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Unravelling cellular anisotropy in human glioblastoma cells via Jones matrix decomposition

Shivam K. Chaubey¹, Mohit Rathor¹, Akash Debnath¹, Rupen Tamang², Biplob Koch² and Rakesh K. Singh^{1*}

Abstract: Quantitative measurement of the Jones matrix is essential for analyzing the polarization behavior of light, especially in anisotropic and birefringent samples. This study presents a label-free, and non-destructive microscopy technique based on digital holography for the spatially resolved measurement of the 2×2 Jones matrix of human glioblastoma cells (U-87 MG). Our approach enables the complete reconstruction of the complex Jones matrix at each pixel, capturing the complete polarization response of the sample. To extract deeper physical insights, we perform a detailed decomposition of the measured Jones matrix, allowing the retrieval of key polarization parameters such as inhomogeneity, diattenuation, and retardance. This comprehensive set of parameters quantitatively characterizes cellular anisotropy, offering a meaningful interpretation of light-matter interactions in complex biological media. Integrating Jones matrix microscopy with advanced polarization analysis establishes a framework for high-resolution, label-free optical characterization of cellular structures, with possible applications in quantitative imaging. In terms of biomedical diagnostics, this work presents a foundational study, and its translation into broader applications is possible through further comparative investigations across multiple cell lines and tissue samples.

Keywords: Jones matrix microscopy; inhomogeneity; retardance; diattenuation; glioblastoma cells

Introduction

Several imaging methods have been developed to gain profound insights into cellular structure and dynamics, playing a crucial role in understanding the morphology and origins of diseases¹. Optical imaging of biological cells is a valuable tool for visually identifying cells, particularly in diagnosing various conditions. In pathology and cytology, tissue biopsy analysis and morphological assessments are essential for detecting diseases like cancer². Continuous advancements in optical microscopy have significantly enhanced medical diagnostic capabilities³. For instance, fluorescence microscopy has been particularly transformative in molecular biology and diagnostics, offering high-contrast imaging through the use of fluorescent dyes^{4–10}. However, limitations such as phototoxicity, photobleaching, and the necessity of labelling agents constrain its effectiveness, particularly for live-cell imaging^{11,12}. The qualitative nature of fluorescence signals also impacts their reproducibility and restricts repeated measurements^{13,14}.

Polarization imaging has emerged as a powerful tool for

label-free imaging and revealing structural details beyond conventional intensity-based methods, as polarization, the orientation of the electric field, provides unique insights into light-matter interactions^{15–18}. In biological imaging, polarization microscopy offers a non-invasive means of analyzing anisotropic fine structures under physiological conditions without needing external dyes or fluorescent markers^{19,20}. A significant challenge in polarization imaging is quantifying anisotropy in biological samples, as tissues exhibit complex, spatially varying birefringence and scattering that limit the use of intensity-based analysis. Accurate characterization requires understanding how anisotropic structures modulate polarization, and polarization-sensitive techniques enable this by revealing intrinsic properties linked to molecular organization, orientation, and structural inhomogeneities. The Jones matrix formalism is widely employed to investigate these interactions, especially for coherent light, as it represents the sample's effect on the incident coherent polarization state through a 2×2 matrix^{21–23}. This mathematical framework describes a sample's optical properties, including how it alters light's

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polarization state^{24,25}. However, precise measurement of the Jones matrix elements remains challenging, particularly in biomedical optics and soft matter physics, where phase sensitivity is crucial²⁶.

Recent advancements in digital holography have enabled the quantitative measurement of polarization. Techniques such as common-path interferometry with 2D gratings allow spatially resolved determination of Jones matrix components in anisotropic samples. However, most applications remain in non-biological media such as optical fibers²⁷, asthenospheric substances^{28,29}, and liquid crystals³⁰. In biology, they are mainly applied to bulk tissues, including cancer^{31,32}, tendon, myocardium^{33,34}, and related structures^{35–37}. At the single-cell level, Jones matrix imaging remains limited by weak birefringence¹⁵. While several studies measured Jones matrices in complex media, their decomposition to extract retardance, diattenuation, and inhomogeneity is still underexplored for biological samples. Such decomposition enhances interpretability and provides a pathway for detailed anisotropy analysis, enabling comprehensive, quantitative cellular characterization beyond conventional intensity or phase imaging.

Achieving clear microscopic visualization of their polarization response requires a suitable measurement technique¹⁵. Our recent work has focused on developing a quantitative microscope capable of extracting the spatially resolved birefringence of cancer cells, particularly glioblastoma cells (U-87 MG), using digital holography³⁸. Expanding on this foundation, the present study provides in-depth investigations and results on the decomposition of Jones matrices to achieve a deeper understanding of the polarization response of the biological cell³⁹. In the present work, we build a digital polarization microscope and focus on the decomposition of Jones matrices measured from glioblastoma cells. Using spatially resolved Jones matrix imaging, we extract and map the polarization parameters to assess local variations in the anisotropic properties. This work contributes to the broader field of biomedical optics by demonstrating how Jones matrix decomposition can enhance the sensitivity and specificity of polarization imaging techniques for biologists and in the diagnosis.

Technique

Jones matrix microscope (JMM)

The experimental configuration of the JMM is depicted in Fig. (1). A vertically polarized light emitted from a He-Ne laser (Newport, N-LHP-925) with a wavelength of 632.8 nm is transformed to 45° linearly polarized light through a half-wave plate (HWP). This linearly polarized light is cleaned using a spatial filter assembly (SF) consisting of a microscope objective (MO1) and a pinhole P. The beam that emerges from the pinhole undergoes collimation through a bi-convex lens (L1) situated at a focal length (f) of 200 mm from the pinhole (P). A beam splitter (BS1) divides the

collimated beam into two equal intensities, directing them into the interferometer's two arms. A mirror (M1) directs the transmitted beam from BS1 towards the sample. After scattering at the sample plane, the beam is relayed to the detector plane by an imaging system comprising a 40× microscopic objective (MO2) with numerical aperture 0.65 from Thorlabs (RMS40X) and a lens L4 (focal length = 200 mm). The microscope's lateral resolution is $\approx 0.59 \mu\text{m}$ for a wavelength $\lambda = 632.8 \text{ nm}$. Simultaneously, the reflected beam from BS1, known as the reference beam, enters a triangular Sagnac geometry. This configuration includes a polarization beam splitter (PBS), a telescopic lens assembly (L2 and L3, both with equal focal lengths of $f = 250 \text{ mm}$), and mirrors (M2, M3). Within the Sagnac geometry, the diagonally polarized reference beam separates into two counter-propagating orthogonally polarized beams. Exiting from the PBS, these beams emerge as a pair of orthogonally polarized reference beams, each possessing a controlled tilt by the mirrors (M2 and M3) and telescopic lens system (L2 and L3). As shown by the dotted line in Fig. 1(b), mirrors (M2 and M3) apply the necessary spatial separations to the emerging orthogonally polarized components, resulting in distinguishable spatial frequencies for orthogonal polarization components. Figure 1(b) highlights an extended part of the dashed rectangle in Fig. 1(a), with a key approach involving the introduction of distinct carrier frequencies into the reference beam's orthogonal polarization components at the camera plane. The orthogonal polarization components, spatially separated at the back focal plane of lens L2, undergo a Fourier transformation through lens L3. Subsequently, the orthogonal polarization components of the reference beam are coherently superimposed with the corresponding object fields. The beams emerging from the reference arm are denoted as

$$R_x(x, y) = \exp[i2\pi(f_{1x}x + f_{1y}y)] \quad (1)$$

$$R_y(x, y) = \exp[i2\pi(f_{2x}x + f_{2y}y)] \quad (2)$$

At the back focal plane of lens L3, $r = (x, y)$ signifies a spatial position. The carrier frequencies corresponding to the horizontal and vertical polarization components of the reference beam are represented by (f_{1x}, f_{1y}) and (f_{2x}, f_{2y}) respectively. The resultant interference pattern is recorded at the charge-coupled detector (CCD) plane, positioned at the back focal plane of lens L3. The CCD employed in this setup is Prosilica GT 1920, with a pixel pitch of 4.54 microns.

Hologram recording and Jones matrix retrieval

We employ a dual-shot polarization-sensitive digital holography approach to characterize the anisotropic samples. The complete 2×2 Jones matrix is retrieved spatially across the field of view by illuminating the sample sequentially with linearly polarized beams oriented at +45° and −45°. The two sequential acquisitions at +45° and −45°

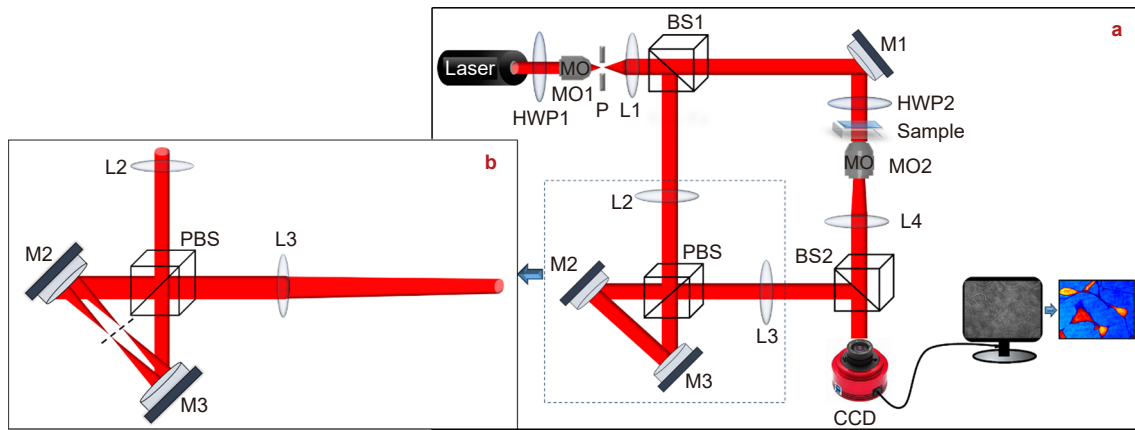


Fig. 1 | (a) shows a schematic of the Jones matrix microscope (JMM), (b) shows the enlarged section of the dashed line (Sagnac Geometry).

orientations were recorded with a time delay of approximately 30 seconds. In the first measurement, the sample is illuminated with a $+45^\circ$ polarized beam, prepared using a half-wave plate (HWP2) placed before the sample. The emergent beam, modulated by the sample's anisotropy, contains x - and y -polarized components. These interfere with corresponding orthogonally polarized reference components, each carrying distinct spatial carrier frequencies, and are recorded in a single hologram. Fourier fringe analysis⁴⁰ is then applied to isolate the corresponding spectrum and then reconstruct the complex field components $Y_{11}(x, y)$ and $Y_{12}(x, y)$. A second hologram is acquired with -45° polarized illumination. The same reconstruction process yields $Y_{21}(x, y)$ and $Y_{22}(x, y)$. Together, these two sets of complex fields allow the direct retrieval of the sample's Jones matrix elements across the field of view.

We first acquire holograms for $+45^\circ$ and -45° polarized input beams without the sample in place to account for systematic errors introduced by the optical setup and reconstruction process. These reference fields are then used to normalize the sample fields by performing a complex field-wise division for fields recovered from the holograms recorded with and without the sample. This procedure effectively compensates for phase offsets, amplitude imbalances, and spatial frequency variations, ensuring accurate retrieval of the sample-induced polarization response.

According to the Jones formalism, the relation between incident and emergent fields under coherent illumination is given by

$$\begin{pmatrix} E_x^{\text{out}}(x, y) \\ E_y^{\text{out}}(x, y) \end{pmatrix} = \begin{pmatrix} J_{xx}(x, y) & J_{xy}(x, y) \\ J_{yx}(x, y) & J_{yy}(x, y) \end{pmatrix} \begin{pmatrix} E_x^{\text{in}}(x, y) \\ E_y^{\text{in}}(x, y) \end{pmatrix}. \quad (3)$$

Beams with $+45^\circ$ and -45° input polarization states are represented by the Jones matrices $\begin{pmatrix} 1 & 1 \end{pmatrix}^T$ and $\begin{pmatrix} 1 & -1 \end{pmatrix}^T$ respectively, and the corresponding output fields yield

$$Y_{11}(x, y) = J_{xx}(x, y) + J_{xy}(x, y), \quad (4)$$

$$Y_{12}(x, y) = J_{yx}(x, y) + J_{yy}(x, y), \quad (5)$$

$$Y_{21}(x, y) = J_{xx}(x, y) - J_{xy}(x, y), \quad (6)$$

$$Y_{22}(x, y) = J_{yx}(x, y) - J_{yy}(x, y). \quad (7)$$

Solving these Eqs. (4–7) provides the spatially resolved Jones matrix elements as follows

$$J_{xx}(x, y) = \frac{1}{2}[Y_{11}(x, y) + Y_{21}(x, y)], \quad (8)$$

$$J_{xy}(x, y) = \frac{1}{2}[Y_{11}(x, y) - Y_{21}(x, y)], \quad (9)$$

$$J_{yx}(x, y) = \frac{1}{2}[Y_{12}(x, y) + Y_{22}(x, y)], \quad (10)$$

$$J_{yy}(x, y) = \frac{1}{2}[Y_{12}(x, y) - Y_{22}(x, y)]. \quad (11)$$

This formulation yields a comprehensive spatial map of the complex-valued Jones matrix, encompassing both amplitude and phase components. In the present study, we apply this method to biological cells, enabling quantitative, label-free mapping of their polarization response for examining the light-matter interaction.

The complete process of retrieving the Jones matrix, from the acquisition of spatially multiplexed holograms to the reconstruction of complex field components, is depicted in Fig. 2. Figure 2(a) shows a hologram recorded for a human U-87 MG under $+45^\circ$ polarized illumination. In contrast, its Fourier transform is shown in Fig. 2(b), revealing five distinct spectral regions corresponding to the DC and off-axis components. The x -polarized spectral element is isolated and shifted to the origin in Fourier space as shown in Fig. 2(c), followed by inverse Fourier transformation to obtain its amplitude and phase as shown in Fig. 2(d,f). As shown in Fig. 2(e,g), a similar reconstruction is performed for the y -polarized component, whose spectrum is shown in Fig. 2(b) and highlighted by a separate red rectangle region. The retrieved fields from both polarization channels, corresponding to $+45^\circ$ and -45° inputs, are

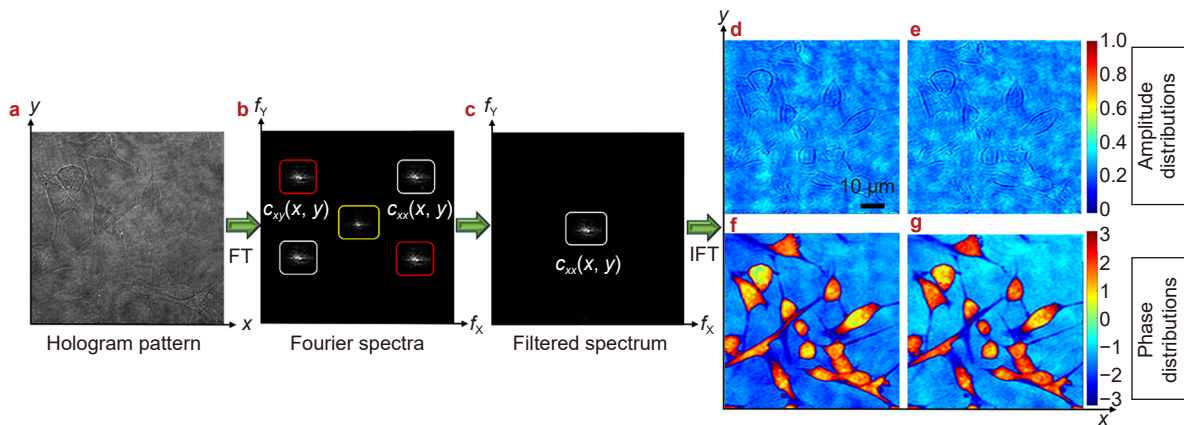


Fig. 2 | Principle of Fourier fringe analysis for extracting a complex field from experimentally recorded hologram patterns. (a) Shows a recorded hologram for +45° polarized input beams, (b) Shows Fourier spectra, and (c) depicts filtered Fourier spectra for the x-polarized component. (d) and (e) Show amplitude and phase distribution for x-component; similarly, (f) and (g) represent amplitude and phase distribution for y-component.

then used to calculate the spatially resolved Jones matrix elements described above.

Jones matrix decomposition and extraction of anisotropic properties

The polarization properties of a sample can be comprehensively described using the Jones matrix formalism, which represents how a coherent polarization state is transformed upon interaction with the sample. For homogeneous polarization elements, such as ideal optical components, the diattenuation and retardance depend solely on the eigenvalues of the Jones matrix. However, biological samples and other complex media often exhibit spatial variations in their optical response, leading to inhomogeneous polarization characteristics^{39,41}.

Lu and Chipman introduced³⁹ a generalized definition of diattenuation (D) and retardance (R) of an arbitrary polarization element which can be expressed as

$$D = \left\{ 1 - \frac{4|\det J|^2}{[\text{tr}(J^\dagger J)]^2} \right\}^{\frac{1}{2}}, \quad (12)$$

$$R = 2\cos^{-1} \frac{\left| \text{tr} J + \frac{\det J}{|\det J|} \text{tr} J^\dagger \right|}{2[\text{tr}(J^\dagger J) + 2|\det J|]^{\frac{1}{2}}}, \quad (13)$$

here $\dagger$ represents the conjugate transpose (Hermitian conjugate). These two relations are more useful than the polar decomposition as they allow us to obtain the diattenuation and retardance directly from the Jones matrix.

As the diattenuation and retardance of a homogeneous polarization element depend only on the eigenvalues of the associated Jones matrices, Eqs. (12) and (13) are reduced to

$$D(J) = \frac{|\xi_q|^2 - |\xi_r|^2}{|\xi_q|^2 + |\xi_r|^2}, 0 \leq D \leq 1, \quad (14)$$

$$R(J) = |\delta_q - \delta_r|, 0 \leq R \leq 2\pi, \quad (15)$$

where $\xi_q = |\xi_q| \exp(i\delta_q)$ and $\xi_r = |\xi_r| \exp(i\delta_r)$ are the eigenvalues. Extending the conventional polar decomposition approach is necessary to analyze inhomogeneous polarization elements rigorously. But in the case of inhomogeneous polarization elements and other complex media, the polarization properties depend not only on the eigenvalues of the Jones matrices as homogeneous elements, but also on an intrinsic parameter called inhomogeneity³⁹. The inhomogeneity of a system (η) is quantified by an additional intrinsic parameter that characterizes deviations from purely homogeneous behaviour. Unlike homogeneous elements, where eigen polarizations remain orthogonal, inhomogeneous systems exhibit variations resulting in a measurable overlap between eigen polarizations. This inhomogeneity parameter provides insight into local variations in the sample's optical response³⁹, which are crucial for understanding biological structures such as cells.

The inhomogeneity is defined as the absolute value of the inner product of eigen polarizations $\hat{E}_q$ and $\hat{E}_r$ as

$$\eta = |\hat{E}_q^\dagger \hat{E}_r|, 0 \leq \eta \leq 1, \quad (16)$$

where $\hat{E}_q$ and $\hat{E}_r$ are orthogonal for homogeneous polarization elements, resulting in an inhomogeneity value of zero. In contrast, when E_q and E_r are parallel (representing degenerate polarization elements), the inhomogeneity parameter equals one. Therefore, this parameter serves as a quantitative measure of the degree of inhomogeneity³⁹. The inhomogeneity can be expressed in terms of the elements of the Jones matrix as

$$\eta^2 = \frac{\text{tr}(J^\dagger J) - \frac{1}{2}|\text{tr} J|^2 - \frac{1}{2}|(\text{tr} J)^2 - 4\det J|}{\text{tr}(J^\dagger J) - \frac{1}{2}|\text{tr} J|^2 + \frac{1}{2}|(\text{tr} J)^2 - 4\det J|}. \quad (17)$$

By introducing inhomogeneity explicitly, we refine the expressions for diattenuation and retardance, incorporating

the spatial variations inherent to biological samples. The modified equations allow direct extraction of these polarization properties while accounting for inhomogeneous effects. To derive expressions for diattenuation and retardance, we express an arbitrary Jones vector as a linear combination of eigen polarizations: $\hat{E} = \alpha \hat{E}_q + \beta \hat{E}_r$ with a constraint $|\alpha|^2 + |\beta|^2 = 1$. Maximizing and minimizing the transmission function, $T(\alpha, \beta) = |\hat{J}\hat{E}|^2$ under this constraint, leads to a quadratic equation for $T_{\max}$ and $T_{\min}$ ³⁹. These extrema help to characterize diattenuation and retardance in terms of the eigenvalues and inhomogeneity of the system by satisfying a quadratic equation

$$(1 - \eta^2) T^2 - \left[|\xi_q|^2 + |\xi_r|^2 - \eta^2 (\xi_q^\dagger \xi_r + \xi_q \xi_r^\dagger) \right] T + (1 - \eta^2) |\xi_q|^2 |\xi_r|^2 = 0. \quad (18)$$

By this quadratic equation, we find $T_{\max}$ and $T_{\min}$ accordingly. Here, ξ_q and ξ_r are the eigenvalues. Diattenuation refers to the differential attenuation of polarization states. So, it shows the polarization-dependent loss, which indicates the material anisotropy. Eq. (12) is modified for inhomogeneous polarization elements and can be rewritten as

$$D = \left\{ 1 - \frac{4(1 - \eta^2) |\xi_q|^2 |\xi_r|^2}{\left[|\xi_q|^2 + |\xi_r|^2 - \eta^2 (\xi_q^\dagger \xi_r + \xi_q \xi_r^\dagger) \right]^2} \right\}^{\frac{1}{2}}. \quad (19)$$

Retardance is introduced to quantify the phase delay between orthogonal polarization components caused by the sample. It is a direct measure of the birefringence of a material, which can arise from differences in refractive index along different axes (structural ordering)⁴². So, Eq. (13) is modified for inhomogeneous polarization elements as follows

$$R = 2\cos^{-1} \left\{ \left[\frac{(1 - \eta^2) (|\xi_q| + |\xi_r|)^2}{\left[(|\xi_q| + |\xi_r|)^2 - \eta^2 (2|\xi_q||\xi_r| + \xi_q^\dagger \xi_r + \xi_q \xi_r^\dagger) \right]} \right]^{\frac{1}{2}} \left| \cos \frac{|\delta_q - \delta_r|}{2} \right| \right\}. \quad (20)$$

Furthermore, to support a more intuitive interpretation of the polarization response, we analyse the spatially resolved polarization ellipse corresponding to each pixel in the sample. Standard parameters such as the orientation angle (ψ) and ellipticity angle (χ) are computed using the retrieved complex fields. These parameters provide insight into the local alignment and birefringence-induced phase shifts within the sample, and collectively describe the shape and orientation of the polarization ellipse. This representation allows us to visualize and quantify how the polarization state of light is modified upon interaction with anisotropic biological structures, such as U-87 MG. The

orientation angle reflects the sample's dominant optical axis at each location. At the same time, the ellipticity indicates the degree of circular or linear polarization. Together, these parameters serve as sensitive indicators of microstructural organization, offering contrast and structural information that extend beyond what is accessible through conventional amplitude or phase imaging.

To apply these principles, we have developed a computational framework using Python to retrieve polarization parameters from inhomogeneous samples, such as U-87 MG. Due to inhomogeneity, spatial variations in the polarization properties of the sample are observed and mapped using Eq. (17). To quantify polarization-dependent attenuation, diattenuation is analysed, revealing anisotropic absorption through differential attenuation of orthogonal polarization states by Eq. (19). With the help of Eq. (20), the retardance of the sample is mapped, which determines the birefringence-induced phase delay between polarization components. Spatial distributions of Jones matrix elements can resolve complex field interactions, linking polarization alterations to sample anisotropy. Polarized light-sample interaction is modelled to relate orientation angle and angle of ellipticity to structural ordering and optical axis alignment. Combining these parameters allows us to achieve a detailed polarization-based analysis beyond conventional imaging techniques.

Sample preparation

Materials

Dulbecco's modified eagle medium (DMEM) and 6-well plates were purchased from CELLclone™ Genetix Biotech Asia Pvt. Ltd. Gibco from Fisher Scientific supplied Fetal bovine serum (FBS), Trypsin-EDTA, and antibiotic antimycotic solution (penicillin and streptomycin). National Centre for Cell Science (NCCS), Pune, India, provided the human glioblastoma U-87 MG cell lines.

Sample preparation

Human U-87 MG were cultured in a T-25 cell culture flask containing DMEM complemented with a heat-inactivated FBS and antibiotic-antimycotic solution following the incubation of the cells at 37°C in a humidified environment with 5% CO₂. Trypsin was used to detach the adherent U-87 MG cells from the surface of the flask when it reached a confluency of 80% – 90%, and further proceeded for slide preparation. To prepare the slides, 1×10^4 U-87 MG cells were seeded on coverslips inserted into a six-well plate. Using forceps, the coverslips were carefully removed from the plate's solution while holding it close to the edge. The coverslips were then washed with phosphate-buffered saline solution, wiping the excess from the coverslips. At last, the coverslips were mounted on the slides using phosphate-buffered saline as a mounting agent. The live U-87 MG cells

were then examined under the microscope and further proceeded to analysis.

Test and validation of the microscopic and computational algorithm

To validate the effectiveness of the developed microscopic technique, we calculated the Jones matrices for known standard samples, such as a linear polarizer and a half-wave plate (HWP) at various orientation angles. The Jones matrices for a linear polarizer (J_{POL}) and an HWP (J_{HWP}) are represented as follows

$$J_{\text{POL}}(\theta) = \begin{pmatrix} \cos^2\theta & \cos\theta\sin\theta \\ \cos\theta\sin\theta & \sin^2\theta \end{pmatrix}, \quad (21)$$

$$J_{\text{HWP}}(\theta) = \begin{pmatrix} \cos 2\theta & \sin 2\theta \\ \sin 2\theta & -\cos 2\theta \end{pmatrix}, \quad (22)$$

where θ is the orientation angle of the fast axis with respect to the horizontal axis. The experimental tests were conducted for a linear polarizer at an orientation angle of 45° , and a half-wave plate (HWP) at 90° . The complex field components denoted as $Y_{11}(x, y)$, $Y_{12}(x, y)$, $Y_{21}(x, y)$, and $Y_{22}(x, y)$ are experimentally retrieved for each sample using the procedures as described previously. Subsequently, we determined the complete Jones matrix elements, and the results are illustrated in Fig. 3. The amplitude images of the Jones matrix elements for a linear polarizer with orientation angle 45° and the HWP are shown in Fig. 3(a,b). Figure 3(c,d) present the corresponding phase images of the Jones matrix elements. In the case of a linear polarizer with a 45° orientation, all the Jones matrix elements exhibit similar amplitudes as depicted in Fig. 3(a). On the other hand, phase values are zero for all four elements of the Jones matrix as represented in Fig. 3(c). For an HWP with θ of 90° , the diagonal elements $J_{xx}(x, y)$ and $J_{yy}(x, y)$ reaches the value unity, while the other off-diagonal elements $J_{xy}(x, y)$ and $J_{yx}(x, y)$ remain at approximately zero value, as shown in Fig. 3(b). The corresponding phase values for these matrix elements are further detailed in Fig. 3(d), confirming that $\angle J_{xx}$ exhibit π phase, while the other elements maintain values close to zero. Plots in Fig. 3(e,f) illustrate the average amplitudes of the Jones matrices, revealing the relative strengths of the matrix elements. These results align closely with the values predicted by Eqs. (21–22). We employed a normalization process to minimize possible errors by capturing reference images in an empty area without the sample. Therefore, the experimental outcomes of the polarizer and HWP agree well with the theoretical values, validating the accuracy of the proposed Jones Matrix Microscope (JMM). While normalization with reference field reduces systematic offsets and aberrations, it does not remove random noise in weak-signal regions. However, in the presence of weak signals and noise, a possible improvement may include temporal averaging of sequential holograms, as well as numerical phase-denoising and speckle-reduction strategies^{43–45}.

To further validate the accuracy of our microscopic technique and algorithms, we measured the Jones matrices for a known inhomogeneous sample, as shown in Fig. (3). The sample was created by cascading a horizontal linear polarizer followed by a quarter-wave linear retarder with its fast axis oriented at 45° ³⁹ (case 1 of Table 1: $p0^\circ q45^\circ$) schematically illustrated in Fig. 3(j). The intrinsic polarization parameters for this configuration are theoretically defined as follows: inhomogeneity = 0.7071, diattenuation = 1.0000, and retardance = 1.5708. Using the experimentally obtained Jones matrix, we calculated these parameters and found an average inhomogeneity of 0.6987, diattenuation of 0.9235, and retardance of 1.5931. Figure 3(g,h,i) show spatially resolved plots of inhomogeneity, diattenuation, and retardance, respectively, across the full field of view (1456×1936 pixels). These maps provide a comprehensive visualization of the sample's optical response, highlighting variations in polarization properties due to inherent anisotropy and experimental conditions.

Furthermore, Fig. 3(k) presents a comparative bar chart illustrating the theoretical and experimentally obtained values of the polarization parameters inhomogeneity, diattenuation, and retardance for a known sample configuration ($p0^\circ q45^\circ$). The blue bars represent the theoretical values, while the orange bars correspond to the experimentally retrieved values. Red error bars indicate the absolute differences between the theoretical and experimental values, providing a direct visual cue for measurement accuracy. Each bar is labelled with its numerical value to aid quantitative comparison. This representation offers a clear and intuitive visualization of the performance of our polarization retrieval technique, highlighting both the precision and any systematic deviations in the extracted parameters.

In addition to the $p0^\circ q45^\circ$ configuration, we evaluated other cases by varying the polarizer's and quarter-wave plate's orientation. The additional configurations include: $p30^\circ q45^\circ$, $p45^\circ q90^\circ$, $q30^\circ p45^\circ$, and $q45^\circ p45^\circ$. For all these cases, a comparative analysis of theoretical and experimental values for inhomogeneity, diattenuation, and retardance is presented in Table 1. These results further reinforce the robustness and accuracy of our methodology across a range of polarization conditions. It is noted that for Case 5 ($q45^\circ p45^\circ$), the measured inhomogeneity (0.1274) deviates from the theoretical value of 0.0000. This offset reflects the instrumental baseline, arising possibly from finite extinction ratios of the polarization elements, slight misalignments, and reconstruction noise. Similarly, across all cases, the measured diattenuation values remain marginally below the theoretical value of 1.0. This may be attributed to imperfect polarization elements and residual depolarizing effects in the optical system. The close agreement between the theoretical and experimental values confirms the reliability of our approach. These findings, along with our previous validation using a linear polarizer and a HWP at various orientations as shown in Fig. (3), demonstrate the effectiveness and accuracy of our JMM. This comprehensive

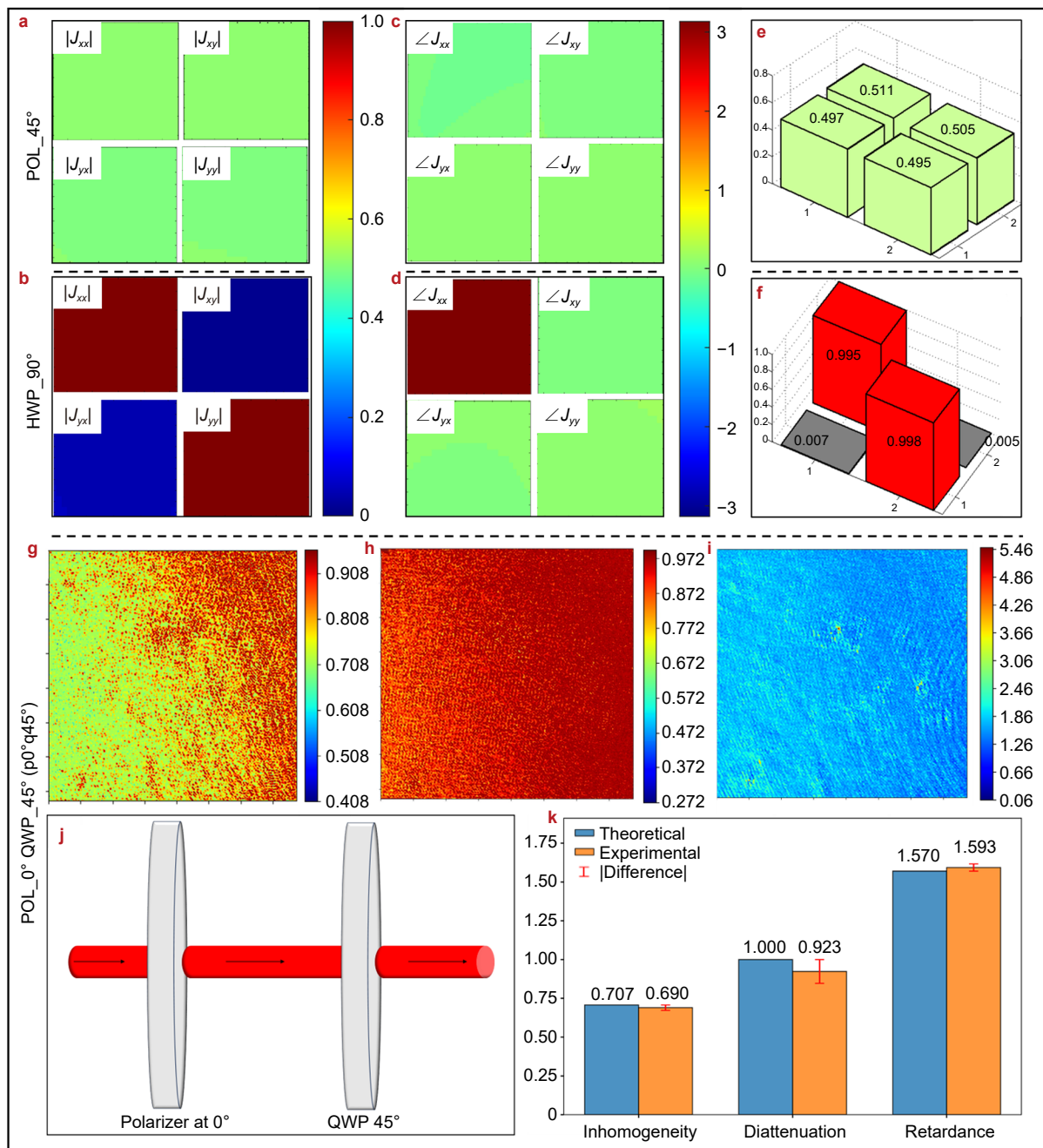


Fig. 3 | Validation of the proposed microscopic technique by using a known standard sample. (a,b) show the amplitudes of the Jones matrix elements of polarization selective images for a linear polarizer at the orientation angle of 45° (POL_45°), and HWP at the orientation angle of 90° (HWP_90°). (c,d) show the corresponding phase distributions of Jones matrix elements for the linear polarizer and HWP at the corresponding orientation angles. (e,f) are the normalized average values of the amplitude of Jones matrix elements. (g) inhomogeneity map. (h) diattenuation map. (i) retardance map. (j) schematic of the sample created by cascading a horizontal linear polarizer (POL_0°) followed by a quarter-wave plate with its fast axis oriented at 45° (QWP_45°), representing the configuration p0°q45°, and (k) comparison of experimentally measured polarization parameters with theoretical values.

validation confirms the robustness of our microscopic technique and computational algorithms for precise anisotropic measurements.

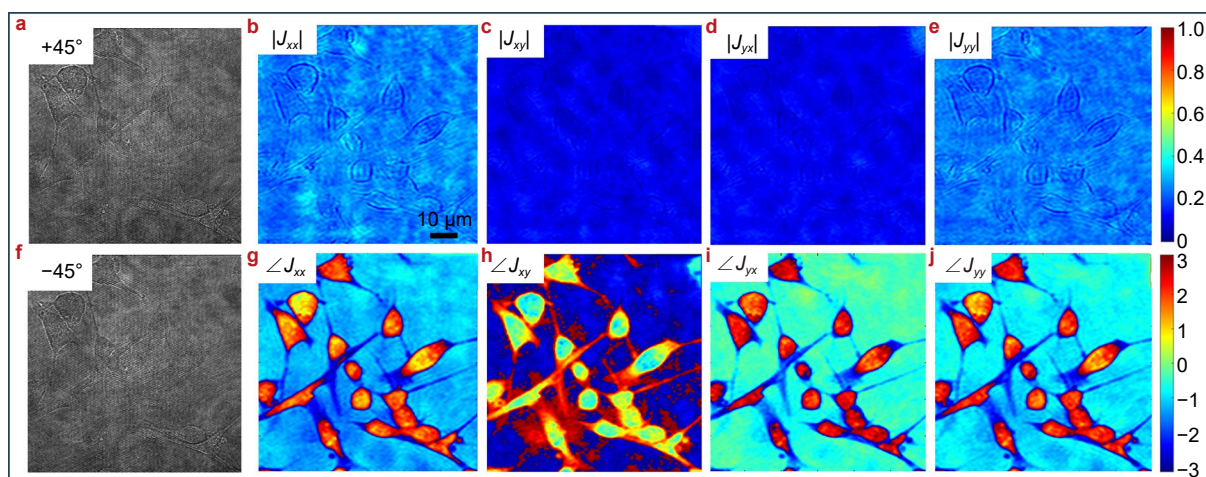
Result & discussion

After validating the accuracy of our microscope and algo-

rithms with standard samples, we acquired the Jones matrix images of the light emerging from U-87 MG as shown in Fig. 4. Figure 4(b,f) present the digital holograms corresponding to the two-shot measurements of the sample beam for illuminations with +45° and -45° beams. The Fourier fringe analysis approach retrieves the complex field components of the light emerging from U-87 MG for each

Table 1 | Comparison of theoretical and experimentally retrieved anisotropic parameters (inhomogeneity, diattenuation, and retardance) for various configurations of cascaded optical elements (polarizer and quarter-wave plate) as shown in Fig. 4(d).

S.No.	Different cases	Inhomogeneity		Diattenuation		Retardance	
		Theoretical	Experimental	Theoretical	Experimental	Theoretical	Experimental
1.	$p0^\circ q45^\circ$	0.7071	0.6987	1.0000	0.9235	1.5708	1.5931
2.	$p30^\circ q45^\circ$	0.3535	0.3689	1.0000	0.9473	1.5249	1.4931
3.	$p45^\circ q90^\circ$	0.7071	0.7267	1.0000	0.9317	1.6694	1.6802
4.	$q30^\circ p45^\circ$	0.3535	0.3426	1.0000	0.9482	1.4714	1.5019
5.	$q45^\circ p45^\circ$	0.0000	0.1274	1.0000	0.9893	1.5708	1.5902

Fig. 4 | (a) and (f) are the two digital holograms corresponding to the sample beam oriented at $+45^\circ$ and -45° with respect to the reference axis. (b–e) show the amplitude of the Jones matrix elements. (g–j) are the corresponding phase distributions.

shot measurement, as shown in Fig. 4. Then from Eqs. (8–11), we retrieve the complete set of Jones matrix elements. Figure 4 shows the amplitude distributions of the Jones matrix elements of the U-87 MG cells, while the corresponding phase distributions are shown in Fig. 4(g–j). Utilizing the JMM optical setup, we capture U-87 MG cells' spatially resolved polarization features. The microscopic image represented through the Jones matrix displays the well-defined cellular structure of U-87 MG cells. The cells display characteristics of epithelial morphology and exhibit distinct processes arising from the cell body. The Jones matrix image of U-87 MG highlights a well-distinct morphology and depicts the anisotropic nature with varied spatial phase distributions. Cells are responsive to the signals from hormones, growth factors, or neighbouring cells, which trigger alterations in metabolism, gene expression, cytoskeletal (actin, intermediate, and microtubule) organization, and membrane dynamics. Therefore, the anisotropic nature of the cells could be attributed to the variation in the cells' organelles, the dynamicity in cells/cell membranes, or the irregular shape of the U-87 MG cells. Since live U-87 MG cells were imaged using a dual-shot approach, the assumption of quasi-static behaviour during the 30-second interval between the $+45^\circ$ and -45° acquisitions is essential. This assumption is supported by prior

reports, where U-87 cell motility and morphological changes were typically analyzed using minute-scale frame intervals over hours-long recordings^{46,47}. Similarly, impedance-based assays show significant changes only across hours to days⁴⁸. Thus, U-87 MG cells can be regarded as stable within the 30-second acquisition window, validating the dual-shot retrieval strategy. This consideration is noted as a methodological limitation.

Following the determination of the Jones matrix elements, we extracted key polarization parameters such as inhomogeneity, diattenuation, and retardance, further to characterize the optical behaviour of U-87 MG cells. These parameters provide insight into the sample's spatial anisotropy and anisotropic response. The inhomogeneity map in Fig. 5 quantifies the spatial variation in the polarization response of U-87 MG cells, revealing a range of values from approximately 0.63 to 0.97. This significant fluctuation in optical properties suggests a complex intracellular organization influenced by the anisotropic distribution of cellular components such as the nucleus, cytoskeleton, and membrane structures, as shown in Fig. 5(a). Higher inhomogeneity values within the cellular region indicate the presence of anisotropic subcellular structures, while the surrounding medium exhibits relatively uniform polarization characteristics. Figure 5(b) presents the statistical

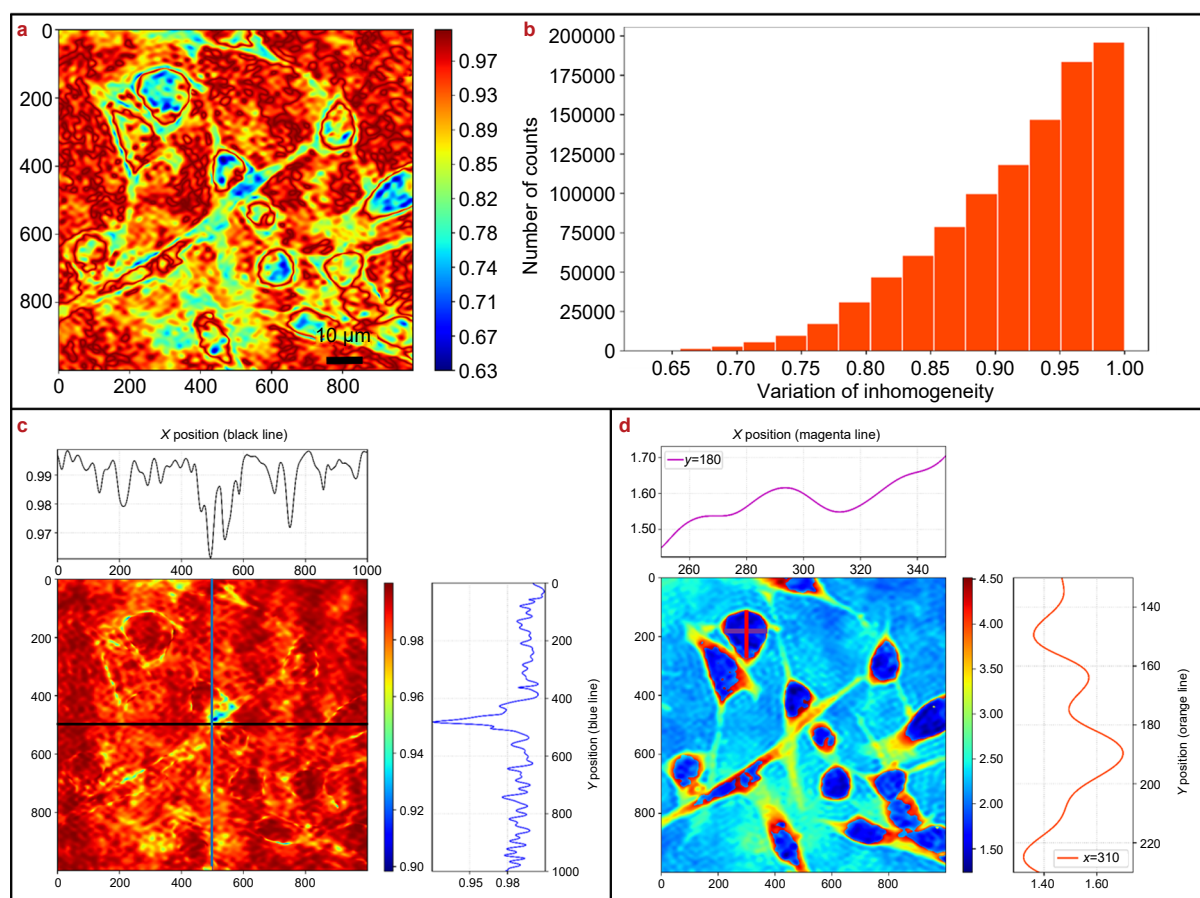


Fig. 5 | (a) Spatially resolved inhomogeneity map of U-87 MG cells, highlighting variations in polarization response across cellular structures. (b) histogram of inhomogeneity values, illustrating the statistical distribution of its variations across all pixels in the field of view. (c) diattenuation map, revealing spatial variations in polarization-dependent transmission. (d) retardance map, highlighting spatial variations in birefringence. The white and black lines indicate the regions analysed for cross-sectional retardance variation.

distribution of inhomogeneity values across all pixels, providing deeper insight into the spatial anisotropy of the sample. The observed inhomogeneity variations directly impact other polarization-dependent properties, making it a crucial parameter for assessing cellular anisotropy.

Diattenuation, a measure of the sample's polarization-dependent transmission or reflection properties, is presented in Fig. 5(c). Ideally, a perfectly polarized beam would exhibit a diattenuation value of $D = 1$. However, as the incident 45° polarized beam interacts with the cellular structures, the observed diattenuation values range from 0.90 to 0.99 across the field of view. This slight deviation in diattenuation is possibly due to system performance limitations and depolarizing effects introduced by light scattering within the cellular environment. Figure 5(c) shows the spatial distribution of diattenuation across the U-87 MG cells, highlighting localized variations. The line profiles provide further insight into these variations, where the black line represents the diattenuation profile along the x-direction ($y = 500$ pixels, $x = 1$ to 1000 pixels), and the blue line shows the variation along the y-direction (blue line, $x = 500$ pixels, $y = 1$ to 1000 pixels). Regions with relatively

lower diattenuation suggest an increase due to subcellular structural complexity. These variations underscore the optical anisotropy of the sample, reinforcing the potential of polarization-based imaging for assessing cellular organization and structural anisotropy.

The retardance distribution in Fig. 5(d) reveals the phase delay introduced between two orthogonal polarization components upon transmission through the sample. The retardance values span from 1.24 to 4.22 radians, indicating birefringence across the cellular region. Notably, the intracellular regions exhibit higher retardance values than the surrounding medium, which remains relatively uniform. This spatial variation in retardance highlights the structural anisotropy of the U-87 MG cells, likely influenced by intracellular molecular organization and refractive index distributions.

Figure 5(d) presents the spatial map of the retardance, with magenta and orange lines indicating the regions analysed for cross-sectional retardance variation. The magenta line shows the retardance variation along the horizontal white line (at $y = 180$ pixels) for a segment of 100 pixels ($x = 250$ –350), capturing fluctuations within a selected cellular

region. Similarly, the orange line illustrates the retardance variation along the vertical black line (at $x = 310$ pixels) for a segment of 100 pixels ($y = 130\text{--}230$), further highlighting the structural anisotropy within the cell. These variations emphasize the complex intracellular organization and the role of birefringence in assessing cellular anisotropy.

These findings underscore the complex optical characteristics of U-87 MG cells, revealing significant spatial variations in polarization properties. The observed inhomogeneity, diattenuation, and retardance provide valuable insights into the cellular microstructure and anisotropic behaviour. The ability to resolve these polarization features at a microscopic scale demonstrates the efficacy of our Jones Matrix-based approach for characterizing biological specimens with intrinsic optical anisotropy.

We superimposed polarization ellipses onto the orientation angle map, as shown in Fig. 6 to further analyze the polarization response at the cellular level. This visualization provides a detailed spatial representation of polarization state variations across the cellular region. The distribution of polarization ellipses highlights highly localized anisotropic domains, exhibiting significant variations in orientation and ellipticity. These U-87 MG cells are invasive and adherent, typically appearing spindle-shaped or irregularly triangular with key cytoplasmic extensions. The variation in the cellular inhomogeneity may be attributed to the organelles and nucleus, which are localized unevenly in

the cytoplasm of cells. Therefore, the cellular inhomogeneity describes the non-uniform distribution of internal structures like the nucleus and organelles. This structural complexity governs the way light interacts with the cells: regions with denser or absorption-prone components produce polarization-dependent differences in transmission, expressed as diattenuation, while fibrous or anisotropic elements such as collagen or microtubules introduce phase delays between orthogonal polarization components, observed as retardance^{49–52}. These findings confirm the polarization sensitivity of U-87 MG cells, underscoring the potential of polarization-based imaging for assessing cellular and structural anisotropy.

Given the high sensitivity of polarization measurements in resolving cellular structures, as demonstrated in Fig. 6, this approach demonstrates the feasibility of mapping anisotropy within cellular structures. However, further comparative studies on different cell types and pathological tissues will extend the scope of this technique for a wider diagnostic application.

Conclusion

In summary, we have presented a quantitative, label-free approach for analysing polarization properties in biological samples through Jones matrix decomposition. Our method enables a comprehensive understanding of light-matter interactions at the microscopic scale by extracting

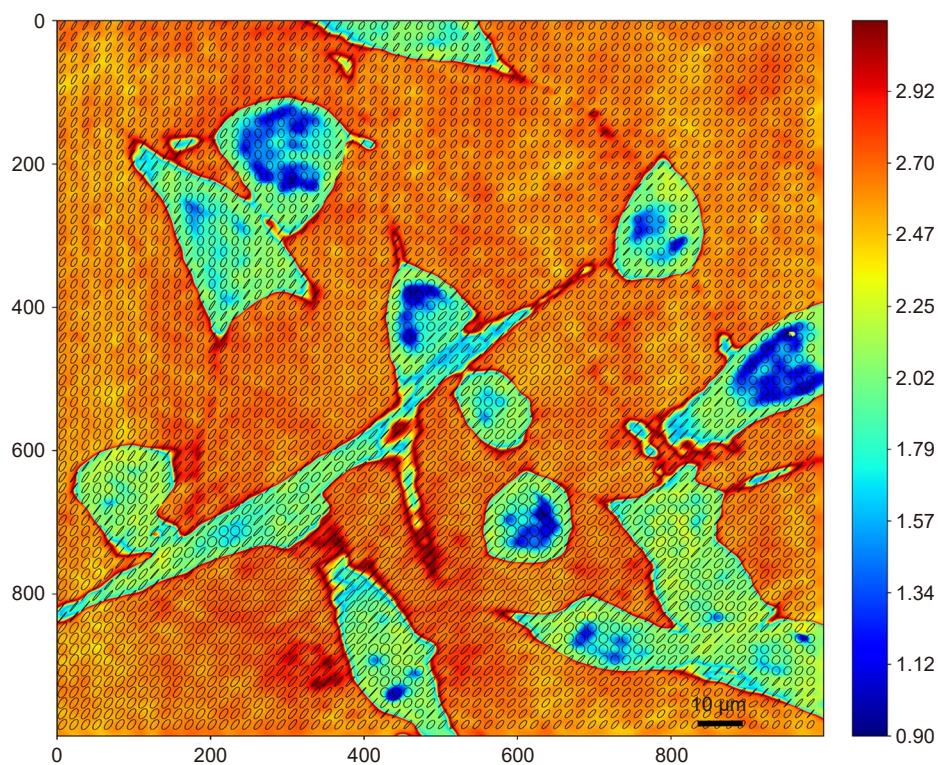


Fig. 6 | Superimposed polarization ellipses on the orientation angle map provide a detailed spatial representation of polarization state variations across neuron cells.

key polarization parameters, including inhomogeneity, diattenuation, retardance, polarization ellipse, and orientation angle. This microscopic technique proves valuable in uncovering the anisotropic properties of biological specimens, presenting immediate applications in quantitative imaging and cell characterization. The accuracy and reliability of our approach were validated using known polarization elements, ensuring its robustness for diverse biomedical applications. Its efficacy was further demonstrated by analyzing human glioblastoma U-87 MG cells, revealing finely resolved complex spatial structures and highlighting its potential for detecting cellular anisotropy. With its ability to unravel the polarization response and light matter interactions, this study serves as a foundational step, which can be extended for multiple cell lines and tissues for non-invasive diagnostics and improved biomedical imaging.

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Author contributions

Shivam K. Chaubey: Conceptualization; Methodology; Investigation; Data curation; Formal analysis; Software; Visualization; Writing original draft; Mohit Rathor: Data curation; Formal analysis; Writing review & editing; Akash Debnath: Data curation; Formal analysis; Writing review & editing; Rupen Tamang: Sample preparation; Formal analysis; Writing review & editing; Biplob Koch: Supervision; Funding acquisition; Resources; Writing review & editing; Rakesh K. Singh: Supervision; Conceptualization; Funding acquisition; Project administration; Resources; Writing review & editing.

Competing interests

The authors declare no competing financial interests.



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