Electronic Supplementary Information (ESI)

A high-accuracy universal polarimeter study of optical anisotropy and optical activity in laminated collagen membranes

Kenta Nakagawa,[a] Heather Harper-Lovelady,[b] Yuji Tanaka,[c] Masahito Tanaka,[d] Masayuki Yamato,[e] and Toru Asahi[a, f]

[a] Graduate School of Advanced Science and Engineering (TWIns), Waseda University, Tokyo, Japan.
[b] Department of Physics, University of South Florida, Florida, USA.
[c] School of Medicine, Tohoku University, Miyagi, Japan.
[d] National Institute of Advanced Industrial Science and Technology (AIST), Ibaraki, Japan.
[e] Institute of Advanced BioMedical Engineering and Science (TWIns), Tokyo Women's Medical University, Tokyo, Japan.
[f] Consolidated Research Institute for Advanced Science and Medical Care (ASMeW), Waseda University, Tokyo, Japan.

1. The generalized HAUP measurement

We briefly explain the principle of the generalized high-accuracy universal polarimeter (HAUP) methods. The optical systems used in the original HAUP[1] and extended HAUP[2-3] methods are the same. The HAUP method employs a simple optical configuration that contains only two optical elements: a polarizer (*P*) and an analyzer (*A*). The axes of *P* and *A* are set in the crossed-Nicols configuration, and a light ray travels through *P*, the sample (*S*), and *A*, successively.

Due to this simple optical configuration, systematic errors, except those relating to the *P* and *A,* are excluded. In the HAUP method, systematic errors originating from parasitic ellipticities of *P* and *A* (*p* and *q*, respectively) and a small error angle (δY, attributed to the displacement of the crossed-Nicols configuration) are evaluated and eliminated.[4] Here, we define θ as an azimuth angle of *P* from an arbitrary origin; *Y* as the azimuth angle of *A* from the crossed-Nicols position of the arbitrary origin of *P*; θ_0 as an extinction position angle (*i.e.,* $\left(\frac{\partial(I/I_0)}{\partial\theta}\right)_{\Upsilon'=0}=0$) of *P* from the arbitrary origin; θ' as the azimuth angle of *P* from θ_0; and Y' as the azimuth angle of *A* from δY. That is, $\theta = \theta_0 + \theta'$ and $Y = \delta Y + Y'$. The values of θ' and Y' can be measured accurately in the practical HAUP experiment.

In the extended HAUP method, the ratio, Γ, of the intensity of transmitted light and intensity of incident light, I and I_0, respectively, is represented as follows:

$$\Gamma(\theta',\Upsilon') = I/I_0 = A''(\theta') + B''(\theta')\Upsilon' + C''\Upsilon'^2 \quad \text{(S.1)}$$

$$A''(\theta') = H''_{11} + H''_{12}\theta' + H''_{13}\theta'^2 \quad \text{(S.2)}$$

$$B''(\theta') = H''_{21} + H''_{22}\theta' \quad \text{(S.3)}$$

$$C'' = H''_{31} \quad \text{(S.4)}$$

where

$$H''_{11} \rightarrow \text{a term independent of } \theta' \text{ and } Y' \quad \text{(S.5)}$$

$$H''_{12} = 0 \quad \text{(S.6)}$$

$$H''_{13} = e^{E} + e^{-E} - 2\cos\Delta \quad \text{(S.7)}$$

$$H''_{21} = -b'_1 p + b'_2 q + a_1\delta\Upsilon - 2c_2(\sin\Delta)k \quad \text{(S.8)}$$

$$H''_{22} = 2\left(e^{E} - \cos\Delta\right) \quad \text{(S.9)}$$

$$H''_{31} = e^{E} \tag{S.10}$$

where

$$a_1 = \frac{2\sin^2\Delta}{e^{E}+e^{-E}-2\cos\Delta} \tag{S.11}$$

$$b'_1 = \frac{2\left(\cos\Delta - e^{-E}\right)\sin\Delta}{e^{E}+e^{-E}-2\cos\Delta} \tag{S.12}$$

$$b'_2 = \frac{2\left(\cos\Delta - e^{E}\right)\sin\Delta}{e^{E}+e^{-E}-2\cos\Delta} \tag{S.13}$$

$$c_2 = \frac{1}{K^2+1} = \frac{1}{\left(E/\Delta\right)^2+1} \tag{S.14}$$

The extinction position angle, θ_0, is represented as follow:

$$\theta_0 = -a_2\left(p+q\right) - b_2\delta\Upsilon + c_1k + c_2k' + N \tag{S.15}$$

where

$$a_2 = \frac{\sin\Delta}{e^{E}+e^{-E}-2\cos\Delta} \tag{S.16}$$

$$b_2 = \frac{e^{E}-\cos\Delta}{e^{E}+e^{-E}-2\cos\Delta} \tag{S.17}$$

$$c_1 = \frac{K}{K^2+1} = \frac{E/\Delta}{\left(E/\Delta\right)^2+1} \tag{S.18}$$

Here, Δ and E represent the retardation and the total LD of the sample, respectively. Using these quantities, linear birefringence (LB), linear dichroism (LD), optical rotatory power (ORP), and circular dichroism (CD) are expressed as follows:

$$LB = \Delta n^{lin} = n_s - n_f \equiv \frac{\Delta\lambda}{2\pi d} \tag{S.19}$$

$$LD = \Delta m^{lin} = m_s - m_f \equiv \frac{E\lambda}{2\pi d} \tag{S.20}$$

$$ORP = \frac{\varphi}{d} = \frac{\pi\left(n_L - n_R\right)}{\lambda} = \frac{\pi\cdot\Delta n^{cir}}{\lambda} = \frac{\pi\cdot CB}{\lambda} \equiv \frac{2\pi LB\cdot k}{\lambda}\ [\mathrm{radm^{-1}}] \tag{S.21}$$

$$CD = \Delta m^{cir} = m_L - m_R \equiv \frac{\Delta k'\lambda}{\pi d} \tag{S.22}$$

Here, n and m represent the refractive index and absorption coefficient, respectively; s and f represent the slow and fast light rays, respectively; L and R represent the left and right circularly polarized light, respectively; and φ corresponds to the rotational angle of the linearly polarized light.

In the extended HAUP method, the values of LB, LD, ORP, and CD are determined using the following procedure. First, the intensities of I are measured as double functions of θ' and Y'. Next, the values of $I_0H''_{ij}$ (i,j = 1, 2, 3) at each θ' position and the extinction position angle θ_0 are determined by least-squares fittings using equations

(S.1)–(S.4). Then, the values of Δ, E, and I_0 are calculated from the θ' dependences of H''_{13}, H''_{22}, and H''_{31}, and equations (S.7) and (S.9)–(S.10). LB and LD are obtained from equations (S.19)–(S.20) using the sample thickness, d. Then, the systematic error parameters q and δY are evaluated to extract ORP and CD from the values of H''_{21} and θ_0 by least-squares fittings using equations (S.8) and (S.15). Since the value of p is independent of the sample settings, the value of p is predetermined by a measurement with an achiral crystal, such as MgF_2 or $LiNbO_3$. In the case of the collagen membranes, we made the following approximations: (i) k is dependent on λ (*i.e.*, $k = s/\lambda + t$) because the ORP dispersion (ORD) spectra of many proteins conform Drude's equation that is inversely proportional to λ^2 by substituting this equation into (S.21) and (ii) k' is nearly zero (*i.e.*, $k' = 0$) in the measurement wavelength region because there is little absorption in the wavelength region used in this study and because the collagen membranes' CD signals are assumed to be almost zero due to the difference of light scattering between left and right circularly polarized light. Finally, the values of k are calculated by eliminating the systematic error parameters determined by equations (S.8) and (S.15), and ORP is obtained from equation (S.21).

As an example, experimental results from the analysis of sample A are shown in Fig. S1. The thickness of the sample is 8.66 μm. The systematic error parameters q and δY determined by least-squares fittings are as follows:

$$q = -1.5\times10^{-3}$$

$$\delta Y = 2.9\times10^{-4}$$

The values of q and δY obtained by least-squares fittings for any other experimental data in this study varied between 10^{-4}–10^{-3} and 10^{-5}–10^{-3}, respectively. In comparison with previous studies, these values are acceptable. Comparisons between the experimental and calculated results obtained by substituting the systematic error parameters into θ_0 and H''_{21}, respectively are shown in Fig. S2. The coefficients of determination of H''_{21} and θ_0 are 0.996 (>0.7) and 0.934 (>0.7), respectively. Thus, the accuracy of our experiments and methodology of evaluating systematic error parameters are guaranteed.

2. Literature values of LB

For comparison, literature values of LB in various fibers and body tissues composed of collagen are listed in Table S1.[6-9]

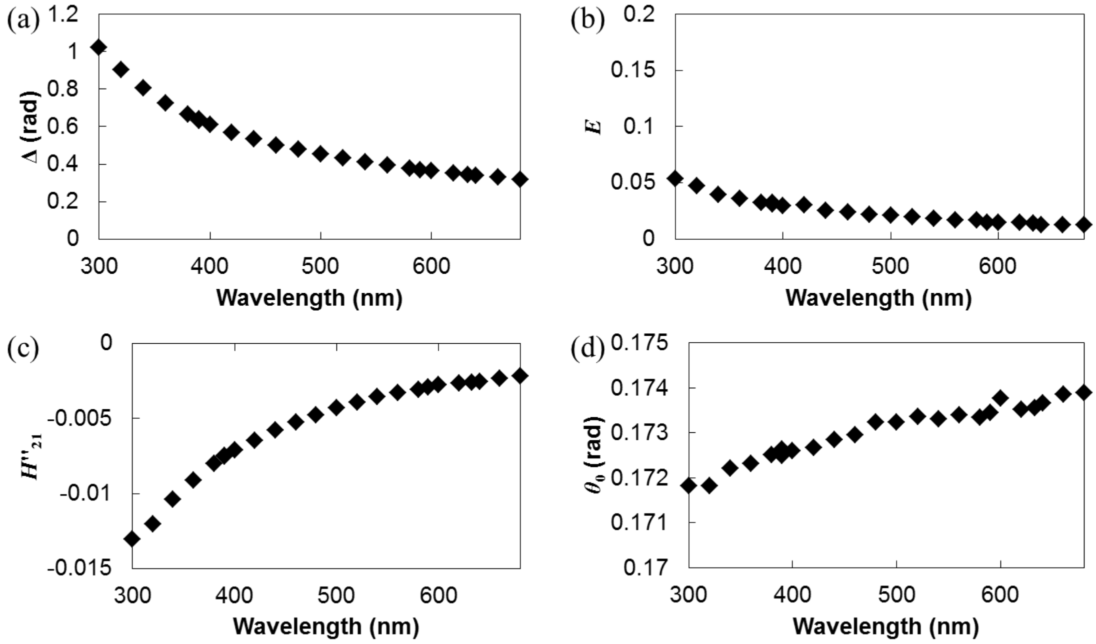

Fig. S1 Wavelength dependences of Δ (a), E (b), H''_{21} (c), and θ_0 (d) of one of the experimental results of the sample A.

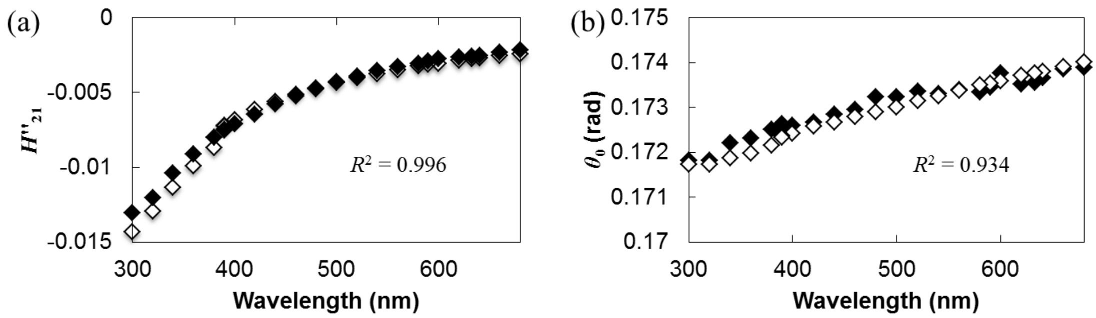

Fig. S2 Comparisons between the experimental and the calculated results of H''_{21} (a) and θ_0 (b).

Solid and open circles are the experimental and calculated results, respectively.

Table S1. LB in various fibers and body tissues composed of collagen.[6-9]

	LB
Nylon66[6]	6.0×10^{-2}
Polyvinyl chloride[6]	1.02×10^{-2}
(Sheep) wool[6]	$1.0–1.1\times10^{-2}$
Rat-tail tendon[7]	$3.0\pm0.6\times10^{-3}$
Cat cornea[8]	3.0×10^{-3}
Human cornea[9]	1.59×10^{-3}

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