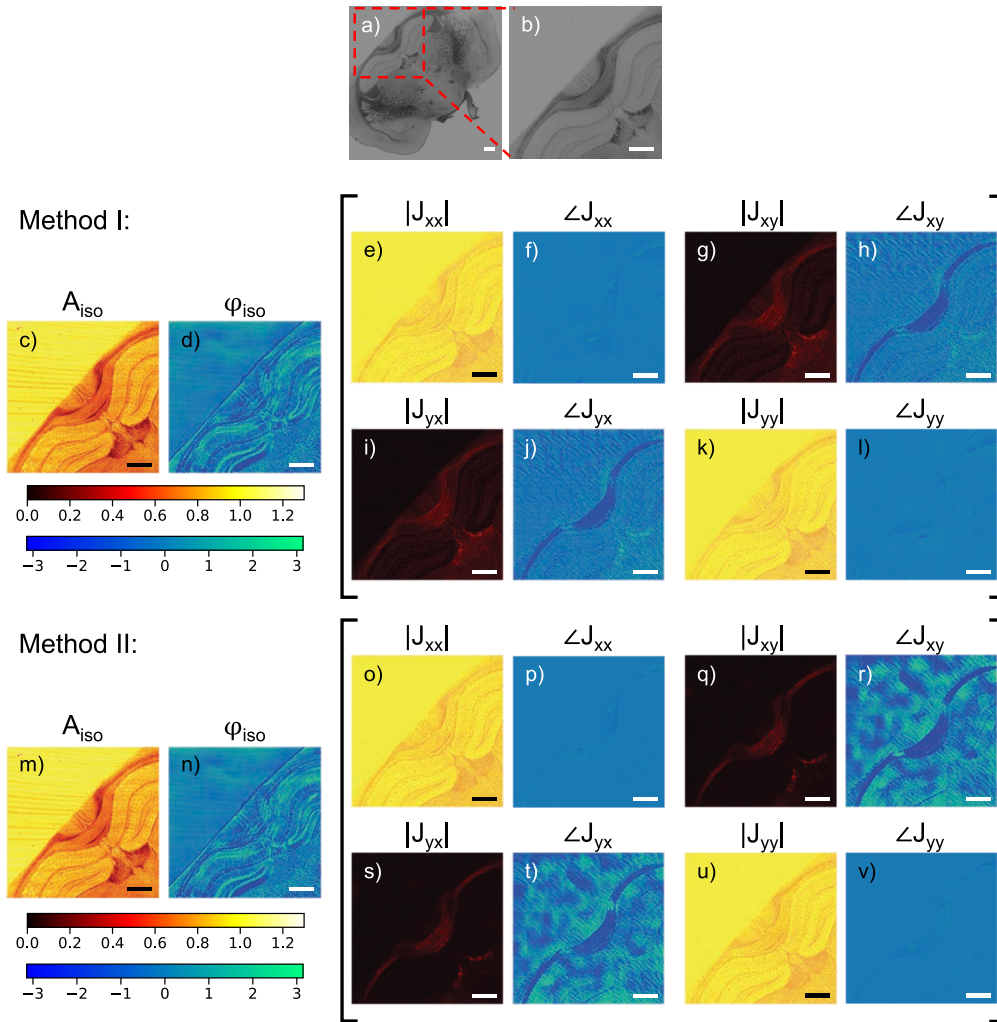


Fig. 6. Stitched Jones matrix maps of a mouse coronal section (20 μm). (a) Bright field mosaic (Zeiss Axio Observer) with the region analyzed by PDHM indicated. (b) Zoom of the dashed box. (c) Stacked isotropic amplitude A_{iso} . (d) Stacked isotropic phase φ_{iso} . (e–l) Jones matrix amplitude and phase maps (Method I). (m–v) Corresponding maps (Method II). Scale bar: 500 μm .

Panels (c, d) and (m, n) show the stitched isotropic amplitude and phase maps of the brain section. These derived maps isolate contributions from isotropic tissue and thus improve the visibility of birefringent structures, making the subsequent Jones element maps easier to interpret across heterogeneous cortex and white matter.

Within the highlighted region, the corpus callosum stands out with elevated off diagonal amplitudes of J_{xy} and J_{yx} (panels g, i, q, s) relative to surrounding gray matter, while the diagonal amplitudes remain comparatively uniform. The corresponding phase maps (panels h, j, r, t) exhibit uniform phase within the tract, consistent with a well aligned bundle of myelinated axons. The same contrast pattern appears in both reconstructions, indicating that tract orientation and retardance are preserved throughout the mosaic.

A higher magnification view of the J_{xy} element (Fig. 7) resolves additional anisotropic pathways, including the cingulum bundle and the dorsal hippocampal commissure. Figure 7(a) shows anatomical annotations from the Allen Reference Atlas – Mouse Brain corresponding to the same slice position [29]. Cingulum bundle (cing), dorsal hippocampal commissure (dhc), and corpus callosum (ccs) are represented by the given abbreviations. These tracts show stronger off diagonal amplitudes (Fig. 7(b, c)) and more uniform phase (Fig. 7(d)) than adjacent tissue, underscoring the ability of full field Jones matrix imaging to delineate white matter architecture. Additionally, the borders of the ccs and dhc are distinguishable from the uniform phase regions in Fig. 7(d).

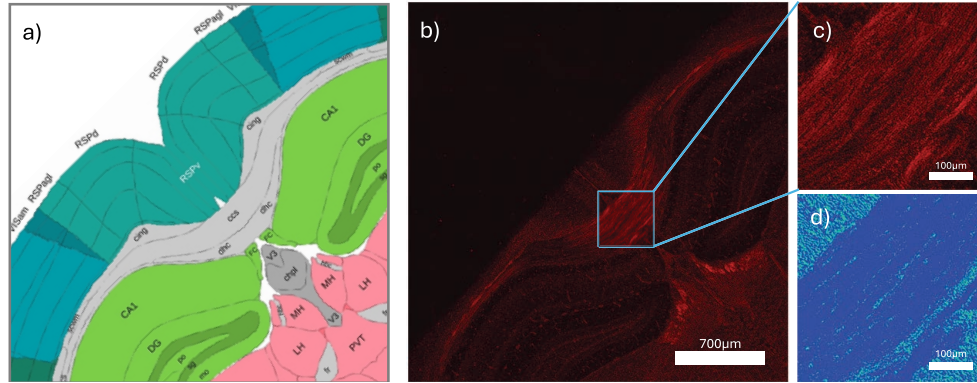
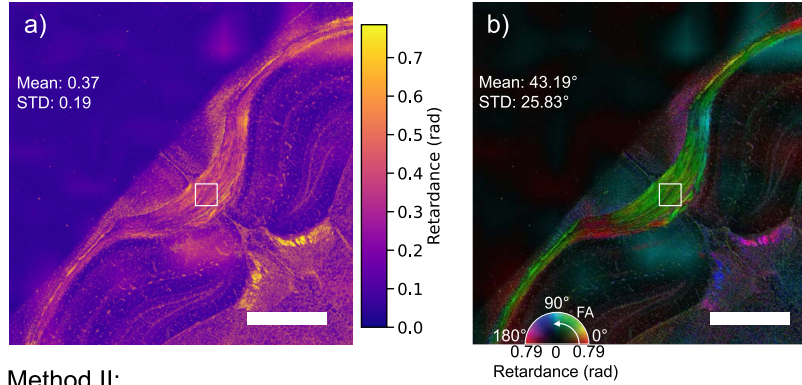


Fig. 7. Anatomical landmarks in the mouse brain. (a) Reference coronal atlas slice (Allen Reference Atlas- Mouse Brain [29]). (b) J_{xy} amplitude (Method I) highlighting anisotropic tracts (e.g. cingulum bundle). (c, d) Zoomed views over the corpus callosum showing J_{xy} amplitude and phase, respectively; corpus callosum and dorsal hippocampal commissure are distinctly visible. Scale bars as indicated.

Using the stitched Jones matrices shown in Fig. 6, we performed a Pauli algebra based Jones matrix decomposition and derived pixel wise retardance and optical-axis orientation maps (Fig. 8) [30,31]. The first row of Fig. 8(a, b) shows the retardance and axis orientation obtained from Method I, while the second row (c, d) shows the corresponding maps from Method II. Both methods delineate the major myelinated white-matter structures, with the corpus callosum exhibiting elevated retardance and coherent axis orientations relative to surrounding regions. To provide quantitative summary values, we selected an ROI within the corpus callosum (white square in Fig. 8). Method I yields a mean retardance of 0.37 ± 0.19 rad and a mean optical axis orientation of $43.2^\circ \pm 25.8^\circ$, while Method II yields 0.41 ± 0.22 rad and $41.5^\circ \pm 25.4^\circ$. Using the laser wavelength and the nominal section thickness (20 μm), these correspond to an effective retardation per unit thickness of approximately 1.86 nm/μm (Method I) and 2.06 nm/μm (Method II), i.e., an effective birefringence (Δn) on the order of $1.9 - 2.1 \times 10^{-3}$ in this ROI. The relatively large standard deviations in both retardance and axis orientation reflect the heterogeneous microstructure within the selected area (partial volume of myelinated fibers, local non-uniformity, and mixed orientations), consistent with the texture visible in the zoomed views of Fig. 7. Finally, we note localized regions (e.g., toward the upper-right portion of Fig. 8) where Method I reports higher retardance than Method II. These differences likely arise from residual method-dependent sensitivity to isotropic-phase removal and/or small tile-wise stitching inconsistencies. Importantly, however, both reconstructions remain consistent in the anatomical localization of anisotropic tracts and the overall orientation trends.

Method I:



Method II:

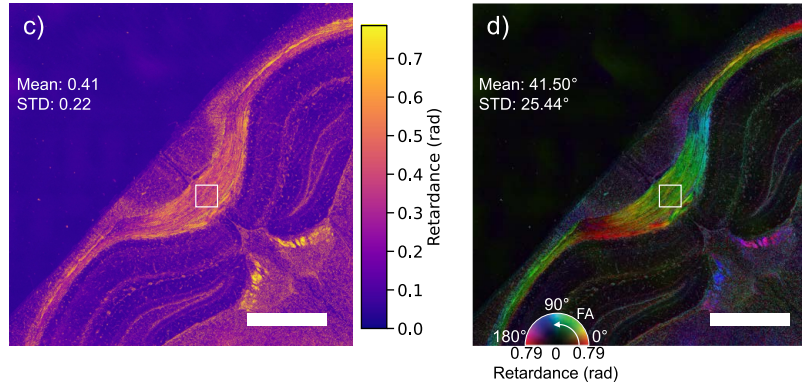


Fig. 8. Polarization properties of the mouse brain derived from the stitched Jones matrices. (a, c) Retardance maps obtained using Method I and Method II, respectively, highlighting myelinated fiber tracts with high birefringence contrast. (b, d) Corresponding optical axis orientation maps derived from Method I and Method II, respectively, show fiber directionality. The corpus callosum and adjacent commissural pathways are clearly delineated. Scale bar: 700 μm .

3.2.2. Cornea sample:

Figure 9 shows stitched Jones matrix maps of a human cornea. The cornea's birefringence arises primarily from the stromal lamellae, highly ordered collagen sheets whose local orientation varies smoothly from the central cornea to the periphery. As in the brain tissue section experiment, we acquired a 10×10 mosaic with 50% overlap and stitched the complex fields to cover both near-central and peripheral regions (Fig. 9(b, c) shows bright field mosaics). The specimen was mounted hydrated and gently flattened between slides. To aid interpretation we first display the isotropic amplitude A_{iso} and the isotropic phase φ_{iso} (Fig. 9(d, e, n, o)). The amplitude map emphasizes stroma, and the isotropic phase primarily reflects residual curvature and thickness variations introduced during flattening. Together, these maps provide a clean background against which the Jones element contrasts can be read. In the Jones element maps, the off-diagonal terms J_{xy} and J_{yx} are elevated where the local fast axis is oblique to the instrument basis, revealing spatial variations in lamellar orientation between central and peripheral stroma (Fig. 9(h, i, j, k, r, s, t, u)). This obliquity is more frequent toward the periphery, where lamellae tend to arc circumferentially. The maps therefore show stronger cross term amplitude there than in the center.

Diagonal terms J_{xx} and J_{yy} are comparatively higher where the local axis aligns with the basis (Fig. 9(f, g, l, m, p, q, v, w)).

Using the same Pauli algebra based Jones matrix decomposition used for the brain maps (Fig. 8), we further derived pixel wise retardance and fast-axis orientation maps for the cornea from the stitched Jones matrices (Fig. 10). The first row of Fig. 10(a, b) reports retardance and axis orientation obtained from Method I, while the second row (c, d) shows the corresponding results from Method II. Both methods reveal a clear regional contrast between the more central field and the paracentral region, consistent with spatially varying collagen lamella organization in the stroma.

Cornea sample:

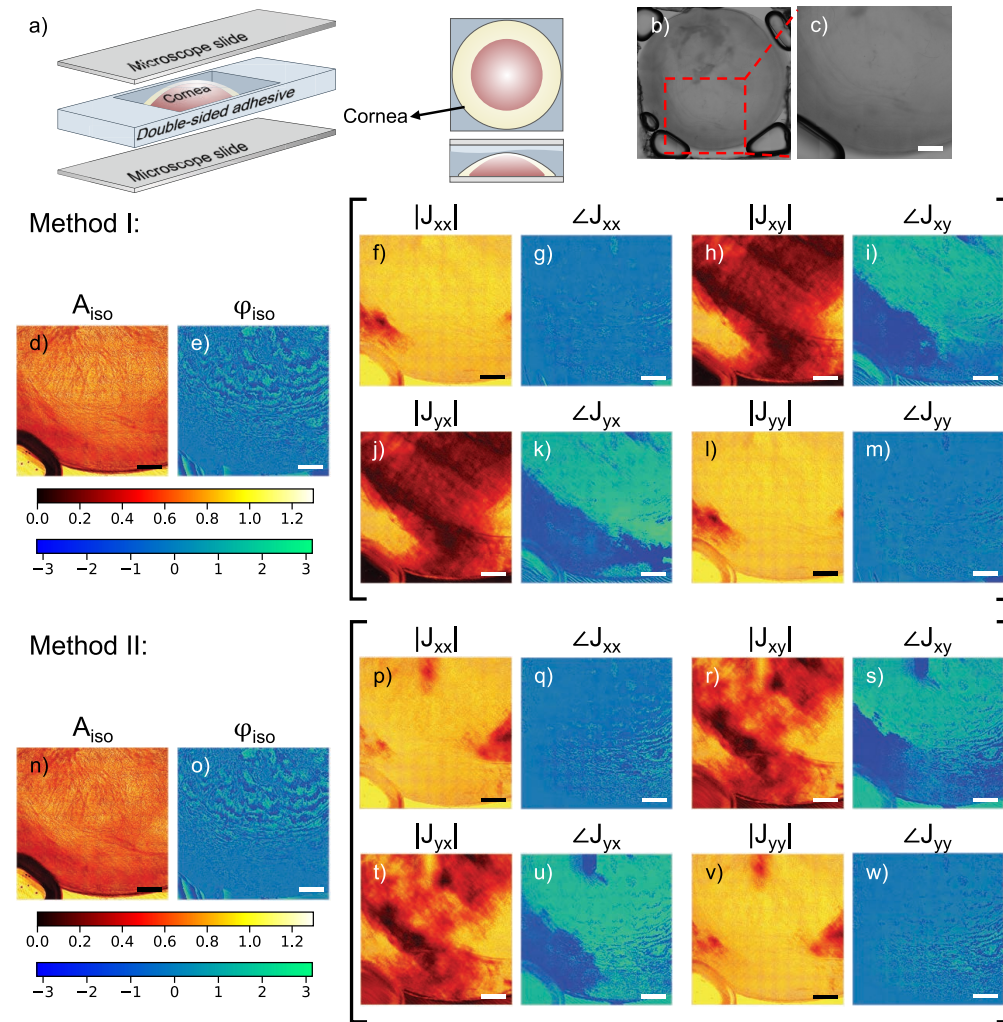
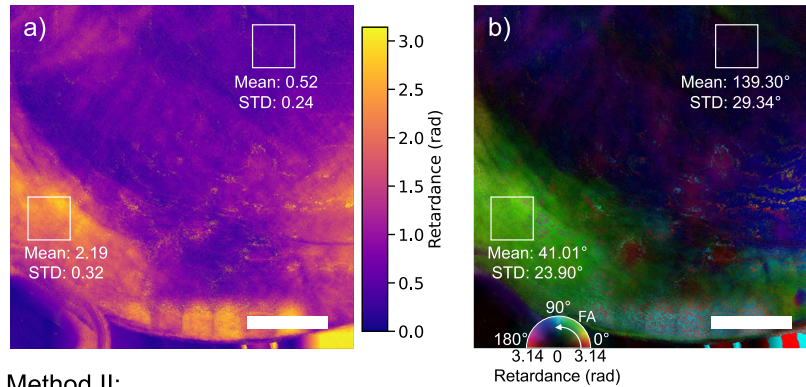


Fig. 9. Human cornea, stitched Jones matrix reconstruction. (a) Schematic showing the cornea mounted using double-sided adhesive tape and placed between two microscope slides. (b) Bright field mosaic (Zeiss Axio Observer 7) with PDHM region indicated; (c) zoom of dashed box. (d) Stitched isotropic amplitude A_{iso} . (e) Stitched isotropic phase ϕ_{iso} . (f–m) Jones element amplitude and phase maps (Method I). (n–w) Corresponding maps (Method II). Scale bar: 500 μm .

Method I:



Method II:

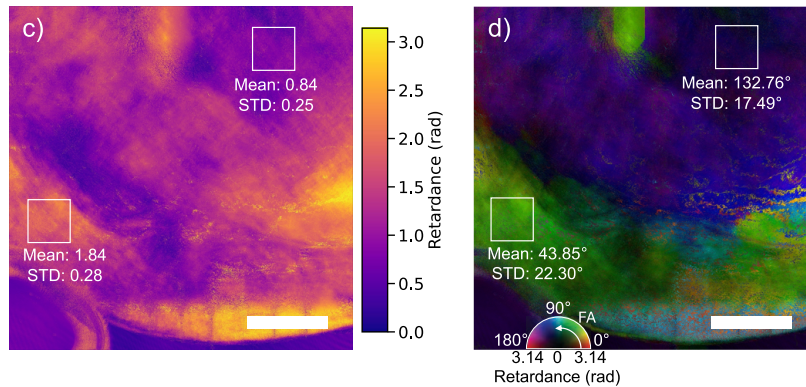


Fig. 10. Polarization properties of the human cornea derived from the stitched Jones matrices. (a, c) Retardance maps obtained using Method I and Method II, respectively. (b, d) Corresponding fast-axis orientation maps derived from Method I and Method II, respectively, showing stromal fiber orientation. The maps highlight the structural distinction between the central cornea and the paracentral region, reflecting regional variations in collagen lamellae organization. Scale bar: 700 μm .

Quantitatively, for the higher retardance paracentral region (lower left ROI), Method I yields 2.19 ± 0.32 rad and a mean axis orientation of $41.01^\circ \pm 23.90^\circ$, while Method II yields 1.84 ± 0.28 rad and $43.85^\circ \pm 22.30^\circ$. In the lower retardance central region (upper right ROI), Method I yields 0.52 ± 0.24 rad and $139.30^\circ \pm 29.34^\circ$, while Method II yields 0.84 ± 0.25 rad and $132.76^\circ \pm 17.49^\circ$. Notably, the dominant axis orientation differs by $\sim 90^\circ$ between the two ROIs in both methods, indicating a substantial change in the prevailing stromal alignment between central and paracentral regions. Because local thickness is not precisely known after hydration and gentle flattening (and residual thickness variation remains), we report retardance directly (rad) rather than converting to birefringence. Method II shows a localized artifact near the top-center of the retardance and optical axis maps, consistent with similar type of artefact observed on Fig. 8. In addition, the fast-axis maps exhibit scattered angular noise in some areas, most prominently near low-retardance pixels and phase unwrap boundaries in the underlying Jones elements. Despite these practical factors, Methods I and II produce consistent regional trends in both retardance contrast and dominant axis orientation, supporting the utility of dual shot PDHM for quantitative polarization mapping in transparent collagenous tissues.

While the presented results from the PDHM system allow robust full-field Jones matrix reconstruction, certain limitations should be noted. As the Jones matrix is reconstructed from two sequential holograms, the temporal resolution is inherently limited by the inter frame acquisition time, and the measurements remain sensitive to any motion between frames, consequently, current implementation is best suited to static or quasi-static specimens. Additionally, the phase bias equalization relies on the presence of non-birefringent (isotropic) region within the field of view, which may be absent in fully anisotropic or dense birefringent samples. In such cases, residual inter-shot phase offsets may remain and can compromise phase consistency across Jones elements. Furthermore, in transmission-based DHM configuration, multiple scattering in biological tissue introduces incoherent background light that reduces fringe visibility and signal-to-noise ratio, while scattering-induced depolarization challenges the deterministic assumptions of the Jones formalism and can limit the quantitative accuracy of the reconstructed Jones matrix. In this work we therefore focused on thin brain cryosections (20 μm) and a hydrated, flattened cornea preparation, for which the cornea remains largely transparent, and fringe contrast is preserved. For thicker or more turbid specimens, one should expect increased error. Mueller matrix modalities may be preferable when depolarization is non negligible.

3.3. Conclusion

We presented a dual shot polarization digital holographic microscopy (PDHM) approach that reconstructs the full field Jones matrix from only two holograms acquired with orthogonal incident linear polarizations. A polarization sensitive CMOS sensor provides four analyzer channels per exposure, eliminating analyzer rotation and specialized multiplexing optics. To compensate the time varying phase drift between the two frames, we introduced a phase bias equalization that uses isotropic reference patches to restore a common phase reference across all Jones elements. In addition, isotropic phase extraction and amplitude normalization were used to suppress background contributions and improve the visibility birefringent contrast, and tile wise phase equalization enabled artifact free mosaics over millimeter-scale fields of view.

The two reconstruction modes exhibit complementary behavior under inter shot drift. Method I (0°/90° inputs; 45°/135° analyzers) preserves amplitudes but biases phases before equalization; Method II (45°/135° inputs; 0°/90° analyzers) can corrupt both amplitude and phase because the sums and differences mix fields from different exposures. The proposed equalization removes these discrepancies, yielding consistent Jones matrix maps and accurate retardance estimates with either mode.

Quantitative experiments on biaxially oriented polypropylene (BOPP) films validated the method. The measured retardance and fast axis maps matched to $\sim\lambda/3$ behavior at 632.8 nm, and the basis dependent transfer of signal between diagonal and off diagonal Jones terms was consistent for both reconstruction routes after equalization. Applications to biological specimens demonstrated robust visualization and quantification of tissue anisotropy. In 20 μm thick mouse brain sections the corpus callosum, cingulum bundle, and dorsal hippocampal commissure were delineated by elevated off diagonal amplitudes and uniform phase. Corpus-callosum expressed retardance values of 0.37 ± 0.17 rad (Method I) and 0.41 ± 0.22 rad (Method II) corresponding to an effective birefringence on the order of $1.9 - 2.1 \times 10^{-3}$ in the selected ROI. In human cornea, retardance and fast-axis maps revealed a clear regional contrast between central and paracentral regions. Dominant axis orientation differed by approximately 90° between the central and paracentral ROIs, consistent with spatially varying stromal lamella organization.

Current limitations stem from the need for an isotropic region within the field of view, which may be absent in fully birefringent or dense fibrous samples. This constraint suggests an avenue for future work to remove the need for isotropic patches. With these developments, the proposed dual-shot PDHM has a clear path toward fast, simple, and quantitative polarization microscopy for neuroanatomy, ocular tissue assessment, and broader biomedical imaging.

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Data availability. Data underlying the results presented in this paper are not publicly available at this time but may be obtained from the authors upon reasonable request.

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