General framework for treating generation, propagation, and polarization of luminescence in anisotropic media

Shane Nichols, Melissa Tan, Alexander Martin, Emily Timothy, and Bart Kahr*

New York University, Department of Chemistry, New York, NY 10003

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Complete polarimeters deliver the full polarization transfer matrix of a medium that relates input polarization states to output polarization states. In order to interpret the Mueller matrix of a luminescent medium at the emission frequency, accountings are required for polarization transformations of the medium at the excitation frequency, the light scattering event, and the polarization transformations at the emission frequency. A general framework for this kind of analysis is presented herein. The fluorescence Mueller matrix is expressed as a product of Mueller matrices of the medium at the excitation and emission frequencies and a scattering matrix, integrated over path length. The Stokes vector for the incident light, evolving according to the Mueller matrix of the medium at the excitation frequency, is multiplied by the scattering matrix to give a Stokes vector of the emitted light along the propagation direction. The scattering matrix is derived from an incoherent orientation ensemble average over a phenomenological scattering tensor that embodies intrinsic molecular scattering proprieties, and dynamical processes that occur during the excited state. The general framework is evaluated for three anisotropic materials carrying luminescent dye molecules including the following: a chiral fluid, a stretched polymer film, and a chiral, biaxial crystal. In the latter case, the most complex, the Mueller matrix was collected in conoscopic illumination and the fluorescence Mueller matrix, mapped in k-space, was fully simulated by the strategy outlined above. Luminescence spectroscopy has typically stood apart from the developments in polarimetry of the past two generations. This need not be indefinite.

I. INTRODUCTION

Polarization properties of fluorescence and the mathematical foundations that underly modern polarimetry have a common origin. In his comprehensive memoir "On the change of refrangibility of light" [1], Stokes coined the term ‘fluorescence' and introduced the so-called Stokes parameters that describe the polarization state of light. He observed that fluorescence from solutions is unpolarized while that from crystals is often polarized. Soleillet later showed that solutions will emit polarized light if excited with polarized light and if the analyte rotates slowly on the time scale of the excited state [2]. He presented four equations that connect the Stokes parameters of the incident excitation to the scattered emission, which depend on the scattering angle and a quantity called fluorescence anisotropy. Soleillet was also the first to invoke what is now called the differential Mueller matrix formalism [3] and used it to consider the effects of anisotropy on the exciting beam [4]. Perrin further developed fluorescence polarization to study anisotropic rotational diffusion [5], and considered scattering by optically active and oriented systems in the context of the Stokes parameters [6]. Despite this history, the field of fluorescence polarization has developed spectacularly outside of the Mueller-Stokes framework.

Fluorescence polarization has since become an important tool in biophysics [7–11]. It has been used to detect binding of small molecules to large biomolecules, and to characterize liquid crystals [12–20], Langmuir-Blodgett films [21, 22], sheared melts [23], and biological membranes [24–26]. Additionally, polarized fluorescence microscopy has shown the local orientation and dynamics in cellular membranes and vesicles [27–34]. Yet, the great shortcoming of the development of fluorescence polarization outside of polarimetry is the failure to explicitly consider polarization transformations in the excitation beam and in the emission beam, save elementary considerations of linear dichroism at the excitation wavelength. While membranes and thin-films have path lengths that likely do not warrant such considerations, liquid crystals, melts, and stretched films most likely do. Accountings of polarization in the Mueller matrix formalism have been reported [35, 36], but with severe approximations.

Recently, researchers in biomedicine have taken a keen interest in implementing Mueller matrix fluorimetry for the characterization of tissues [37–40]. In such cases, developing models of light generation and propagation are less important than establishing robust fingerprints to distinguish one tissue from another, usually for disease recognition. Fluorescence Mueller matrix (FMM) measurements have also been used to characterize scattering from collagen fibers [41], polycrystalline films of biological origin [42], polymeric nanoparticles [43], and supramolecular assemblies [44]. Many of these works and others [4, 45] suggest various phenomenological parameters to quantify effects of propagation and scattering, but are not firmly rooted in the differential Mueller matrix formalism. Our aim here is to develop robust methods to handle the effects of propagation at both the excitation and emission wavelengths, and to substantiate the methods proposed with simple, illustrative measurements which correspond to practical optical systems.

* bart.kahr@nyu.edu
The three examples include a fluorophore in an optically active isotropic medium (glucose), a fluorophore in an anisotropic medium (a stretched polymer film), and a fluorophore in a bianisotropic medium, (a dyed, chiral crystal).

II. THEORY

While the differential Mueller matrix formulation was introduced nearly 40 years ago [46] and has since been well studied [47–49], its form in the presence of luminescent sources has not been considered. In a continuous medium with luminescent sources throughout, the evolution of the Stokes vector at the emission wavelength \( s_e(z) \) at a point \( z \) along the propagation direction is governed by the nonhomogeneous differential equation

\[
ds_e/dz = m_e s_e + s_s
\]

where \( s_s \) is a \( z \)-dependent source term that represents the Stokes vector of light emitted at \( z \) that is directed along the propagation direction, and \( m_e \) is the differential Mueller matrix at the emission wavelength. Using the method of variation of parameters, the general solution to Eq. (1) is

\[
s(z) = M_e(z)M_e^{-1}(0)s(0) + M_e(z)\int_0^z M_e^{-1}(z')s_s(z')\,dz'
\]

where \( M_e \) is the Mueller matrix describing propagation at the emission wavelength and is the solution to the corresponding homogeneous system, i.e., it satisfies \( dM_e/dz = m_eM_e \). Physically, the first term on the right of Eq. (2) propagates incident light at the emission wavelength through the medium whereas the second term accounts for the generation and propagation of luminescence within the medium. The integral in Eq. (2) represents an incoherent superposition and is only appropriate for light generation processes in which the phases of light emitted from different points in the medium are random, e.g., spontaneous emission and vibrational Ramon scattering [50]. Coherently driven processes, such as nonlinear mixing and stimulated emission, should be described by an analogous formulation of Eq. (1) in terms of the differential Jones formalism [51].

Evaluation of Eq. (2) requires a model for \( s_s \). In the case of fluorescence (or any kind of spontaneous photoluminescence), the source term is derived from the excitation field, described by its incident Stokes vector \( s_s(0) \) and the Mueller matrix \( M_s(z) \) by which it propagates, according to

\[
s_s(z) = S(z)M_s(z)s_s(0).
\]

\( S \) is a scattering matrix that relates the instantaneous value of \( s_s(z) \) to the net polarization of fluorescence that it generates. To obtain the overall FMM \( F \) that connects the incident Stokes vector at the excitation wavelength to the outgoing Stokes vector at the emission wavelength, we substitute Eq. (3) into Eq. (1), introduce the path-length \( d \) over which fluorescence is integrated, and drop \( S_s(0) \), giving

\[
F(d) = M_e(d)\int_0^d M_e^{-1}(z)S(z)M_s(z)\,dz. \quad (4)
\]

Note that the first term on the right of Eq. (2) has vanished because we assume that no light enters the medium at the emission wavelength. The form of Eq. (4) is valid for media in which \( m_e \) may be continuously varying in \( z \), for example a twisted nematic liquid crystal, but hereafter we will consider only homogeneous media, where \( m_e \) and \( S \) are \( z \)-independent. Such media have the simplifying properties \( M(a) = \exp(am) \), and \( M(a)M(b)^{-1} = \exp((a-b)m) \) that permit rewriting Eq. (4) as

\[
F(d) = \int_0^d \exp((d-z)m_e)S\exp(zm_e)\,dz, \quad (5)
\]

in which \( m_s \) is the differential Mueller matrix at the excitation wavelength. Equation (5) is intuitive in that the integrand expressly gives the FMM for light generated at \( z \); the excitation propagates to \( z \) according to \( \exp zm_e \), generates fluorescence according to \( S \), which then propagates over the remaining path length according to \( \exp((d-z)m_e) \). The overall FMM is then the integral over the path length.

Incorporation of boundaries requires a more general formulation than Eq. (5). Rigorous accountings of light generation in arbitrary homogeneous media typically begin with incorporating source terms into Maxwell’s equations. Solutions are given in terms of dyadic Green’s functions, in which the net electric field in the exit medium is written as a volume integral [52–54]. We question, however, whether this approach is appropriate for luminescence because an integral over field amplitudes is fundamentally a coherent superposition, analogous to casting Eq. (2) as Jones matrices. Moreover, deriving the requisite Green’s functions is usually a formidable task [55]. Here we introduce a simple, approximate method to incorporate interfaces that is based on the reduction of propagation in plane parallel media to partial waves [56, 57].

Consider a medium bounded by plane parallel interfaces normal to \( z \) at positions \( z = 0 \) and \( z = d \), as shown in Fig. 1. The excitation wave vector makes an angle \( \phi_x \) with the substrate normal and is contained in the \( xz \) plane. In general, the incident field excites in the slab two forward and two backward propagating plane waves. If we ignore reflections from inside the slab, the excitation propagation is fully described by three \( 2 \times 2 \) Jones matrices; \( T_0^{12} \) maps the electric field in the incident ambient medium, described in the \( sp \) basis, to the amplitudes of the forward propagation eigenwaves within the layer; \( T_0^{12} \) maps the forward eigenwaves inside the layer to the \( sp \) basis in the ambient exit medium; \( D_x \) is the diagonal matrix that propagates the eigenwaves across the layer and...
we achieved nearly identical results by simply nor-
malizing $T^{01}$ and $T^{12}$ such that the reflection losses that they innately describe are mitigated, and their effect is only to map the fields into an $sp$ basis. The normal-
ized matrices are defined by $T_i^{N12} = T^{12}_i / ||T^{12}_i||$ and $T_i^{N01} = T^{01}_i / ||T^{01}_i||$ where $i$ is a row index and $j$ is a column index. We can now define the overall Jones matrices that handle propagation before and after the scattering event as

$$J_e(z) = T_x^{N12} D_x(z) T_x^{01}, \quad (6a)$$

$$J_e(d - z) = R(\theta) T_e^{12} D(d - z) T_e^{N01} R(-\theta) \quad (6b)$$

where

$$R(\theta) = \begin{bmatrix} \cos \theta & \sin \theta \\ -\sin \theta & \cos \theta \end{bmatrix} \quad (6c)$$

is a rotation matrix that brings the emission field into the global coordinate system. Both Jones matrices are converted to Mueller matrices by $M_n = A(J_n \otimes J'_n) A^{-1}$, where $\otimes$ is the Kronecker product, and

$$A = \begin{bmatrix} 1 & 0 & 0 & 1 \\ 1 & 0 & 0 & -1 \\ 0 & 1 & 1 & 0 \\ 0 & i & -i & 0 \end{bmatrix}. \quad (7)$$

Finally, the Mueller matrices are inserted into Eq. (4) to obtain the FMM.

We now turn our attention to the scattering matrix $S$. To provide the most general results, we make no assump-
tions as to the scattering geometry in its derivation. Properties of the fluorophores such as electronics, rotatio-
nal dynamics, and energy transfer are embodied in the scattering events that convert excitation to emission. However, $S$ is not a standalone property of the medium but corresponds to a particular scattering geometry in which $S_x$ and $S_e$ propagate along arbitrary directions described by the respective wave vectors $k_x$ and $k_e$. As shown in Fig. 1, the electric fields of both rays are pro-
jected onto unit vectors in $\hat{p}$ and out (\hat{s}) of the scatter-
ing plane. In terms of this geometry, we introduce a $9 \times 9$ matrix $Q$ that contains the three-dimensional scattering properties of the medium, from which $S$ is extracted according to

$$S = A \left( [\hat{p}_x \otimes \hat{p}_e \ \hat{p}_x \otimes \hat{s}_e \ \hat{s}_x \otimes \hat{p}_e \ \hat{s}_x \otimes \hat{s}_e]^{T} \times Q [\hat{p}_x \otimes \hat{p}_x \ \hat{p}_x \otimes \hat{s}_x \ \hat{s}_x \otimes \hat{p}_x \ \hat{s}_x \otimes \hat{s}_x] \right) A^{-1} \quad (8)$$

where each Kronecker product produces a 9-element column vector.

In essence, $Q$ represents an ensemble average over all molecular scattering events that can occur in the medium. Herein we assume that the scattering process is electric-field mediated, thus a single scattering event can be described by a $3 \times 3$ tensor $P$ that relates the three-dimensional incident and scattered electric fields according to $E_s = PE_i$. [50]. For elastic (coherent) scat-
tering, an orientation ensemble of scatterers is described
by direct integration over $\mathbf{P}$, however, because fluorescence is intrinsically incoherent, any ensemble average must be performed on second order moments [59–61]. In this case, $Q = \langle \mathbf{P} \otimes \mathbf{P}^* \rangle$, which may be formally written as

$$Q = \frac{\int_\Omega w(\Omega)\mathbf{P} \otimes \mathbf{P}^* \, d\Omega}{\int_\Omega w(\Omega) \, d\Omega}$$

(9)

with $d\Omega$ being a volume element of orientation space, and $w(\Omega)$ a weighting function giving the probability density of a fluorophore being in a particular orientation. There may be multiple tensors $\mathbf{P}$ that describe different possible scattering events, for example multiple electronic transitions at the excitation frequency, or emission from multiple excited states. Because fluorescence is incoherent, $\mathbf{P}$ describing each unique scattering event should be mapped to a tensor $Q$, and the $Q$’s summed. As molecules are much smaller than the wavelength of light, molecular scattering tends to be dipole-like [62], in which case $\mathbf{P}$ becomes the dyad $\mathbf{P} = \hat{\mathbf{u}}_x \otimes \mathbf{u}_x$, where $\hat{\mathbf{u}}_x$ and $\mathbf{u}_x$ are respectively unit vectors along the directions of the absorption and emission dipole moments of the fluorophore. For dipole scattering, circular polarization in the excitation field is inconsequential because the last row and column of $\mathbf{S}$ are zero for any orientation distribution of dipole scatterers.

As a final note, we have neglected terms that relate to the absolute quantity of light emitted, such as the quantum yield, because polarimetric measurements are typically normalized by the overall light intensity. We have also neglected the usual $1/\nu^2$ dependence of radiation intensity under the assumption that the thickness of the specimen is much smaller than the distance between the specimen and the detector.

### III. EXPERIMENTAL

Measurements in Sections IV A and IV C were performed with a previously described spectroscopic Mueller matrix polarimeter that uses four photelastic modulators [63]. A broadband light source was used for transmission studies, while either a 441 nm laser (4 W, CW direct-diode, Lasertack GmbH) or a 532 nm laser (300 mW, CW DPSS, Lasermate) was used for fluorescence excitation. Low strain birefringence microscope objectives with 4x magnification and a numerical aperture of 0.1 were positioned on both sides of the specimen to focus the excitation and gather emission. Emission was separated from excitation using two high-contrast long-pass filters, and the emission was spectroscopically analyzed with a scanning grating monochromator. All measurements were performed at normal incidence in the forward scattering configuration using an instrument coordinate system in which $\hat{\mathbf{p}}_x = \hat{\mathbf{p}}_c = \mathbf{x}$ and $\hat{\mathbf{s}}_x = \hat{\mathbf{s}}_c = \mathbf{y}$.

The measurement in Section IV D was performed with a Mueller matrix microscope that uses continuous rotating retarders before and after the specimen to modulate the polarization state of light. Its operational principles are similar to others [64, 65], and its particular details are given elsewhere [66]. A collimated 450 nm laser (3.5 W, CW direct-diode, Wicked Lasers) was used for excitation. Emission was gathered with a polarization grade 40x objective and passed through a 550-570 bandpass filter. The rear focal plane of the objective was imaged on a camera sensor to acquire $k$-space ( conoscopic) Mueller matrix images, similar to the device described in Ref. [67].

### IV. RESULTS AND DISCUSSION

Three illustrative homogeneous media are shown to substantiate the equations of the previous section, and to examine some particular forms of $\mathbf{S}$. Knowledge of the materials are used to either predict $\mathbf{S}$, or reduce its form to a few parameters that may be fitted from the data. Application of Eq. (5) requires knowing $\mathbf{m}_x$ and $\mathbf{m}_c$. In a linear homogeneous medium, $\mathbf{m}$ has only seven parameters and takes the form

$$\mathbf{m} = \frac{1}{d} \begin{bmatrix} -A & -LD & -LD' & CD \\ -LD & -A & CB & LB' \\ -LD' & -CB & -A & -LB \\ CD & -LB' & LB & -A \end{bmatrix},$$

(10)

in which $LD$ and $LD'$ are, respectively, on and off axis linear dichroism, $LB$ and $LB'$ are, respectively on and off axis linear birefringence, $CD$ and $CB$ are respectively circular dichroism and circular birefringence, and $A$ is the mean absorbance [68]. These parameters are obtained either from an optical model of the medium in terms of the constitutive relations that solve Maxwell’s equations, or from a measurement of the transmission Mueller matrix (TMM). In the latter case, however, care must be taken to ensure that the correct branch is selected from the multivalued function $\mathbf{m} = \ln(\mathbf{M})/d$ [48]. Although each branch maps to the same Mueller matrix of the homogeneous solution $\mathbf{M} = \exp(2\mathbf{m})$, the same is not true for Eq. (5) due to the integral. Equations to obtain $\mathbf{m}$ from either optical models or transmission data are provided in Appendix A.

#### A. Isotropic distributions

We previously considered the form of $\mathbf{F}$ for an isotropic distribution of fluorophores [4], in which we recast Soleillet’s classical results on fluorescence scattering [2] as the matrix

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

(11)

where $\phi$ is the scattering angle, and $a$, $b$, and $c$ are properties independent of $\phi$. We previously remarked that $c$
is related to optical activity [4], but this statement was made in error. Because \( c \) appears on the diagonal of \( S \), it does not induce asymmetry with respect to any state of polarization. Rather, parameters \( b \) and \( c \) respectively establish the extent to which linear and circular polarizations are transferred from the excitation to the emission. To relate these parameters to a tensor \( P \) that describes a scattering event, we evaluated Eqs. (8) and (9) with \( w(\Omega) = 1 \) and obtained, \( a = (6t_1 + t_2 + t_3)/10 \), \( b = (3(t_2 + t_3)/2 - t_1)/10 \), and \( c = (t_1 - t_2)/4 \), where \( t_1 = \text{tr}(PP^\dagger) \), \( t_2 = \text{tr}(PP^\dagger) \), \( t_3 = \text{tr}(P) \text{tr}(P^\dagger) \), and \( \text{tr} \) denotes the trace. While this result suggests the possibility of \( c \neq 0 \), such an observation has yet to be made in any fluorescence experiment, to our knowledge, due to the dipole nature of molecular scattering, for which \( c = 0 \). Higher multipoles in \( P \) do produce \( c \neq 0 \), however. For instance, if \( P \) takes the form of an arbitrary quadrupole moment, we find that \( S \propto \text{diag}(7,1,1,-2.5) \) in the forward scattering direction. Perhaps emissive nanoparticles may offer a route to obtain \( c \neq 0 \). Such a system may be useful as it would exhibit emission that “remembers” the handedness of the excitation light.

For dipole scattering, where \( P = u_\varepsilon \otimes u_x \), the parameters \( a \) and \( b \) are more conveniently cast in terms of the angle \( \delta \) between the absorption and emission dipole moments, i.e., \( \cos \delta = u_\varepsilon \cdot u_x \). It is known that \( \delta \approx 0 \) for typical fluorophores [62], but a molecule may behave as if \( \delta \) is larger than its intrinsic value due to rotational diffusion of molecules while in the excited state. In that sense, the vectors \( u_\varepsilon \) and \( u_x \) should be evaluated at the time of absorption and emission, respectively. Thus, rotational diffusion leads to a distribution in \( \delta \), and the definitions \( a = \frac{1}{16}(2 \langle \cos^2 \delta \rangle + 6) \), and \( b = \frac{1}{16}(3 \langle \cos^2 \delta \rangle - 1) \) where pointed braces refer to an ensemble value. From a single steady-state measurement, one cannot determine the extent to which nonzero \( \langle \cos^2 \delta \rangle \) arises by an intrinsic misalignment of the absorption and emission dipole moments or by rotational diffusion, however, time resolved measurements or steady-state measurements at various viscosities can be used to elucidate the relative proportions [62]. Although we find \( \delta \) to be more intuitive, \( a \) and \( b \) are more often cast in terms of the more abstract angle \( \theta \), defined as the angle between the emission dipole and the azimuth of the excitation polarization [4, 62]. In terms of this angle, \( a = (1 + \langle \cos^2 \theta \rangle)/2 \), and \( b = (3 \langle \cos^2 \theta \rangle - 1)/4 \). Another often used parameter is fluorescence anisotropy \( r \), which is directly \( r = 2b/4 \) [4], and can be obtained from the scattering matrix in Eq. (11) by

\[
r = \frac{2(S_{22} - S_{21})}{3(S_{11} - S_{12}) + S_{21} - S_{22}},
\]

which is independent of the scattering angle, and insensitive to uniform scaling of \( S \). In the trivial case of a medium that does not impart anisotropy on propagation at either the excitation or emission wavelengths, \( F \propto S \), and thus Eq. (12) may be applied to the elements of \( F \) to obtain the fluorescence anisotropy, which is the only parameter for such a medium.

Although it is possible for a medium that imparts general anisotropy on wave propagation to have an isotropic distribution of fluorophores, we will consider only the more simple case of an optically active medium (nonzero \( A, CD, \) and \( CB \)) at both the excitation and emission wavelengths. In this case, the Mueller matrix has the analytic form

\[
M(z) = e^{-A}
\begin{bmatrix}
\cosh(CD) & 0 & 0 & \sinh(CD) \\
0 & \cos(CB) & \sin(CB) & 0 \\
0 & -\sin(CB) & \cos(CB) & 0 \\
\sinh(CD) & 0 & 0 & \cosh(CD)
\end{bmatrix}.
\]

\( F \) is obtained by combining Eqs. (5), (10), (11) and (13). An analytical solution is readily obtained when \( \phi = 0 \), and \( CD_e = 0 \) because the generators of \( CB \) and \( A \) commute with \( S \) in the forward scattering direction, and thus \( S \) can be moved to the left of the integral in Eq. (5).

Furthermore, because the generators of \( A, CB, \) and \( CD \) commute, the law of exponentials can be used to solve the integral, giving

\[
F = S(\exp(dm_e) - \exp(dm_e))(m_e - m_e)^{-1}.
\]

Expanded equations are provided in Appendix B. There has been much interest in probing \( CD_e \) by measuring the differential intensity of fluorescence upon excitation with left and right circularly polarized light, a quantity usually called fluorescence detected circular dichroism (FDCD). Circular dichroism spectropolarimeters actually measure the ratio \( M_{14}/M_{11} \), which is approximately \( CD \) when \( CD \) is small. Likewise, FDCD polarimeters measure \( F_{14}/F_{11} \), but this quantity is a more complicated function. However, in the limit that \( A_e \to 0 \), and \( \exp(-A_e) \to 0 \) (that is, no absorption at the emission wavelength and total absorption at the excitation wavelength), then \( F_{14}/F_{11} \to CD_e/A_e = g_{abs}/2 \) where \( g_{abs} \) is the so-called dissymmetry factor. This particular result is independent of the scattering angle and does not depend on fluorescence anisotropy.

### B. Dye in a viscous, optically active medium

To test Eq. (14), a \( 1 \times 10^{-4} \) M solution of rhodamine 6G in corn syrup (concentrated aqueous glucose) was prepared and placed in a 2 mm path length cuvette. The solution had small \( CB \) at both the excitation and emission wavelengths but showed no \( CD \) because rhodamine 6G, the sole absorbing molecule, is achiral. An unnormalized TMM measurement was used to recover \( A \) and \( CB \), as shown in Fig. 2a. The FMM emission spectrum was measured at \( \phi = 0 \) using 441 nm excitation; the solution showed bright fluorescence despite being at the edge of the absorption band. Nonzero elements of the FMM, normalized by \( F_{11} \), are shown in Fig. 2b along with predicted values that were calculated.
FIG. 2. Transmission and fluorescence polarization properties of $1 \times 10^{-4}$ M solution of rhodamine 6G in corn syrup. (a) Absorbance and CB computed from the TMM. Data was not normalized so as to recover $A$. The emission spectrum is also given, in arbitrary units. (b) Red and blue spectra show, respectively, the measured and predicted FMM elements corresponding to the central block of the Mueller matrix; other elements are zero or one.

using equations in Appendix B and the measured quantities in Fig. 2a. The calculation also requires knowing the fluorescence anisotropy. Because $F_{23}$ and $F_{32}$ are very small, we assumed that Eq. (12) could be used to a good approximation. The same line shape of $r$ was observed in non-optically active solutions of rhodamine 6G in glycerol, but with smaller values due to the lower viscosity of glycerol compared to corn syrup. Although propagation has a weak effect here, the $F_{23}$ and $F_{32}$ elements agree well with the expected values.

C. Dye in an anisotropic polymer film

This section demonstrates recovering $S$ of a more general distribution of dye. Congo red (CR, 400 µl of $1 \times 10^{-3}$ M) was added to polyvinyl alcohol (PVOH, 7.38 g in 80.6 g deionized H2O) and the mixture poured into a rectangular mold and left to dry in open air for several days. The resultant polymer was ca. 100 µm thick after drying. The normalized TMM of the film was very close to the identity matrix, and the forward scattering FMM was $\approx \text{diag}(1, 0.5, 0.5, 0)$, which corresponds to a frozen isotropic distribution of dyes having collinear absorption and emission dipole moments. Unidirectional stretching of the film induced linear anisotropy, and induces a probability distribution that has a mean value along, and is symmetric about, the stretching direction. The stretching direction was oriented along the instrument $x$ axis by minimizing $LD'$ in transmission. By numerically integrating Eq. (9) for such a distribution with its mean value along $x$, and evaluating Eq. (8) for forward scattering, we determined that $S$ in this case has the general form,

$$S = \begin{bmatrix} 1 & S_{12} & 0 & 0 \\ S_{32} & S_{22} & 0 & 0 \\ 0 & 0 & S_{33} & 0 \\ 0 & 0 & 0 & 0 \end{bmatrix}$$  \tag{15}$$

where $S$ has been normalized by $S_{11}$. This result agrees with others [69] as well as our intuition that $S$ in this case should resemble the Mueller matrix of a partial polarizer with its transmission direction along $x$, except $S_{14} = 0$ due to the incoherent nature of the scattering process. By this analogy, we can anticipate that all parameters in Eq. (15) will be positive numbers.

Parameters $S_{12}$, $S_{22}$, and $S_{33}$ were obtained by fitting Eq. (5) to measured values of $F$, and order-corrected values of $m$ obtained from the measured TMM. The top panel of Fig. 3 shows the TMM and $m$ as a function of wavelength, from which $m_x$ and $m_y$ are obtained.
D. Dye in a bianisotropic crystal

While molecules are only partially aligned within stretched films and membranes, growth impurities incorporated into the facets of crystals are often highly aligned [70, 71]. Impurities may incorporate homogeneously in space under suitable conditions, thereby providing an ideal medium in which to study the anisotropy of photophysical properties [72–74]. Such a medium is considered here. Crystals of ethylenediammonium sulfate (EDS)
were grown from aqueous solution in the presence of sunset yellow FCF (SY), a disulfonated azo dye. EDS forms large, transparent, and optically clear crystals in the chiral, tetragonal point group 422 (D4), and exhibits excellent (001) cleavage. We previously reported the optical functions of pure EDS [75]. From polarized absorbance measurements of dyed crystals cut along different directions, we established that SY selectively incorporates into the 102 growth sectors and is oriented with its molecular plane perpendicular to the α component of the growth direction. Dyes are highly aligned in that plane such that the dominant absorption band is polarized perpendicular to the emission wavelength [57, 67] and multiplying the matrix by a linearly polarized Stokes vector with its azimuth of polarization aligned along the emission dipole moment of SY. The result of this calculation is shown in Fig. 5, which closely resembles the first column of the FMM maps in Fig. 4c. To a good approximation, the other columns of the FMM are related to the first column by overall scale factors that are given by the first row of the normalized TMM at the excitation wavelength. For this crystal, in which light was normally incident, the first row of \( M_x \) is \[ [1 \ 0.95 \ -0.22 \ -0.06] \], whereas the average values along the first row of the calculated FMM map in Fig. 4 are \[ [1 \ -0.91 \ 0.22 \ 0.06] \]. We see that the scale factors for the second, third, and fourth columns of the FMM are directly the negative of the corresponding elements in the first row of \( M_x \). In this way, it is possible to approximate \( F \) without evaluating the integral in Eq. (5).

V. CONCLUSION

To summarize, we have extended the differential Mueller matrix formalism by adding a source term that represents the Stokes vector of light generated within the medium by an incoherent scattering process. The general solution contains an intuitive integral term that propagates and sums newly generated light from all points along the propagation direction. This result was applied herein to fluorescence by connecting the source term to a scattering process driven by the Stokes vector at the excitation wavelength. An approximate approach to include boundaries was also given. Scattering and propagation were analyzed for several representative systems and the methods validated by measurement. While this work expressly considered fluorescence, all manner of luminescence or other incoherent scattering processes are encompassed within the general scope of this work. We expect these results to be illuminating to those researchers working in the newly emerging field of fluorescence polarimetry, as well as researchers involved in more classical fluorescence polarization analyses. With regards to possible future directions, this work provided analytical solutions to the integral equations for optically active isotropic media only, but it would be useful to have analytical solutions for general homogeneous media or other relevant media, such as continuously varying liquid crystalline phases, or elastically scattering media.

Appendix A

Methods to obtain \( m \) are discussed here. The Jones formalism is used for convenience, in which a Mueller matrix given by \( \mathbf{M} = \exp(\mathbf{L}) \), where \( \mathbf{m} = \mathbf{L}/d \) with \( \mathbf{m} \)
having the form of Eq. (10), has the equivalent Jones matrix representation \( \mathbf{J} = \exp(-i\mathbf{N}) \). The so-called differential Jones matrix \( \mathbf{N} \) is [51]

\[
\mathbf{N} = \frac{1}{2} \left[ \begin{array}{cc} A + L & L' + iC \\ L' - iC & A - L \end{array} \right],
\]

(A1)

in which \( L = LB - iLD, L' = LB' - iLD, C = CB - iCD \). Equations will be given for the parameters of \( \mathbf{N} \), from which \( \mathbf{m} \) is obtained by \( \mathbf{m} = \mathbf{A}(\mathbf{N} \oplus \mathbf{N}^*)\mathbf{A}^{-1}/d \), where \( \oplus \) is the Kronecker sum and \( \mathbf{A} \) is given in Eq. (7).

1. Obtaining \( \mathbf{m} \) from optical constants

Homogeneous, non-magnetic, and Lorentz-reciprocal media can be described by an electric permittivity tensor \( \mathbf{e} \) and an optical activity tensor \( \mathbf{\alpha} \) that enter into the Tellegen constitutive relations [55] \( \mathbf{D} = \mathbf{eE} - i\mathbf{\alphaH} \), and \( \mathbf{B} = \mathbf{H} + i\mathbf{\alpha}^{T}\mathbf{E} \), in Gaussian units. Light propagating in an unbounded medium in the direction of the unit vector \( \mathbf{k} \) accumulates

\[
\begin{align*}
A &= -k_0d \text{Im} \left( 1/\sqrt{U_{11}} + 1/\sqrt{U_{22}} \right) \quad (A2a) \\
L &= k_0d \left( 1/\sqrt{U_{11}} - 1/\sqrt{U_{22}} \right) \quad (A2b) \\
L' &= k_0d \left( 1/\sqrt{(U_{11} + U_{22} + U_{21} + U_{12})/2} \right. \\
& \left. -1/\sqrt{(U_{11} + U_{22} - U_{21} - U_{12})/2} \right) \quad (A2c) \\
C &= k_0d(k^T[\text{tr}(\mathbf{\alpha})\mathbf{I} - \mathbf{\alpha}[\mathbf{k}]) \quad (A2d)
\end{align*}
\]

over a distance \( d \) where \( k_0 \) is the wavenumber, \( \mathbf{I} \) is the identity matrix and, \( \mathbf{U} = (\hat{\mathbf{p}} \hat{\mathbf{s}})^T e^{-1}(\hat{\mathbf{p}} \hat{\mathbf{s}}) \). Unit vectors \( \hat{\mathbf{p}} \) and \( \hat{\mathbf{s}} \) are orthogonal directions perpendicular to \( \mathbf{k} \) along which the electric field is projected.

2. Obtaining \( \mathbf{m} \) from a TMM measurement

Assuming interfaces do not have a large effect, \( \mathbf{m} \) can in principle be obtained directly from a TMM measurement by \( \ln(\mathbf{M})/d = \mathbf{m} \), but the physical solution must be selected from the generally infinite number of solutions to the matrix logarithm [48]. By definition, \( \mathbf{m} \) is unambiguously the matrix that satisfies \( d\mathbf{M}/dz = \mathbf{mM} \), but usually \( \mathbf{M} \) cannot be measured as a function of the path length. Our approach is to compute various possible solutions, and use other available information to select the physical branch of the matrix logarithm.

Analytical solutions are straightforward if \( \mathbf{M} \) can be represented by a Jones matrix. Because experimental data contains noise, \( \mathbf{J} \) is usually estimated from \( \mathbf{M} \) by matrix filtering [76], but formally we can write

\[
\begin{align*}
J_{11} &= \sqrt{(M_{11} + M_{12} + M_{21} + M_{22})/2} \quad (A3a) \\
J_{12} &= (M_{13} + M_{23} - i(M_{14} + M_{24}))/2J_{11} \quad (A3b) \\
J_{21} &= (M_{41} + M_{42} + i(M_{43} + M_{44}))/2J_{11} \quad (A3c) \\
J_{22} &= (M_{33} + M_{44} + i(M_{34} - M_{33}))/2J_{11}. \quad (A3d)
\end{align*}
\]

From the elements of \( \mathbf{J} \), the parameters of each possible solution to \( \mathbf{N} \) are

\[
\begin{align*}
A &= -\text{Re}(2\ln(1/K)) \quad (A4a) \\
L &= i\Omega(J_{11} - J_{22}) \quad (A4b) \\
L' &= i\Omega(J_{12} + J_{21}) \quad (A4c) \\
C &= \Omega(J_{12} - J_{21}) \quad (A4d)
\end{align*}
\]

where,

\[
\begin{align*}
K &= 1/\sqrt{\det(\mathbf{J})} \quad (A4e) \\
T &= 2\cos(K(J_{11} + J_{22})/2) \quad (A4f) \\
\Omega &= K(T + 2\pi n)/(2\sin(T/2)) \quad (A4g)
\end{align*}
\]

which are equivalent to published relations [77] except that we have added the factor \( 2\pi n \) in Eq. (A4g) where \( n \) is any integer. As anisotropy is uniformly increased from 0, the value of \( n \) increments as 0, -1, 1, -2, 2 . . . .
If M is measured spectroscopically with sufficiently high resolution, n only needs to be known at one wavelength if m(λ) is assumed to be continuous. Our approach is to assign an initial value of n at the longest measured wavelength and then use curve continuity to determine n elsewhere. Comparing spectra for different initial values of n can in some cases elucidate the physical value. Such a comparison is made in Fig. 6 using the TMM data in Fig. 3. All but the initial value of n = −1 lead to large values of CD and CD around 466 nm that are unphysical. This approach should be used with caution, however. While it is robust for inverting simulated (i.e., perfect) Mueller matrices of absorbing media, even small amounts of added noise (0.1%) can create instabilities in Eq. (A4) that generally grow with n. A numerical approach to order correction suggested by others is likewise very sensitive to measurement errors [48]. Our polarimeter operates with systematic errors of ≈ 0.0005 in transmission mode, and thus we were confident that our assignments of m were correct. Yet generally solving m = ln(M) for imperfect data with due regard for multivaluedness remains a challenge.

**Appendix B**

In a medium with nonzero $A_x, A_e, CB_x, CB_e,$ and $CD_x$, the FMM $F$ in the forward scattering direction ($\phi = 0$) has the nonvanishing elements,

\[
F_{11} = S_{11} \frac{(A_x - A_e)(e^{-A_x} - e^{-A_e} \cos CD_x) - CD_x e^{-A_x} \sinh CD_x}{(A_x - A_e)^2 - CD_x^2} \\
F_{14} = S_{11} \frac{(A_e - A_x)e^{-A_x} \sinh CD_x + CD_x (e^{-A_e} - e^{-A_x} \cos CD_x)}{(A_x - A_e)^2 - CD_x^2} \\
F_{22} = F_{33} = S_{22} \frac{(A_x - A_e)(e^{-A_x} \cos CB_x - e^{-A_e} \cos CB_e) + (CB_x - CB_e)(e^{-A_x} \sin CB_x - e^{-A_e} \sin CB_e)}{(A_x - A_e)^2 + (CB_x - CB_e)^2} \\
F_{23} = -F_{32} = S_{22} \frac{(CB_x - CB_e)(e^{-A_e} \cos CB_x - e^{-A_x} \cos CB_e) + (A_x - A_e)(e^{-A_e} \sin CB_x - e^{-A_x} \sin CB_e)}{(A_x - A_e)^2 + (CB_x - CB_e)^2}
\]

in which the scattering matrix elements $S_{ii}$ are given by Eq. (11).

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