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Probing intrinsic anisotropies of fluorescence: Mueller matrix approach

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Probing intrinsic anisotropies of fluorescence: Mueller matrix approach

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Abstract. We demonstrate that information on “intrinsic” anisotropies of fluorescence originating from preferential orientation/organization of fluorophore molecules can be probed using a Mueller matrix of fluorescence. For this purpose, we have developed a simplified model to decouple and separately quantify the depolarization property and the intrinsic anisotropy properties of fluorescence from the experimentally measured fluorescence Mueller matrix. Unlike the traditionally defined fluorescence anisotropy parameter, the Mueller matrix-derived fluorescence polarization metrics, namely, fluorescence diattenuation and polarizance parameters, exclusively deal with the intrinsic anisotropies of fluorescence. The utility of these newly derived fluorescence polarimetry parameters is demonstrated on model systems exhibiting multiple polarimetry effects, and an interesting example is illustrated on biomedically important fluorophores, collagen. © 2015 Society of Photo-Optical Instrumentation Engineers (SPIE) [DOI: 10.1117/1.JBO.20.8.085005]

Keywords: polarization; fluorescence anisotropy; Mueller matrix; polarimetry.

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1 Introduction

Fluorescence spectroscopy is a well-established and widely explored spectroscopic method. The parameters of fluorescence that have been investigated include static spectra, decay kinetics, and polarization. Polarization of fluorescence is traditionally studied using the so-called fluorescence anisotropy parameter (A), which is the ratio of the polarized component of fluorescence to the total fluorescence intensity.¹ Experimentally, the polarization anisotropy of fluorescence is determined using linear polarization measurements alone and is given by

$$A = \frac{I_{VV} - I_{VH}}{I_{VV} + 2I_{VH}}, \quad (1)$$

where I_{VV} and I_{VH} are the intensities measured with the detection polarizer (analyzer) oriented along the vertical and horizontal directions, respectively, while the incident light is vertically polarized.¹ Note that in polarization optics, the terminology “anisotropy” implies either anisotropic absorption (difference in the amplitude between orthogonal polarizations known as diattenuation or dichroism) or birefringence (difference in phase between orthogonal polarizations).^{2–4} Either of the anisotropy effects are related to the anisotropic polarizability of matter. Depolarization is the other prominent polarimetry effect, which arises due to randomization of the field vector.^{4,5} Traditionally defined fluorescence anisotropy parameter [Eq. (1)] encodes both (a) any possible depolarization effects arising from random orientation of fluorophore molecules and (b) any possible presence of “intrinsic” anisotropy (resulting from anisotropic orientation/organization of fluorophore molecules). The resulting anisotropy parameter should thus represent a “lumped” effect and it does not exclusively refer to the intrinsic anisotropy of the system.⁵ Yet quantification of the intrinsic anisotropy is

valuable, because this may provide useful information on the anisotropic organization/orientation of fluorophore molecules. A Mueller matrix (a 4×4 matrix, transfer function of an optical system in its interaction with polarized light) appears to be an ideal tool for the quantification of all the complex polarization effects associated with fluorescence emission.^{5–7} However, a Mueller matrix is conventionally defined for reflection/refraction and elastic scattering process. Recently, there have been preliminary theoretical⁶ and experimental attempts^{8,9} for defining and measuring the Mueller matrix for inelastic scattering processes such as fluorescence. Note that fluorescence is a strongly depolarizing process and cannot be modeled using Jones matrix formalism as it deals with nondepolarizing interactions only. On the other hand, the Stokes–Mueller formalism deals with both depolarizing and nondepolarizing interactions and hence is best suited for this purpose. In this paper, we demonstrate that information on intrinsic anisotropies originating from preferential orientation/organization of fluorophore molecules can be probed using the Mueller matrix of fluorescence. We build upon the existing approaches on fluorescence Mueller matrix modeling⁶ and develop an approach to separately extract and quantify the depolarization and intrinsic anisotropies of fluorescence via additional polarization metrics (derived from the fluorescence Mueller matrix). We illustrate the utility of these newly derived parameters by performing fluorescence Mueller matrix measurements on model systems exhibiting multiple polarimetry effects and provide an important example of such measurements on biologically relevant fluorophores, namely, collagen. The novel ability to decouple and extract/quantify the intrinsic anisotropy properties of fluorescence and depolarization may prove to be useful for biological tissue characterization/diagnosis and for characterizing a wide variety of other complex fluorescent systems.

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2 Theory

In order to define the Mueller matrix of fluorescence, we begin with the dipole approximation. Under this approximation, where the fluorophore molecules are treated as randomly oriented dipoles, the major contributing polarimetry effect is depolarization. The depolarization primarily originates from (a) random orientation of the fluorophore molecules, leading to the so-called photoselection process when excited with polarized light and (b) other extrinsic causes such as fast Brownian rotation, radiationless energy transfer, etc., leading to further changes in the relative orientation between the excitation and the emission dipoles.^{1,3,4} The corresponding polarization transfer matrix can be written as⁶

$$\mathbf{M}_d = \begin{bmatrix} a - b \sin^2 \phi & -b \sin^2 \phi & 0 & 0 \\ -b \sin^2 \phi & b(1 + \cos^2 \phi) & 0 & 0 \\ 0 & 0 & 2b \cos \phi & 0 \\ 0 & 0 & 0 & 2c \cos \phi \end{bmatrix}, \quad (2)$$

where ϕ is the scattering angle and the parameters a and b are defined as

$$a = \frac{1}{2}(1 + \langle \cos^2 \theta \rangle), \quad b = \frac{1}{4}(3\langle \cos^2 \theta \rangle - 1), \quad (3)$$

where θ is the angle between the emission dipole and azimuthal angle of the polarization of the excitation light. Note that the conventionally defined anisotropy parameter A from Eq. (1) can be obtained from the Mueller matrix of Eq. (2) with an appropriate combination of the linear polarization descriptor Stokes vector of light (when operated on the Mueller matrix). In fact, using this definition, the limiting values of A can also be obtained as $-0.2 \leq A \leq 0.4$.

As we stated in the beginning, the fluorescence process may also additionally exhibit intrinsic anisotropies resulting from the anisotropic orientation/organization of fluorophore molecules. Such an anisotropy effect occurring during both the excitation and the emission processes can be included via additional polarization transfer matrices. So the resultant transfer matrix in the presence of all the fluorescence polarimetry effects can be written as

$$\mathbf{R} = \mathbf{M}_1 \mathbf{M}_d \mathbf{M}_0, \quad (4)$$

where \mathbf{M}_0 accounts for the absorption (excitation) anisotropy of the ground molecular state, representing the differential excitation of fluorescence by orthogonal polarization states (both linear and circular). The matrix \mathbf{M}_1 on the other hand, accounts for the emission anisotropy of the excited molecular state, representing the differential emission of orthogonally polarized light. In principle, the matrices \mathbf{M}_0 and \mathbf{M}_1 should include effects associated with both amplitude and phase anisotropies (differences in amplitude and phase between orthogonal polarizations).¹⁰ Note that in Mueller matrix formalism, either of these effects is encoded in characteristic intensity elements of the Mueller matrix.¹¹ However, for fluorescence, the phase anisotropy effects may be ignored because of the considerably longer lifetime involved in the fluorescence emission as compared to the characteristic time period of a light wave. This eventually leads to complete randomization of the phase information.

In absence of the phase anisotropy effects, the matrices \mathbf{M}_0 and \mathbf{M}_1 can be written as

$$\mathbf{M}_i = \begin{bmatrix} 1 & m_i & n_i & p_i \\ m_i & r_i' & 0 & 0 \\ n_i & 0 & r_i'' & 0 \\ p_i & 0 & 0 & r_i''' \end{bmatrix} \quad i = 0, 1, \quad (5)$$

where the parameters (m_i , n_i , and p_i) encode amplitude anisotropies (differential amplitude between orthogonal polarization states). Specifically, m_0 and m_1 correspond to linear (XY -horizontal/vertical) diattenuation (differential excitation of fluorescence with orthogonal polarization states) and linear (XY -horizontal/vertical) polarizance (differential emission of fluorescence with orthogonal polarization states) respectively; n_0 and n_1 , corresponding to $+45$ deg / -45 deg (linear) diattenuation and $+45$ deg / -45 deg (linear) polarizance, respectively; and p_0 and p_1 related to the left/right circular diattenuation and left/right circular polarizance, respectively. According to this definition, the fluorescence diattenuation and polarizance parameters deal with anisotropies of the ground and the excited molecular states, respectively. In Eq. (5), the other set of parameters (r_i' , r_i'' , r_i''') is also inter-related to the (m_i , n_i , and p_i) parameters.¹¹ For practical purposes, we now use the small parameter approximation, i.e., m_i , n_i , $p_i \ll 1$, which holds for most real systems. Under this approximation, r_i' , r_i'' , and r_i''' will be taken to be unity in both the matrices \mathbf{M}_0 and \mathbf{M}_1 .

For our experimental configuration of exact backscattering fluorescence measurements, we will take the scattering angle $\phi = \pi$. Note that other excitation-detection geometries may also be modeled with an appropriate choice of ϕ . We now make the following important assumption involving the coefficients a , b , and c of Eq. (2): $2c \ll 2b \ll a$. This assumption $2b \ll a$ follows from the strong depolarization nature of fluorescence. The parameter c , on the other hand, is related to optical activity and is usually much smaller than a and b .⁶ With these approximations, the elements of the resultant Mueller matrix \mathbf{R} can be obtained following a few simple algebraic steps, the approximated forms of which are given as

$$\begin{aligned} R_{11} &\approx a, & R_{12} &\approx am_0, & R_{13} &\approx an_0, & R_{14} &\approx ap_0 \\ R_{21} &\approx am_1, & R_{31} &\approx an_1, & R_{41} &\approx ap_1. \end{aligned} \quad (6)$$

Here, we have provided approximate expressions for only those elements which are subsequently used to determine the amplitude anisotropy parameters of fluorescence (diattenuation and polarizance). The other elements, R_{23} , R_{24} , R_{32} , R_{34} , R_{42} and R_{43} , indirectly depend upon the (m_i , n_i and p_i) parameters and may also yield nonzero values (expressions not provided here). Nevertheless, the approximate expressions given in Eq. (6) allow one to directly link the elements of the recorded fluorescence Mueller matrix to the intrinsic anisotropy parameters (first row and first column of \mathbf{R} represent anisotropy during excitation and emission processes, respectively, of the ground and excited molecular states).

$$\begin{aligned} R_{12}/R_{11} = m_0 \quad \text{and} \quad R_{13}/R_{11} = n_0 \\ R_{21}/R_{11} = m_1 \quad \text{and} \quad R_{31}/R_{11} = n_1 \\ R_{14}/R_{11} = p_0 \quad \text{and} \quad R_{41}/R_{11} = p_1 \end{aligned}$$

Clearly, R_{12}/R_{11} and R_{13}/R_{11} directly yield the horizontal/vertical and $+45$ deg/ -45 deg, respectively, linear anisotropy of the excitation process (fluorescence linear diattenuation); the circular anisotropy of the excitation process (circular diattenuation) is given by R_{14}/R_{11} . Similarly, R_{21}/R_{11} and R_{31}/R_{11} yield the linear anisotropy of the emission process (linear polarizance) for horizontal/vertical and $+45$ deg/ -45 deg linear polarization states, respectively, while R_{41}/R_{11} gives the circular anisotropy of the emission process (circular polarizance).⁶

Based on this, we can now define the fluorescence diattenuation (α) and polarizance (β) parameters:

$$\begin{aligned} \alpha_t = \frac{\sqrt{R_{12}^2 + R_{13}^2 + R_{14}^2}}{R_{11}}, \quad \alpha_L = \frac{\sqrt{R_{12}^2 + R_{13}^2}}{R_{11}}, \\ \alpha_c = \frac{R_{14}}{R_{11}} \end{aligned} \quad (7)$$

as fluorescence total, linear, and circular diattenuations, respectively.

$$\begin{aligned} \beta_t = \frac{\sqrt{R_{21}^2 + R_{31}^2 + R_{41}^2}}{R_{11}}, \quad \beta_L = \frac{\sqrt{R_{21}^2 + R_{31}^2}}{R_{11}}, \\ \beta_c = \frac{R_{41}}{R_{11}} \end{aligned} \quad (8)$$

as fluorescence total, linear, and circular polarizance, respectively.

Finally, we define another parameter, intrinsic polarization, which is independent of the intrinsic anisotropy properties and exclusively deals with depolarization:

$$P_{\text{int}} = \frac{|R_{22}| + |R_{33}| + |R_{44}|}{3} = 1 - \Delta, \quad (9)$$

where Δ is the net depolarization coefficient. Note that the above definition of the intrinsic anisotropy and depolarization parameters of fluorescence is strictly valid under the approximation ($2c \ll 2b \ll a$).^{6,11} In the general case, determination of these parameters would also, however, involve the other elements of the recorded fluorescence Mueller matrix **R**.

3 Experimental Methods

The experimental setup (Fig. 1) to record full 4×4 fluorescence spectroscopic Mueller matrix has been reported earlier in detail.^{8,9} The excitation source is a 405 nm line of a diode laser (Pegasus, Shanghai, China). Four different polarization states are generated (at the excitation wavelength) and analyzed (at emission wavelengths) using the polarization state generator (PSG) unit and a polarization state analyzer (PSA) unit, respectively. Following excitation with polarized input light (generated by the PSG unit), the emitted fluorescence signal from the sample is collected and relayed to a CCD spectrometer (Shamrock imaging spectrograph, SR-303i-A, ANDOR technology) for spectrally resolved signal detection (450 to 800 nm). The PSG unit comprises a fixed linear polarizer (Thorlabs) oriented horizontally with respect to the laboratory reference frame, followed by rotatable achromatic quarter wave retarders (Thorlabs) mounted on a computer controlled rotational mount (PR < 1/M-27E, Thorlabs). The PSA unit consists of the same components but placed in reverse order with the analyzer in a crossed

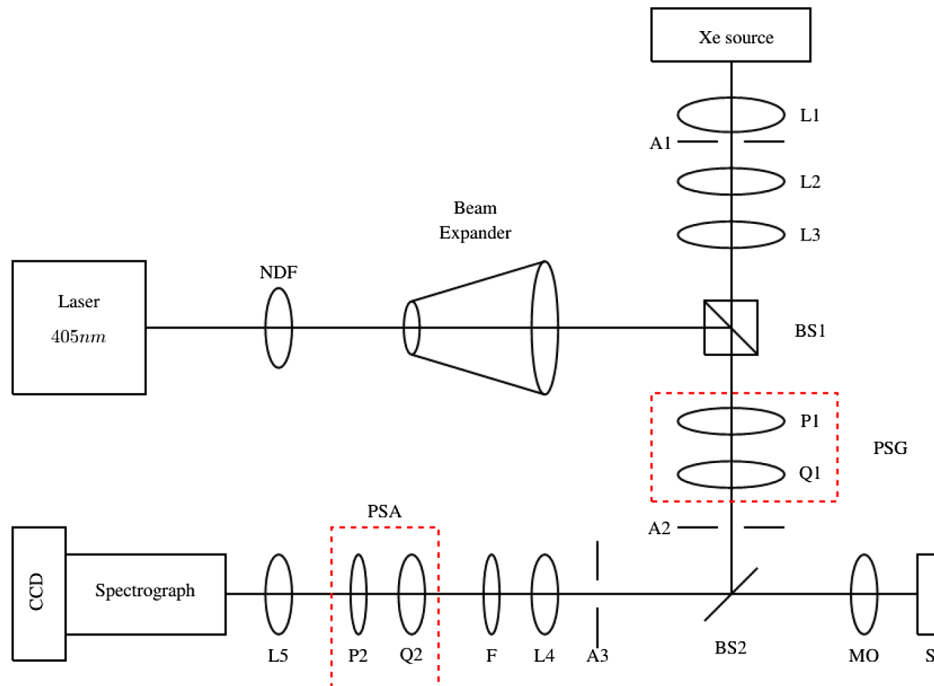


Fig. 1 Schematic of the experimental set-up for spectroscopic Mueller matrix measurement. Here, P1 and P2 are linear polarizers; Q1 and Q2 are rotatable achromatic quarter wave retarders; L1, L2, L3, L4, and L5 are lenses; A1, A2, and A3 are variable apertures; BS1 and BS2 are beam splitters; MO is microscope objective; NDF is neural density filter; P1 and Q1 form PSG unit; and P2 and Q2 form PSA unit.

state with the polarizer of the PSG unit. Sixteen elements of the fluorescence Mueller matrix are recorded by four optimized combinations (orientation angle with respect to the axis of the polarizer) of the quarter waveplates of the PSG and PSA units. The complex polarization responses of the source and the detector are taken care of in our set-up by fixing the polarizer (analyzer) throughout the Mueller matrix measurement. Further, the system was eigenvalue calibrated to yield the exact nature of the experimental PSG and PSA matrices (\mathbf{W} and \mathbf{A} , respectively), which also corrected the wavelength and polarization response of each optical element present in the set-up.^{8,12} The generator matrix $\mathbf{W}(\lambda)$ for a fixed excitation wavelength ($\lambda_{\text{ex}} \sim 405$ nm) and analyzer matrix $\mathbf{A}(\lambda)$ for varying emission wavelengths ($\lambda_{\text{em}} \sim 450 - 800$ nm) are used in combination with the sixteen recorded spectra to construct the spectroscopic fluorescence Mueller matrices.^{8,9}

4 Results and Discussion

The experiments were carried out on two types of samples exhibiting (a) primarily depolarization effect and (b) simultaneous depolarization + intrinsic anisotropy effects of fluorescence. The ability of the proposed approach to decouple and separately quantify the contributing fluorescence polarimetry effects were tested on these model systems. The first set of samples were Coumarine 152 dye (C-152) in two different solvents having different viscosities; they accordingly exhibited strong (and variable) depolarization effect and negligible “intrinsic” anisotropy effects. The second sample was a C-152 dye doped with poly-methyl methacrylate (PMMA) which exhibited significant intrinsic anisotropy (along with depolarization). Following successful demonstration of the fact that information related to anisotropic organization/orientation of fluorophores can be extracted using the Mueller matrix model, we illustrate an important example of a biologically relevant fluorophore, namely, collagen.

4.1 Model System I: C-152 in Solvents Having Different Viscosities (Exhibiting Primarily Depolarization Effect)

Full 16-element spectroscopic Mueller matrices were recorded for two solutions of Coumarine 152 (C-152), prepared in glycerol (viscosity $V_1 = 1.412$ Pa · s) and in ethylene glycol (viscosity $V_2 = 1.61 \times 10^{-2}$ Pa · s). Since the viscosities of the two solvents are different, a change in the depolarization in the two cases is expected. We have shown the Mueller matrix-derived intrinsic polarization parameter (P_{int} , which is a measure of depolarization) of C-152 for two different solvents in Fig. 2.

Since the viscosity of glycerol is greater than that of ethylene glycol, the rotational degree of freedom is less for C-152 molecules in glycerol, hence the depolarization is lower (manifested as a higher magnitude of P_{int}) in glycerol solutions compared to that in ethylene glycol solution.

In Fig. 3, the wavelength variation of fluorescence total diattenuation (α_t) and fluorescence total polarizance (β_t) for the above two samples is shown. The values of the fluorescence diattenuation and polarizance parameters are observed to be relatively small (~ 0.06 to 0.09). The small magnitudes of these parameters indicate that the emitting C-152 molecules (and accordingly the molecular dipoles) are randomly oriented in the solution phase, exhibiting no significant orientational/organizational anisotropy.¹³ The corresponding polarization

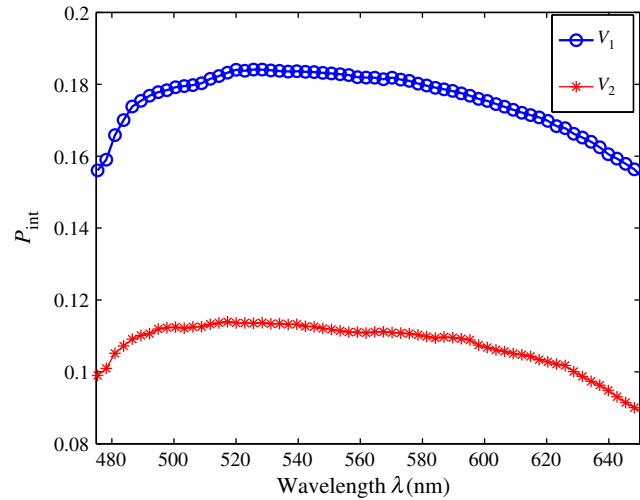


Fig. 2 The wavelength dependence of the fluorescence Mueller matrix derived intrinsic polarization parameter P_{int} [using Eq. (9)] for C-152 solution prepared in two solvents with different viscosities (V_1 for glycerol and V_2 for ethylene glycol). The wavelength of excitation was 405 nm for this and all subsequent figures.

transfer is primarily determined by the matrix \mathbf{M}_d of Eq. (4). Importantly, no prominent changes in the values of the two intrinsic anisotropy parameters are observed for the two different solvents. This indicates that the change in the solvent viscosity does not cause any appreciable changes in the intrinsic anisotropy.

The results presented above clearly demonstrate that the prominent polarimetry effect here is depolarization, which is subsequently determined by the intrinsic polarization parameter P_{int} . By contrast, in a conventional fluorescence polarization measurement, the nonzero magnitude of intrinsic polarization (P_{int}) would have been estimated as fluorescence anisotropy (A). Accordingly, the change in the rate of depolarization as a result of changing viscosity would have been misinterpreted

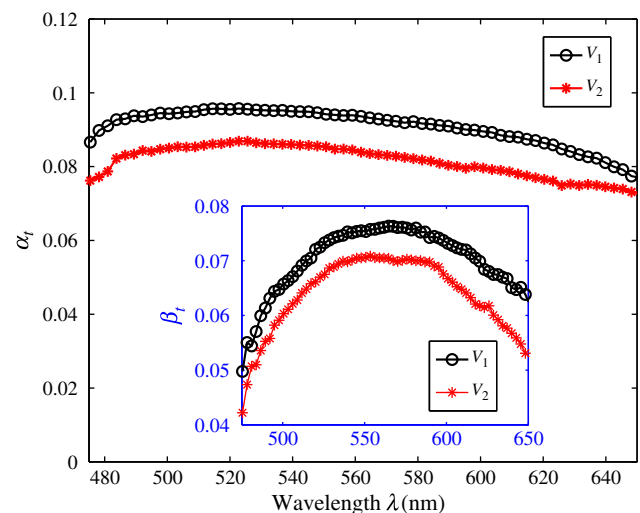


Fig. 3 Spectral dependence of the fluorescence total diattenuation parameter [α_t , derived using Eq. (7)] for C-152 solution prepared in two solvents with different viscosities (V_1 for glycerol and V_2 for ethylene glycol, where $V_1 > V_2$). The inset shows the fluorescence total polarizance parameter [β_t , derived using Eq. (8)] for the same samples.

as changing anisotropy of fluorescence, despite the fact that there is, in fact, no significant change in the true anisotropy of the fluorescent system.

4.2 Model System II: C-152 Doped with Polymethyl Methacrylate (Exhibiting Simultaneous Depolarization + Intrinsic Anisotropy Effects)

A thin film of 7-cm diameter was formed by using C-152 dye doped with PMMA. Acetone was used as a common solvent for both dye and PMMA. The film forming solution C-152/PMMA/acetone was poured into a Petri dish filled with mercury (Hg). The solution was completely dried with a controlled evaporation rate and a C-152 doped PMMA transparent film was formed at the top of the mercury. PMMA was used as a doping material because it is an optically highly transparent in the wavelength region of interest and its chemical and physical properties are well known.¹⁴ Here, mercury was used to improve the optical quality of the surface morphology. In Fig. 4, we have shown the recorded fluorescence spectroscopic Mueller matrix from this sample. As in the previous samples (whose Mueller matrices were not shown), the three diagonal elements of the Mueller matrix (R_{22} , R_{33} , and R_{44}) are of relatively smaller magnitudes as compared to the R_{11} element, implying the strong depolarizing nature of fluorescence.

Importantly, the considerable magnitude of the elements in first row of \mathbf{R} implies the presence of significant intrinsic amplitude anisotropies. Moreover, the excitation anisotropy appears to be higher (first row of \mathbf{R}) as compared to the emission anisotropy (first column of \mathbf{R}) (discussed subsequently). Figure 5 shows the spectral variation of the fluorescence linear and circular diattenuation parameters. The corresponding fluorescence linear and circular polarizance parameters are shown in

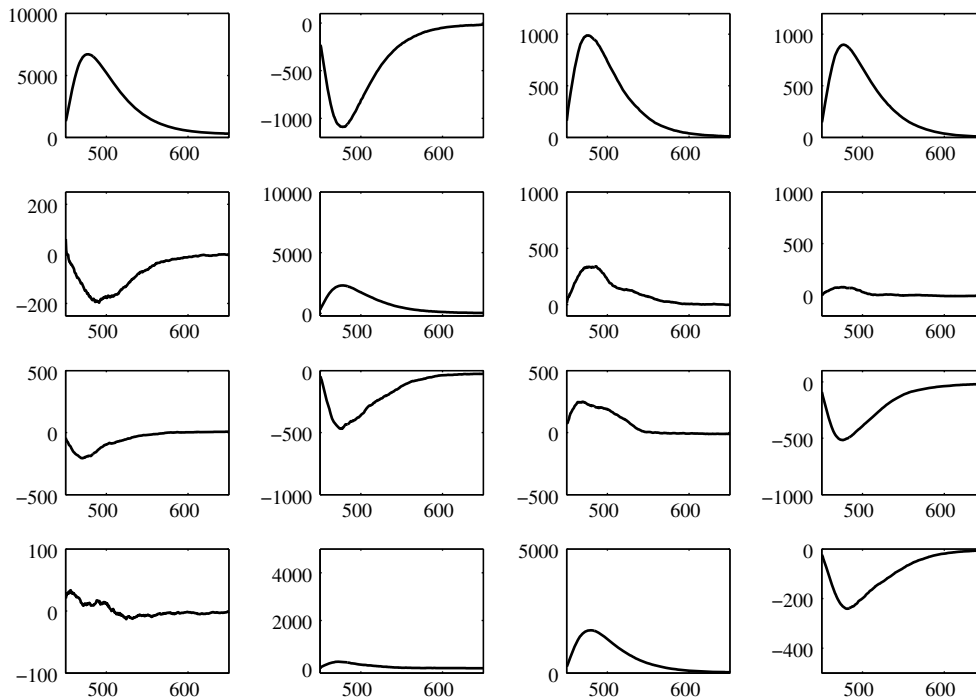


Fig. 4 The spectral variation of the different elements of the recorded fluorescence Mueller matrix (\mathbf{R}) of thin film of C-152 doped with polymethyl methacrylate (PMMA). The fluorescence spectroscopic Mueller matrix shown here is un-normalized (not normalized by the R_{11} element). Y-axes represent fluorescence intensity values in arbitrary unit.

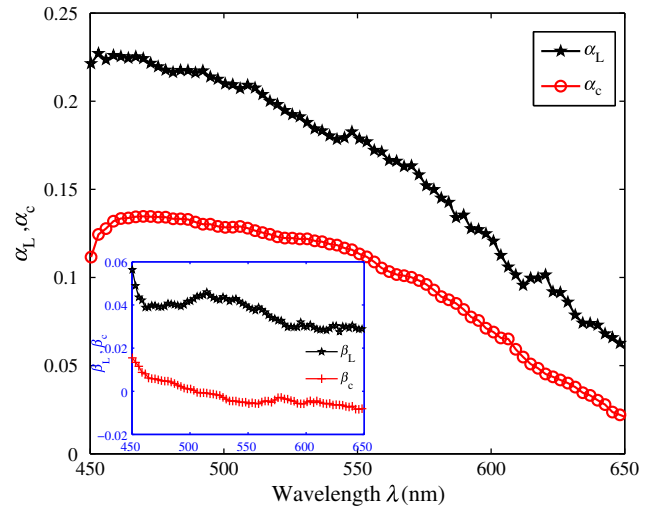


Fig. 5 Spectral variation of the fluorescence linear (α_L) and circular diattenuation (α_c) for C-152 doped PMMA (corresponding to Fig. 4). The inset shows the linear (β_L) and circular (β_c) polarizance parameters for the same sample.

the inset. As explained earlier, the fluorescence diattenuation parameters account for the anisotropy of the excitation process and imply the differential excitation of fluorescence using orthogonally polarized light (both linear and circular). The fluorescence polarizance parameters, on the other hand, are related to the anisotropy of the emission process (polarization selective differential emission).

The enhanced fluorescence linear diattenuation is attributed to the fact that the addition of PMMA polymer leads to

anisotropic molecular organization and ordering of molecular dipoles, resulting in enhanced linear polarization selective excitation as compared to the C-152 dye in solution phase, where molecular dipoles were randomly oriented (Fig. 3).¹⁴ The magnitude of the fluorescence polarizance parameters is weaker compared to the fluorescence diattenuation parameters, implying less prominent molecular dipolar ordering in the excited state as compared to the ground state. This may originate from the fact that a fraction of the C-152 molecules may exist in the twisted internal charge transfer state upon excitation due to the relatively low local microviscosity of the solid polymer host.¹⁵ Note that the same mechanism may also contribute to the observed stronger depolarization of fluorescence for the C152 molecules in thin solid films (where one would usually expect lower depolarization due to less degree of rotational freedom). These, however, require further investigations. Finally, note that both the circular diattenuation and polarizance parameters exhibit smaller values as compared to their linear counterparts, implying a weak (or negligible) chiral property of the sample (see Fig. 4). These results provide concrete evidence that intrinsic anisotropies originating from anisotropic organization of fluorophore molecules can indeed be probed and quantified via the fluorescence Mueller matrix-derived diattenuation and polarizance parameters.

4.3 Model System III: Bovine Collagen with Acetic Acid Treatment

Collagen is a major fluorophore in connective tissues, which is known to possess an anisotropically organized/oriented molecular structure arising from the crosslinks between individual collagen molecules.^{16,17} Collagen is the main component of the connective tissue and plays an important role in tissue regulation and infrastructure. Many types of tissue abnormalities (including precancerous/cancerous alterations) are associated with characteristic biochemical and morphological changes in collagen molecular structure and organization.^{18,19} Quantification of the intrinsic anisotropy parameters from the fluorescence Mueller matrix of collagen may provide a novel route for probing subtle morphological/biochemical alterations in connective tissue as a signature of preinvasion/invasion of diseases such as cancers.

In order to explore whether the organization/orientation of collagen molecules can be probed using the newly derived fluorescence polarimetry parameters, we performed fluorescence Mueller matrix measurements and analysis on Bovine collagen

samples (purchased from Sigma Aldrich). The collagen samples were treated with acetic acid, which is known to break molecular crosslinks of collagen structure.²⁰ Fluorescence Mueller matrices were recorded from the collagen samples before and after acetic acid treatment. The Mueller matrix-derived fluorescence diattenuation and polarizance parameters for the collagen samples, before and after acetic acid treatment, are shown in Fig. 6. These parameters are observed to be significantly reduced following acetic acid treatment, implying reduced molecular organization/ordering. The fluorescence diattenuation and polarizance parameters thus capture useful information on the biochemical and structural changes occurring due to the breakage of collagen's molecular crosslinks and subsequent degradation of the collagen fibrous network. These parameters, therefore, show early promise as potentially useful biomarkers and may be explored for diverse biomedical applications including tissue characterization/diagnosis. We note here that the reduction in these intrinsic fluorescence parameters with acetic acid treatment was also accompanied with an overall decrease of the fluorescence intensity because breakage and degradation of the collagen molecular crosslinks also lead to reduction in the fluorescence quantum yield.²⁰

5 Conclusion

To summarize, we have demonstrated that intrinsic anisotropies associated with anisotropic organization of fluorescent molecules can be probed via fluorescence spectroscopic Mueller matrix. In this regard, we have shown that the traditional fluorescence anisotropy parameter does not exclusively probe such intrinsic anisotropies. We have developed a simplified model to separately decouple and quantify the depolarization property and the intrinsic anisotropy properties of fluorescence from the experimentally measured fluorescence Mueller matrix and demonstrated the utility of these parameters on controlled model systems. The biomedical potential of the derived intrinsic anisotropy parameters of fluorescence has initially been explored on collagen, an important tissue fluorophore. The initial results show the considerable promise of these newly derived fluorescence polarimetry parameters as potentially useful biometrics and warrant further exploration of this approach in tissue diagnosis/assessment. Finally, the demonstrated novel ability of this approach in delineating otherwise hidden information on intrinsic anisotropy of fluorescence should, in general, prove to be valuable for probing/characterizing a wide variety of complex fluorescent systems of biological and/or nonbiological origin.

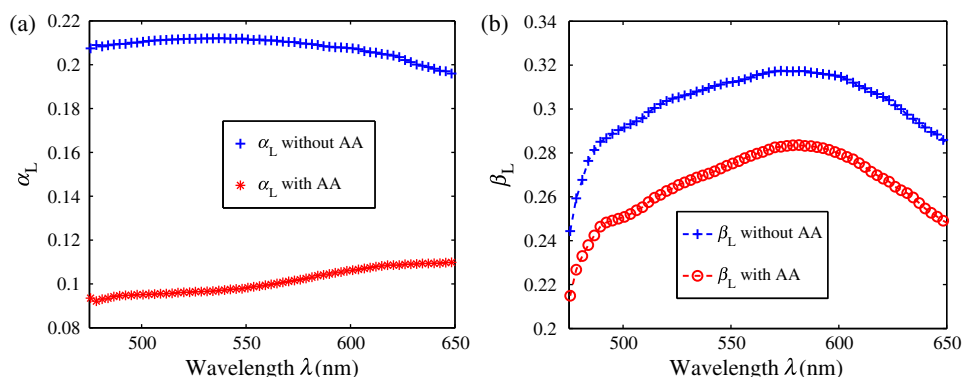


Fig. 6 Spectral variation of the fluorescence (a) linear diattenuation (α_L) and (b) linear polarizance (β_L) parameters for the bovine collagen sample with and without the acetic acid (AA) treatment.

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