Simple multi-wavelength imaging of birefringence: case study of silk

Reo Honda¹, Meguya Ryu¹, Jing-Liang Li², Vygantas Mizeikis³, Saulius Juodkazis⁴,⁵, and Junko Morikawa¹,∗

¹Tokyo Institute of Technology, Meguro-ku, Tokyo 152-8550, Japan
²Institute for Frontier Materials, Deakin University, Geelong, Victoria 3220, Australia
³Research Institute of Electronics, Shizuoka University, Naka-ku, 3-5-3-1 Johoku, Hamamatsu, Shizuoka 4328561, Japan
⁴Swinburne University of Technology, John st., Hawthorn, 3122 Vic, Australia
⁵Melbourne Center for Nanofabrication, Australian National Fabrication Facility, Clayton 3168, Melbourne, Australia
⁶Correspondence: morikawa.j.aa@m.titech.ac.jp

ABSTRACT

A polarised light imaging microscopy with an addition of liquid crystal (LC) phase retarder was implemented to determine the birefringence of silk fibers with the high ∼ 2 µm spatial resolution. The measurement was carried out with silk fiber (the optical slow axis) and the slow axis of the LC retarder set parallel (a perpendicular alignment can also be used). The direct fit of the transmission data provides a high fidelity determination of birefringence, Δn ≈ 1.63 × 10⁻³ (with ∼ 2% uncertainty) of the brown silk fiber (Antheraea pernyi) averaged over the wavelength range λ = (425 – 625) nm. By measuring retardance at four wavelengths it was possible to determine the true value of the birefringence of a thick sample when an optical path may include large number of wavelengths (2π cycles in phase). The numerical procedures and required hardware are described for the do-it-yourself assembly of the imaging polariscope at a fractional budget compared with commercial units.

1 Introduction

Optical imaging of metasurfaces for definition of the engineered birefringence and its orientational pattern is gaining interest due to capability of direct evaluation of the fabrication quality and phase retardance in a fast growing field of flat optical elements¹. Determination of the slow-axis orientation and retardance can be made for an arbitrary sample using transmission polariscopy². This principle was commercially implemented as a side-port addition onto a microscope (Abrio). However, a wider use of this technique was hampered by a comparatively high price; moreover, production of the unit was discontinued. There is a need for the in situ monitoring of birefringence in complex micro-fluidic flows³ and cell division microscopy where optical detection of a cell division could be monitored in real time⁴ using a simple instrumentation. Measurements of birefringence are highly required in microscopy and material science fields, however, quite expensive dedicated microscopes or bulky add-on microscopy units have to be used and usually works at fixed wavelengths⁵. Birefringence can be inferred from Stokes polarimetry which is realised by different principles of phase delay or polarisation rotation, e.g., based on photoelasticity⁶ or using liquid crystal (LC) retarders⁶,⁷. Crossed polariser-analyser setup with a rotating quarter-waveplate compensator was used for determine a 3D orientation maps of birefringent fibre structures in a brain tissue⁸. When waveplates are utilised together with rotating elements and lock-in amplifiers, setups of high sensitivity and resolution can be realised, however, they become bulky, complex and, frequently, wavelength specific.

Simplification of the optical retardance measurement at a broader spectral range is still strongly required especially for the flat optical elements and bio-materials with high orientation anisotropy and domain structure. For example, birefringence of silk is usually measured by a shear interferometry⁹,¹¹, which does not provide a high resolution imaging capability. Emerging optical applications of transparent wood¹² needs better understanding of optical properties of the micro-tubular wood structure. Stress induced birefringence in crystals/glasses/polymers¹³⁻¹⁵, volume phase transitions¹⁶, and complex topological structures for volumetric stress control¹⁷, or patterning of absorbance in transparent materials¹⁸ all produce complex optical anisotropy which needs high resolution, ∼ 1, imaging.

A set of crossed polariser and analyser is used to reveal qualitatively the birefringence, Δn, of a sample placed between them using optical imaging at the selected wavelength, λ. The retardance Δnd is defined by sample’s thickness d and birefringence. For the quantitative determination of the birefringence and optical retardance, transmittance is measured (Fig. 1). The transmittance, T, through the birefringent medium of thickness, d, when reflectance and absorbance are negligible for the
crossed polariser and analyser is given by (Fig. 1(a)):

\[ T_\theta = I_\theta / I_0 = \sin^2 \frac{\theta - \theta_R}{2} \sin^2 \left( \pi \frac{\Delta n d}{\lambda} \right), \tag{1} \]

where \( I_{0,0} \) are the transmitted and incident intensities, respectively, \( \theta \) is the angle between the transmission axis of analyser and the horizontal x-direction of the view field and it is positive for the anti-clockwise rotation (looking into the beam), \( \theta_R \) is the slow (or fast) axis direction with the slow axis usually aligned to the main molecular chain or along the polymer stretch or a silk fiber direction as in this study. Equation 1 represents the Maltese cross pattern shown in Fig. 1(a).

By placing a sample on a LC cell retarder (Fig. 1(a)) which has an electrically controlled birefringence (Fig. 1(b)), it is possible to determine the birefringence of the sample by compensating it with an opposite sign at the chosen orientation of the LC-retarder. Transmission vanishes at the regions with zero birefringence (Eqn. 1). This is the principle used, e.g., in Berek compensator where, instead of a liquid crystal, a tilting of the birefringent quartz plate is used.

Here, we use a simple LC-cell as a birefringence compensator for determination of silk fiber birefringence. The sample is placed directly on the LC-cell window and aligned with slow-axis of the LC retarder. No any sample nor retarder rotation was required during measurements. By using a standard microscopy imaging at the freely chosen wavelength, the birefringence of a single strand silk fiber was determined with high fidelity and resolution (which can be comparable with the wavelength \( \lambda \) at tight focusing. This method is applicable to measure birefringence of any transparent materials over the visible 400-800 nm spectral range determined by transparency of LC cell. Data acquisition and analysis were fully automated using Labview and Matlab codes. Due to virtue of multi-wavelength measurement capability, the proposed method allows to determine birefringence even when the retardance has \( 2\pi \) phase changes.

## 2 Method and samples

### 2.1 Polarisation change due to birefringence

The used setup is based on a linearly polarised light illumination of the sample (Fig. 1(a)). Correspondingly, a simpler Jones matrix calculus (as compared to a more general Mueller calculus) is applicable to calculate the evolution of E-field of light as outlined next. The x-polarised (horizontally) incident light is defined by the E-field Jones vector (Fig. 1):

\[ E_H = \begin{pmatrix} 1 \\ 0 \end{pmatrix}. \]
Figure 2. Optical micro-images of white silk (Bombyx mori) through the crossed polarizer-analyser under the white light illumination. Liquid crystal (LC) retarder voltage is marked. Slow-axis of the LC retarder was at $\theta = 45^\circ$ and silk fiber was perpendicular to the slow-axis of the LC-retarder.

The analyser is crossed and transmits only $y$-polarised (vertically) light. The corresponding Jones matrix is given by:

$$ J_V = \begin{pmatrix} 0 & 0 \\ 0 & 1 \end{pmatrix}. $$

A generic Jones matrix of the retarder with the phase delay, $\phi = k\Delta n d$, wavevector $k = 2\pi / \lambda$, and with slow-axis at angle, $\theta$, with respect to the x-axis is given:

$$ J_R(\phi, \theta) = \begin{pmatrix} \cos \frac{\phi}{2} + i \sin \frac{\phi}{2} \cos(2\theta) & i \sin \frac{\phi}{2} \sin(2\theta) \\ i \sin \frac{\phi}{2} \sin(2\theta) & \cos \frac{\phi}{2} - i \sin \frac{\phi}{2} \cos(2\theta) \end{pmatrix}. $$

In experiments, the LC-retarder was inserted with silk fiber (the sample) oriented in parallel to the slow-axis of the LC-retarder (Fig. 1). The Eqn. 4 is used for the LC-retarder, $J_{LC}(\phi, \theta)$. Figure 1 shows setup and calibration curves of the retardance vs. voltage. The silk fiber (sample) contributes to the phase retardance as $J_s(\phi_s, \theta_s)$, where $\theta_s$ is calculated from x-axis ($\theta_s = 0$).

The overall transmission through the setup (Fig. 1(a)) is then:

$$ E_t = (J_V J_{LC}) E_i = \begin{pmatrix} 0 & 0 \\ A & B \end{pmatrix} E_i = \begin{pmatrix} 0 \\ A \end{pmatrix}, $$

where $A = a_1 + ia_2$ and $B = b_1 + ib_2$ are given in the Supplement; such simplification of the cumulative matrix is due to the $J_V$. Intensity of the transmission image detected on CCD (Fig. 1(a)) is then $I = AA^*$, where $A^*$ is the complex conjugate. Further simplification of the trigonometric expressions $A, B$ takes place at $\theta_s = \pi/4$ and allows a simple calculation of the intensity at each $[x, y]$ pixel $I(x, y)$. By matching $I(x, y)$ with the experimentally measured retardance $\text{Ret}_{exp} \equiv \arcsin \sqrt{T}$ (Eqn. 1) allows to access the retardance $\Delta n \times d$. When the length of birefringent region $d$ is known (measured independently), the birefringence $\Delta n$ at each pixel can be calculated.

2.2 Samples and measurements

For imaging, a Nikon Optophot-POL microscope with an Olympus LMP PlanFLN objective lens with 20× magnification and numerical aperture $NA = 0.4$ was used. The CCD images with VGA resolution 480 × 640 pixels where captured (CCD camera BU030C Toshiba teli) for processing at $N = 718$ number of points (voltage values of the LC-retarder cell). The LC-retarder (LCC1223T-A, Thorlabs) was used with the TC200 controller and temperature stabilizer LCC25; the latter was used for the quantitative determination of the retardance. Factory calibration of retardance vs. applied voltage at selected wavelengths was provided by vendor (Fig. 1(a)), however, we applied a different calibration procedure suitable for any wavelength selected by
Figure 3. (a) Optical image of a brown silk (Antheraea pernyi) fiber at the liquid crystal cell retarder voltage of 15 V. The band pass filter at 635 nm wavelength was used for the white light condenser illumination. The birefringence of silk is determined by the difference of the extraordinary and ordinary refractive indices $\Delta n = n_e - n_o$. The dark region (out off the fiber sample) is where only the LC-retarder is between crossed polariser-analyser, marked as region of interest (ROI): "Air". (b) The intensity integrated over selected “Air” (see, (a)) vs. the voltage (rms) of the retarder. (c) Digitised retardance of the LC cell $\text{Ret}_{\text{exp}} \sim \arcsin \sqrt{T_T}$, where $T_T = I_T/I_0$ (Eqn. 1). Temperature of the LC-retarder was set 24.7-24.9°C, exposure time 0.85 s.

Two types of silk white (Bombyx mori) and brown (Antheraea pernyi) were used in this study. Both were degummed, i.e., a sericin cladding desolved as described earlier $^{20}$ and single strands were used for imaging. Both silk types have similar composition and structure, hence, birefringence $^{20}$. Brown silk strands have on average a slightly larger diameter. Image acquisition was carried out at room conditions and took several minutes for $N = 718$ frames.

Figure 4. Experimental measurement of the transmission. (a) Integrated transmission intensity vs. retardance $\text{Ret}_{\text{exp}} \sim \arcsin \sqrt{T_T}$ for the marked region (see the inset) without silk fiber (marked square: “Air”). Inset shows the image of the brown silk (Antheraea pernyi) fiber at 15 V with highlighted regions of the birefringence measurement $2 \times 2$ pixels ($0.66 \times 0.66 \mu m^2$); note, the resolution of the used objective lens was $\sim 2 \mu m$. (b) Integrated transmission through the silk fiber (dots) and the best fit (line). The fit function $f(x) = a \sin^2(\pi[x + b]) + o$, where the best fit was achieved for selected amplitude, phase delay, and offset $a, b = \Delta nd$, and $o$, respectively, and $\Delta n = 2.17 \times 10^{-2}$ when $d = 30 \mu m$. 

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Figure 5. Retardance $\Delta n d / \lambda$ map at four different wavelengths selected by interference filters (635, 575, 525, 425 nm) measured through the brown silk (Antheraea pernyi) single strand. Since silk is naturally made from two strands, after degumming an asymmetry of the strand is revealed (cross section has a trapezoidal or triangular shape). The average $2 \times 2$ pixels was used for numerical processing of the original VGA 480 $\times$ 640 pixels CCD images. The optical resolution can be estimated as the radius of Airy disk $w = 0.61 \lambda / NA = 0.95 \, \mu m$ for the used $NA = 0.4$ objective lens. The silk fiber was placed on the LC-retarder which had the slow axis orientation parallel to the fiber. The ROI region was used to determine birefringence.

3 Results and discussion

First, we show a qualitative method of retardance imaging using silk fibers. Then, the quantitative method is demonstrated using a simple LC retarder cell without waveplates (Sec. 2.1).

3.1 Qualitative imaging of retardance

Phase retardance is used in polarisation microscopy to create color contrast under a white light (condenser) illumination. It is useful for the qualitative distinction of regions with different birefringence (phase thickness) in the image. Figure 2 shows images of a single white silk *Bombyx mori* fiber after degumming at different LC-retarder voltages. The slow-axis of the LC-retarder was perpendicular to the silk fiber in order to compensate the birefringence by decreasing retardance at larger voltages (Fig. 1(a)). At the perfect compensation of birefringence, $\Delta n$, the dark region is formed in the image as in the Maltese cross (Fig. 1(a)). The most dark region is changing its location in the image of the fiber recognisable at large voltages (Fig. 2). The fiber strands are, in fact, with a triangular or trapezoidal cross section which is causing a non uniform color appearance across the fiber. However, only a qualitative estimate of the birefringence can be made using this method, even when imaging is carried at one wavelength or at a spectrally narrow bandwidth.

3.2 Quantitative imaging of retardance

Using CCD imaging at different LC-retarder voltages (number of points $N = 718$) at different $\phi_{LC}$ values and by applying formulas Eqns. 2-5, it is possible to determine the birefringence with high fidelity as described next. We used a $\sim$10-nm-bandwidth filter to select a narrow spectral window from the white light condenser illumination. Silk fiber was set at 45 deg angle between the polarizer and analyzer (Fig. 3). Also, the fiber was parallel to the slow-axis of the LC-retarder ($\theta - \theta_R = \pi / 4$). A region of interest (ROI) “Air” was selected outside the silk fiber (Fig. 3(a)) where only a reference retardance of the LC-cell was in the optical path. The $N = 718$ number of measurement points of transmittance was selected in equidistant steps of retardance over the entire range of LC-retarder voltages as shown in (b). To establish the relation between the average intensity on CCD (b), which is proportional to the measured transmittance $T_{exp} = I_{Air} / I_0$, and to calculate the retardance using Eqn. 1, the intensity, $I_{Air}$, at the “Air” ROI (out of sample) was measured. The incident light intensity, $I_0$, was controlled by electrical current not to cause saturation over the entire $2\pi$ LC-retarder cycle. The minimum intensity corresponded to the 0-phase (or $2\pi$) while the maximum to $\pi$ (Fig. 4(a)). Since $\sin^2(2(\theta - \theta_R) = 1$ by the selection of LC-retarder orientation $\theta_R = \pm \pi / 4$, the reference retardance of the LC-cell is found from $T_{exp} = \sin^2(\pi \Delta n_{LC} d_{LC} / \lambda)$ (Eqn. 1, where $n_{LC}, d_{LC}$ are the birefringence and thickness of the liquid crystal cell, respectively (Fig. 3(c)).

The measured intensity averaged over ROI $2 \times 2$ pixels (see the inset in Fig. 4 from regions on the LC-retarder and on silk fiber are plotted in Fig. 4(a) and (b), respectively. Smaller ROIs were necessary due to a non uniform thickness of the}
Figure 6. Retardance $\Delta n d/\lambda$ vs $1/\lambda$ at four wavelengths (Fig. 5) 635, 575, 525, 425 nm presented by corresponding color markers. Each point is average over ROI (see, Fig. 5). Thickness of the brown silk (Antheraea pernyi) fiber was $d \approx 30 \mu m$, which defines the birefringence $\Delta n \approx (1.63 \pm 0.05) \times 10^{-2}$. The linear fit equation $y = p_1 x + p_2$, coefficient and 95% confidence interval are $p_1 = 487.5$ (from 450.6 to 524.5), $p_2 = -0.3929$ (from -0.4639 to -0.3219), $R^2 = 0.9994$.

Figure 7. Wavelength-averaged retardance ($\Delta n d$ [nm]) map (averaged over the four wavelengths; Fig. 5). Thickness of the brown silk (Antheraea pernyi) fiber was $d \approx 30 \mu m$, which defines average $\Delta n = (1.63 \pm 0.05) \times 10^{-2}$ at the center of the silk fiber.
axis of fiber affect the offset of the sinusoidal curve in Fig. 4(b). The variation of \( d \) is the variation of retardance, therefore it changes the horizontal shift of the curve. Since the orientation of the slow axis is the first sinusoidal part of the Eqn. 1, it affects only amplitude of the curve in Fig. 4(b). The only explanation of the offset is the depolarization of the light due to the scattering at the surface of the fiber sample.

Retardance averaged over 2 \( \times \) 2 pixels was determined for the entire image using the fitting shown in Fig. 4(b). It is presented in Fig. 5 for the four different wavelengths selected by interference filters with 10 nm bandwidth. The 30-\( \mu \)m-thick silk fiber is effectively a half-waveplate at 525 nm wavelength. When thickness of the birefringent object is exceeding one wavelength (\( 2\pi \) in phase), there is an ambiguity in calculation of birefringence. To obtain the exact \( \Delta n \) value, retardance was measured at four wavelengths and fitted by \((\Delta nd/\lambda \pm m)\) where \( m = 0, 1, 2, \ldots \) as shown in Fig. 6. A good linear fit was obtained for the retardance averaged over ROI (Fig. 5) for \( m = 0 \) plotted in Fig. 6. For the central part of the fiber the birefringence \( \Delta n \approx (1.63 \pm 0.05) \times 10^{-2} \) was determined.

To obtain the map of the averaged retardance \( \Delta nd \) [nm] over the spectral range from 425 nm to 625 nm, the same procedure as for the Fig. 6 was carried out for each 2 \( \times \) 2 pixels of the image at four wavelengths. From the slope of the linear fit, the \( \Delta n \) was calculated (as in Fig. 6) and is plotted in Fig. 7. Edges of the silk fiber scattered light stronger which resulted in a higher detected light intensity \( T_{\theta} \) (Eqn. 1) and correspondingly up to twice larger effective retardance. Noteworthy, the thickness of silk fiber is smaller at the edges.

4 Conclusions and outlook

In summary, a simple LC-retarder addition to polarisation microscopy provides a highly sensitive method to image birefringence as demonstrated for silk fibers. The proposed method relies on a large data set (sampling) of images obtained at different LC-retarder voltages (phase delays) used for the best fit (a minimum of four images with \( \pi/4 \) LC-phase delays are required for the sin-fit). It is shown that birefringence of silk \( \Delta n \approx 1.6 \times 10^{-2} \) was determined with an uncertainty of \( \sim \pm 2\% \) from the area of just 2 \( \times \) 2 pixels at the center of 30 \( \mu \)m-thick brown silk fiber with a high fidelity. Smaller number of images or integration over larger ROI areas can be flexibly applied to achieve a better spatial resolution or an average birefringence, respectively. Measurements at several wavelengths were made to establish the absolute phase retardance. This is one of the strong features of the proposed method since most of commercial microscopy-based realisations of birefringence measurements are usually carried out at one wavelength. The multi-wavelength measurement allows to extend retardance range beyond the \( 2\pi \) in phase.

The proposed method is much simpler and requires a fractional budget of \( \sim S2k \) as compared with the established birefringence measurement microscopes. When the slow axis of the sample is unknown or it is changing orientation over the image area, the axial alignment can be made by an additional measurement at four points (the minimum number required for the fit) of the angular orientation of the sample and to implement calculations for the corresponding \( A, B \) values Eqns. 1 5. This functionality can be easily added. Also, an absorption anisotropy (diattenuation) can be measured using transmission with adequately high resolution \( \sim \lambda \) using this simple technique for analysis of molecular alignment\(^21\).

The proposed technique could also find application in bio-medical field for cell monitoring and optical detection of cell division exploiting a new dimension - the birefringence - in addition of the usual set of the big-data dimensions of the lateral \( xy \)-position of the cell, time, intensity and shape of the object. The enhanced light scattering at the edges would be beneficial for determination of the outline of the dividing cells.

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Additional Information

The authors declare no competing interests. J.M. and S.J. come up with the idea of experiments, R.H. developed numerical analysis, wrote the program for image acquisition, and carried out experiments together with M.R.; J.L.L. and V.M. made birefringent samples for testing. All the authors participated in discussion and analysis of the results and contributed to editing of the manuscript.

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5 Supplement

Analytical expressions for the coefficients $A = a_1 + ia_2$ and $B = b_1 + ib_2$ of Eqn. 5 are following:

\[
\begin{align*}
    a_1 &= \sin \frac{\phi}{2} \sin \frac{\phi_c}{2} (\cos(2\theta_s) \sin(2\theta_{LC}) - \sin(2\theta_s) \cos(2\theta_{LC})) \\
    a_2 &= \sin \frac{\phi}{2} \cos \frac{\phi_c}{2} \sin(2\theta_s) + \cos \frac{\phi}{2} \sin \frac{\phi_c}{2} \sin(2\theta_{LC}) \\
    b_1 &= -\sin \frac{\phi}{2} \sin \frac{\phi_c}{2} \sin(2\theta_s) \sin(2\theta_{LC}) + \cos \frac{\phi}{2} \cos \frac{\phi_c}{2} - \sin \frac{\phi}{2} \sin \frac{\phi_c}{2} \cos(2\theta_s) \cos(2\theta_{LC}) \\
    b_2 &= -\cos \frac{\phi}{2} \sin \frac{\phi_c}{2} \cos(2\theta_{LC}) - \sin \frac{\phi}{2} \cos \frac{\phi_c}{2} \cos(2\theta_s).
\end{align*}
\]