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Theoretical Foundation for Electric-Dipole-Allowed Chiral-Specific Fluorescence Optical Rotary Dispersion (F-ORD) from Interfacial Assemblies

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Abstract

Fluorescence optical rotary dispersion (F-ORD) is proposed as a novel chiral-specific and interface-specific spectroscopic method. F-ORD measurements of uniaxial assemblies are predicted to be fully electric-dipole-allowed, with corresponding increases in sensitivity to chirality relative to chiral-specific measurements in isotropic assemblies that are commonly interpreted through coupling between electric and magnetic dynamic dipoles. Observations of strong chiral sensitivity in prior single-molecule fluorescence measurements of chiral interfacial molecules are in excellent qualitative agreement with the predictions of the F-ORD mechanism and challenging to otherwise explain. F-ORD may provide methods to suppress background fluorescence in studies of biological interfaces, as the detected signal requires both polar local order and interfacial chirality. In addition, the molecular-level descriptions of the mechanisms underpinning F-ORD may also potentially apply to aid in interpreting chiral-specific Raman and surface-enhanced Raman spectroscopy measurements of uniaxially oriented assemblies, opening up opportunities for chiral-specific and interface-specific vibrational spectroscopy.

Graphical abstract

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Absolute chirality plays a critical role in biological interactions, governing enzyme–substrate interactions.^{1–3} To design a functioning pharmaceutical therapeutic agent, target chirality is typically a prerequisite. However, few probes are available to definitively characterize chirality. Vibrational circular dichroism (VCD) and Raman optical activity (ROA) are selective techniques commonly utilized for chiral sensing but are limited by low sensitivity.^{4,5} Electronic circular dichroism (ECD) offers higher sensitivity, but the broad-band nature of electronic transitions reduces specificity.⁶ Fully electric-dipole-allowed chiral-specific effects including circular extinction are reasonably well-established in measurements of crystalline materials. In studies of achiral dyes incorporated during crystal growth, Kahr and coworkers have nicely demonstrated the observation of chiral specificity for achiral dyes incorporated into chiral lattices.⁷ In this instance, the circular dichroism observed arose from an interplay between birefringence in the crystal and linear dichroism from preferred crystal orientation and is thus limited to dye-doped crystalline and polycrystalline materials.⁸ Alternative methods employing second-order nonlinear optical (NLO) spectroscopy have been shown to be highly sensitive to chirality, also through an electric-dipole-allowed mechanism.^{9–11} Near 100% dichroic ratios have been observed for oriented binaphthol monolayer films using second harmonic generation (SHG) circular dichroism (CD).¹² In addition, Fourier transform microwave spectroscopy has been employed for electric-dipole-allowed enantiomer-specific detection methods via triply resonant microwave nonlinear wave mixing.^{13,14} However, the accessibility of NLO approaches is reduced by the requirement of a pulsed laser source at optical frequencies and the complexity of the experiments in general. One-photon fluorescence (1PF), in contrast, does not require a coherent or pulsed light source and can be easily implemented into existing fluorescence microscopes. Here we propose a new spectroscopic method based on 1PF that is selective to chirality at interfaces.

A simple physical model explaining the prediction of electric-dipole-allowed chiral-specific fluorescence in uniaxial assemblies is shown in Figure 1 in the limiting case of orthogonally polarized absorption (blue arrow) and fluorescence (red arrow) transition moments. A

nonzero net rotation in the primary axis of polarization can be observed between the projections of $\vec{\mu}^a$ and $\vec{\mu}^f$ on the X - Y plane. Excitation with linearly polarized light will result in fluorescence that, on average, yields a maximum in the polarized emission intensity that is rotated relative to the excitation axis. This rotation is the origin of the chiral-specific signal. From inspection of the graphic, the F-ORD process is nonreciprocal such that flipping the surface through a 180° rotation about the X axis will result in the opposite sense of rotation.

The mathematical framework for chiral-specific fluorescence builds most directly on the electric-dipole-allowed orientational mechanism underpinning chiral-specific four-wave mixing described by Davis et al.¹⁵ In this mechanism, a chiral-specific nonlinear optical field can be produced even in the absence of intrinsic chirality within the chromophore through chiral orientational arrangement in an ensemble. The macroscopic chirality arises purely through packing effects and does not require any coupling between chromophores or chiral distortion. Furthermore, because both the chiral and achiral responses are fully electric-dipole-allowed, they are often comparable in magnitude, yielding dichroic ratios approaching unity under favorable conditions. A qualitatively similar mechanism is well-established for describing the high sensitivity of chirality in surface second harmonic and sum frequency generation.

The connection between the experimental observables and the molecular properties can be constructed by starting with fundamental light matter interactions at the molecular level. The process of absorption can be described either by a transition dipole moment μ^i or more formally by the imaginary component of the polarizability matrix α^{ij} , where i, j represents the polarization of the emission and excitation light, respectively. The two formalisms are connected by the following equation describing the excitation from state I to state n . The damping term Γ describes the half width at half-maximum of the line shape function.

$$\alpha^{ij}(-\omega; \omega) = \frac{1}{\hbar} \sum_n \frac{\mu_{In}^i \mu_{nI}^j}{\omega_n - \omega - i\Gamma_n} + \frac{\mu_{In}^j \mu_{nI}^i}{\omega_n - \omega - i\Gamma_n} \quad (1)$$

For a molecule initially in the ground state such that $I=0$, the first term in the summation will dominate because the excited state lies at an energy higher than the initial state. In the case of fluorescence emission, the second term dominates, as the initial state is higher in energy than the state contributing to the resonance enhancement.¹⁶ Only the imaginary contributions to the polarizability impact the overall attenuation or amplification of the optical field, corresponding to absorption or emission, respectively. The polarization dependence of absorption and emission is dictated by the terms in the numerator. In a molecular coordinate system in which the z' axis parallels the fluorescence emission transition moment and the absorption transition moment lies in the $x'-z'$ plane, the fluorescence transition moment has only one nonzero term $\mu_{z'}^f = |\vec{\mu}^f|$, and the absorption transition moment has two components: $(\mu_{nI}^{x'})^a = |\vec{\mu}_{nI}^a| \sin \delta$ and $(\mu_{nI}^{z'})^a = |\vec{\mu}_{nI}^a| \cos \delta$, where δ defines the angle between the fluorescence and absorption transition moments consistent with Figure 1.

Using the relation $\vec{\mu}_{nm} = \vec{\mu}_{mn}^*$, the expression for the linear polarizability describing absorption in eq 1 can be rewritten as follows for a molecule initially in the ground state $I=0$.

$$\alpha^{x'x'} \cong S(\omega) |\vec{\mu}_{n0}|^2 \sin^2 \delta$$

$$\alpha^{z'z'} \cong S(\omega) |\vec{\mu}_{n0}|^2 \cos^2 \delta$$

$$\alpha^{x'z'} = \alpha^{z'x'} \cong S(\omega) |\vec{\mu}_{n0}|^2 \sin \delta \cos \delta \quad (2)$$

where the $S(\omega)$ describes the resonance line-shape function. If it is assumed that molecular rotation is slow relative to the fluorescence lifetime, the orientational averages connecting the molecular frame to the laboratory frame are performed over the combined coordinates describing absorption and emission.

In a general sense, the intensity of pure linearly polarized fluorescence as a function of the polarization rotation angle γ for linearly polarized excitation light depends on the following three trigonometric polynomial coefficients $A-C$.

$$I_n(\gamma) = A_n \cos^2 \gamma + B_n \sin \gamma \cos \gamma + C_n \sin^2 \gamma \quad (3)$$

In the above equation, n may refer to the Stokes frame horizontal polarization (H) or vertical polarization (V). These three coefficients are in turn related back to the molecular orientation distribution and the transition moments within the molecular frame. Furthermore, if the angle between the absorption and emission angles is defined to be δ described above, the expressions for the polynomial coefficients can be rewritten in terms of δ , as shown below for H polarized detection.

$$\begin{aligned} A_H = K (|\vec{\mu}^f|^2 |\vec{\mu}^a|^2) \{ & \cos^2 \delta \langle (R_{Xz'} R_{Xz'})^f (R_{Xz'} R_{Xz'})^a \rangle \\ & + 2 \sin \delta \cos \delta \langle (R_{Xz'} R_{Xz'})^f (R_{Xx'} R_{Xz'})^a \rangle \\ & + \sin^2 \delta \langle (R_{Xz'} R_{Xz'})^f (R_{Xx'} R_{Xx'})^a \rangle \} \end{aligned} \quad (4)$$

$$B_H = K (|\vec{\mu}^f|^2 |\vec{\mu}^a|^2) \{ \cos^2 \delta \langle (R_{Xz'} R_{Xz'})^f (R_{Xz'} R_{Yz'})^a \rangle + \sin^2 \delta \langle (R_{Xz'} R_{Xz'})^f (R_{Xx'} R_{Yx'})^a \rangle \}$$

(5)

$$C_H = K(|\vec{\mu}^f|^2 |\vec{\mu}^a|^2) \left\{ \cos^2 \delta \langle (R_{Xz'}, R_{Xz'})^f (R_{Yz'}, R_{Yz'})^a \rangle \right. \\ \left. + 2 \sin \delta \cos \delta \langle (R_{Xz'}, R_{Xz'})^f (R_{Yx'}, R_{Yx'})^a \rangle \right. \\ \left. + \sin^2 \delta \langle (R_{Xz'}, R_{Xz'})^f (R_{Yx'}, R_{Yx'})^a \rangle \right\} \quad (6)$$

The degeneracy of the input photon polarizations ($\alpha^{x'y'} = \alpha^{y'x'}$) produces two orientational averages equal in magnitude but opposite in sign, yielding a net zero $\sin(\delta)\cos(\delta)$ term for the B coefficient. Expressions for each of the eight orientational averages in eqs 4–6 for a uniform distribution in ϕ have been explicitly evaluated previously.¹⁵ The orientational averages for the contribution in B_H are given by the following expressions.

$$\langle (R_{Xz'}, R_{Xz'})^f (R_{Xz'}, R_{Yz'})^a \rangle = 0 \quad (7)$$

$$\langle (R_{Xz'}, R_{Xz'})^f (R_{Xx'}, R_{Yx'})^a \rangle = \frac{1}{4} \langle \sin^2 \theta \cos \theta \sin \psi \cos \psi \rangle \quad (8)$$

In the presence of a mirror plane containing the unique axis, the B term goes to zero due to the corresponding even symmetry in the distributions in ψ . Thus only ensembles exhibiting chiral order will display this response; the B coefficient contribution to detected signal is hereby referred to as the chiral-specific signal. The origin of the chiral-specific signal can be understood from the nonzero orientational average in eq 8. Note that a chiral uniaxial assembly alone is thus insufficient to observe F-ORD experimentally; a nonzero orientational average $\langle \sin^2(\theta)\cos(\theta) \rangle$ is required and implied by the term polar order. Using a lipid monolayer on an air/water interface as an example, the aliphatic moiety for the ensemble is virtually always oriented away from the bulk liquid, giving the whole interface a polar orientation. In contrast, a bilayer of those same lipids can exhibit alignment about a unique axis but no preferred orientation (i.e., polar order) and no corresponding F-ORD response. The B_H coefficient is given by the following equation as a function of orientational parameters and the transition moments of the fluorophore.

$$B_H \propto \langle \sin^2 \theta \cos \theta \sin \psi \cos \psi \rangle (|\mu_z^f|^2 |\mu_x^a|^2) = |\vec{\mu}^f|^2 |\vec{\mu}^a|^2 \langle \sin^2 \delta \sin^2 \theta \cos \theta \sin \psi \cos \psi \rangle \quad (9)$$

From eq 9, three requirements must be met to exhibit chiral specificity in the fluorescence produced from uniaxial assemblies: (i) the assembly exhibits polar order, such that the expectation values over the polar tilt angle θ are nonzero, (ii) the arrangement of the plane containing the absorption and fluorescence transition dipoles must exhibit a chiral twist such

that the expectation values over ψ are nonzero, and (iii) the transition moments for absorption and fluorescence are nonparallel such that δ is nonzero. In the limit of a uniform distribution in ψ , both orientational averages in eq 8 approach zero.

The magnitude of the F-ORD signal can be interpreted in the context of a linear intensity difference (LID) measurement most analogous to circular intensity difference measurements with circularly polarized excitation. In LID measurements, the polarized fluorescence intensity is measured for $+45$ and -45° linear polarizations, with the difference in the detected intensity directly connected to the F-ORD rotation angle.

$$\text{LID} \equiv \frac{I_{\text{H}}(+45) - I_{\text{H}}(-45)}{I_{\text{H}}(+45) + I_{\text{H}}(-45)} = \frac{B_{\text{H}}}{A_{\text{H}} + C_{\text{H}}} \quad (10)$$

There are an infinite set of angles which generate a maximal dissymmetry parameter due to the interplay between the functions of θ and δ . For $\delta = 90^\circ$, explicit evaluation for the theoretical maximum using eqs 10 and 4–6 yields an LID maximum of 25% with $\theta = 65.8^\circ$ and $\psi = 22.3^\circ$. The azimuthal distribution in molecular orientation results in a reduction in the LID values relative to the theoretical maximum of 1 for an infinitely sharp azimuthal orientation distribution or for single molecules. Note, however, that typically reported electronic CD g values are on the order of 10^{-3} . It is posited that because the F-ORD mechanism arises purely from electric dipole contributions the generated signal may be much greater.

Evidence supporting the potential relevance of this proposed mechanism can be found in single-molecule fluorescence studies of chiral molecules first reported by Barnes and coworkers, reproduced with permission in Figure 2. In that study, Barnes and coworkers observed dissymmetry parameter g related to differences in fluorescence intensity for right versus left circularly polarized excitation.¹⁷ Clear statistically significant differences were observed in the histograms of g values produced for the two senses of helicity for the chiral fluorophores used.

In follow-up work by Cohen and coworkers,^{18,19} it was suggested that the dichroic mirrors altered polarization incident to the sample. It was demonstrated that the dichroic mirror induced phase shifts and had different reflectivity efficiencies. Furthermore, corrections to ensure pure circular polarizations at the sample resulted in significant decreases in the spread and magnitude of g values obtained. Cohen and coworkers concluded that the observation of nonzero dissymmetry could be attributed to the presence of linear dichroism rather than circular dichroism. In subsequent studies by Barnes and coworkers, a reduction in dissymmetry was confirmed when particular attention was taken to remove potential artifacts from linear dichroism, although the underlying sensitivity to absolute chirality remained.²⁰

Interestingly, no subsequent work has to our knowledge appeared to address what are arguably the most exciting observations in the original study, namely, the observed chiral specificity in the single-molecule linear dichroism contributions to the Barnes and

coworkers' measurements. The results shown in Figure 2 demonstrate clear differences in the mean values of the dissymmetry factors for the two diastereomers at the interface. These observations can potentially be explained through the F-ORD mechanism proposed herein.

The presence of unanticipated F-ORD contributions in the prior experimental design can be understood by considering the influence of the Jones matrices for the optical path in the original study, as subsequently detailed by Cohen and coworkers. In brief, the Jones matrix describing the polarization state of the incident circularly polarized light upon reflection from a dichroic mirror can be broken up into a complex component describing elliptically polarized light along the eigenpolarizations of the mirror and a purely real component corresponding to $\pm 45^\circ$ linear polarization.

$$\frac{\rightarrow a}{e} = \begin{bmatrix} r_H^a & 0 \\ 0 & r_V^a \end{bmatrix} \frac{1}{\sqrt{2}} \begin{bmatrix} 1 \\ \pm i \end{bmatrix} = \begin{bmatrix} |r_H| & 0 \\ 0 & |r_V|e^{i\Delta} \end{bmatrix} \frac{1}{\sqrt{2}} \begin{bmatrix} 1 \\ \pm i \end{bmatrix} = \frac{1}{\sqrt{2}} \begin{bmatrix} |r_H^a/r_V^a| - \sin \Delta \\ \pm i \cos \Delta \end{bmatrix} + \frac{\sin \Delta}{\sqrt{2}} \begin{bmatrix} 1 \\ \pm 1 \end{bmatrix} \quad (11)$$

In eq 11, r_H^a and r_V^a refer to the complex-valued amplitude reflection coefficients for the dichroic mirror (assuming the p and s eigenpolarizations coincide with the H and V reference frames of the image), and Δ is the phase shift in the polarization state induced by the mirror, given by $\Delta = \arg(r_H^a/r_V^a)$. From eq 10, the difference in detected intensity for $\pm 45^\circ$ is directly connected to the chiral-specific coefficients B_H and B_V . For detection of horizontally polarized light, the following equality emerges.

$$I_H(+45^0) - I_H(-45^0) = 2 \left(\frac{1}{2} B_H \right) |t_H^f|^2 \quad (12)$$

Taking advantage of the identity $B_H = -B_V$, the integrated fluorescence intensity difference arising from residual linear dichroism can be connected to the transmission coefficients of the dichroic mirror along the return path at the fluorescence wavelength through the following relationship.

$$I_{\text{tot}}(+45^0) - I_{\text{tot}}(-45^0) = B_H |t_H^f|^2 + B_V |t_V^f|^2 = B_H (|t_H^f|^2 - |t_V^f|^2) \quad (13)$$

From previous measurements of dichroic mirrors performed by the Cohen group,¹⁸ reasonable values of $\Delta = 0.79$ were recorded for one dichroic mirror, corresponding to as much as $\sim 1/2$ the incident light potentially contributing to linear dichroism. From eq 13, the presence of a significant difference in transmissivity for the eigenpolarizations of the dichroic mirror prior to detection could provide one possible explanation for the observations of large dissymmetry factors directly tied to the sense of chirality. Conversely, unpolarized detection of the total fluorescence should yield no selectivity for chirality,

consistent with the two transmissivity terms in eq 13 being equal. It would also explain the reductions in this sensitivity when using pure circular polarizations, corresponding to the case of $\theta \cong 0$. Note that the reduction in signal observed with circularly polarized light is entirely consistent with the Cohen response in 2009, where it was noted that careful treatment of polarization purity to ensure pure CPL at the sample resulted in sharp collapse of the distribution of g about zero. Finally, it suggests that the overall sensitivity to chirality could be significantly improved yet further through optimization of the experiment to explicitly target F-ORD measurements.

It is interesting to compare and contrast the proposed mechanism with previous work by Cohen and coworkers, describing “superchiral” light arising in the vicinity of nodes present at highly reflective interfaces.^{18,21,22} In brief, the electric dipole contributions approach zero at a node, while the same effect does not arise for magnetic dipole contributions. As such, suppression of the electric-dipole-induced fluorescence results in a relative enhancement in the chiral-specific magnetic dipole contributions and a correspondingly large dissymmetry factor. This mechanism produces a greater g value with a maximum signal detected for pure circularly polarized light and zero signal for linearly polarized light. In contrast, the F-ORD mechanism, as detailed above, yields a minimum g value for circularly polarized light, as evidenced by the 2009 response to the original Barnes manuscript.¹⁸ In a followup manuscript by Barnes and coworkers in 2012, it was shown that careful treatment to reduce linear polarization contamination resulted in a reduction in dissymmetry parameters g , consistent with the predictions of F-ORD and contrary to those of the “superchiral” light mechanism.²³

It is worthwhile to compare and contrast the chiral-specific response predicted for fluorescence with the fully electric-dipole-allowed chiral-specific Raman from oriented assemblies first considered several years ago by Efrima and subsequently refined by Hecht and Barron.^{24,25} In brief, symmetry arguments indicated the possibility of electric-dipole-allowed chiral-specific Raman optical activity (ROA) in chiral assemblies exhibiting polar order. In that work, electric-dipole-allowed ROA was predicted to be possible from macroscopic symmetry arguments, with the ROA scaling with the antisymmetric component of the Raman tensor. Because antisymmetry is enhanced near electronic resonance, ROA was predicted to be enhanced in resonance Raman measurements. These predictions were subsequently confirmed experimentally and put to great advantage in surface-enhanced Raman measurements.²⁶ This confirmation has led to a recent growth in SEROA arising from molecules assembled at the surfaces of metal nanoparticles. Despite the successes of SEROA, few detailed molecular level interpretations have emerged for the origins of the chiral specificity in terms of the molecular orientation distribution at the interface, such that *ab initio* interpretations of SEROA measurements for absolute chirality determination remain challenging.

More recent independent studies of coherent four-wave mixing of uniaxial assemblies¹⁵ may potentially help clarify the origin of the chiral specificity of spontaneous Raman in SEROA measurements and their possible connection to chiral-specific fluorescence. In that work, explicit expressions were derived for the orientational averages connecting the molecular frame to the interfacial frame for all coherent four-wave mixing measurements. Fortunately,

both fluorescence and the spontaneous Raman processes can be treated mathematically with the same framework as four-wave mixing, although with two of the four fields produced by the sample rather than one as in CARS. These general expressions are equally valid for spontaneous Raman and fluorescence, providing a route for clarifying the molecular-level requirements for both electric-dipole-allowed chiral-specific Raman and F-ORD.

In summary, a framework for the theoretical description of chiral-specific fluorescence was described. The proposed model imposes the following molecular constraints: (i) the presence of a significant difference between the absorption and emission transition moment orientations within the molecular frame, (ii) polar order of a uniaxial interface, and (iii) ensemble chirality. The F-ORD chiral-specific signal was mathematically connected to the B polynomial coefficient in polarization rotation measurements and to linear intensity difference measurements of chiral assemblies. The model provides a novel electric-dipole-allowed mechanism for chiral-specific fluorescence, which may explain the remarkable linear dissymmetry parameters previously reported by Barnes and Cohen. Furthermore, the described framework lends itself to future extension into the theoretical treatment of SEROA measurements, providing increased generality over previous treatments.

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Figure 1.

Graphic illustration of planar fluorophores (represented by gray plates) with uniaxial symmetry in the laboratory X - Y - Z frame. The Euler angle ϕ is uniformly sampled for the assembly. Detected signal is defined in the projection onto the laboratory X - Y plane. Y -polarized excitation results in fluorescence detected at an angle rotated clockwise, as shown in the projection.

Figure 2.

Normalized fluorescence-detected circular dichroism (FD CD) histogram published by Barnes and coworkers.¹⁷ The blue and red traces represent the signal from right (*P2*) and left (*M2*) handed helical structures, respectively. Mean *g* values reported were 0.52 and -0.63, respectively, two orders of magnitude greater than the bulk response. Reproduced with permission from ref¹⁷. © 2006 AAAS.