Investigation Of The Orientation Dependence On Chiroptical Properties Of Single Molecules

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INVESTIGATION OF THE ORIENTATION DEPENDENCE ON CHIROPTICAL PROPERTIES OF SINGLE MOLECULES

A Dissertation Presented

by

AUSTIN J. CYPHERSMITH

Submitted to the Graduate School of the University of Massachusetts Amherst in partial fulfillment of the requirements for the degree of

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INVESTIGATION OF THE ORIENTATION DEPENDENCE ON CHIROPTICAL PROPERTIES OF SINGLE MOLECULES

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ABSTRACT

INVESTIGATION OF THE ORIENTATION DEPENDENCE ON CHIROPTICAL PROPERTIES OF SINGLE MOLECULES

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Optical activity is the defining property of chiral materials that is essential for characterization in biology, chemistry, and physics. While a substantial body of research has provided a strong theoretical framework of the origin of optical activity, we still know very little by way of experiment about an individual molecule’s contribution to the bulk optical activity. The chiroptical response of a single molecule can depend on molecular orientation and local molecular environment, information which is lost in ensemble averaging.

This thesis focuses on establishing methods for a priori determination of chiral molecule orientations and refining measurements to probe the chiroptical response of a single molecule using a generalization of well-known defocused emission pattern imaging. Recent experiments probing the chiroptical response of single helicene dimer molecules offer new insight into the relationship between local molecular environment and coupling between chiral moieties. New experiments, such as probing the chiroptical response of an achiral, non-centrosymmetric molecular systems and polarization resolved spectral measurements which probe the Davydov splitting of coupled chromophore systems offer promising new avenues for understanding the connection between the polarization properties of single molecules and the ensemble.
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACKNOWLEDGEMENTS</td>
<td>iv</td>
</tr>
<tr>
<td>ABSTRACT</td>
<td>v</td>
</tr>
<tr>
<td>LIST OF FIGURES</td>
<td>vi</td>
</tr>
<tr>
<td>INTRODUCTION</td>
<td>1</td>
</tr>
<tr>
<td>CHAPTER</td>
<td></td>
</tr>
<tr>
<td>1: INTRODUCTION TO OPTICAL ACTIVITY</td>
<td>5</td>
</tr>
<tr>
<td>2: SUMMARY OF PRIOR WORK</td>
<td>9</td>
</tr>
<tr>
<td>3: ORIENTATION AND POLARIZATION IN CDSE-ZNS QUANTUM DOTS: A Prototype System for Chiral Molecules</td>
<td>12</td>
</tr>
<tr>
<td>4: ORIENTATION IMAGING OF CHIRAL MOLECULES BY DEFOCUSED EMISSION PATTERN IMAGING</td>
<td>17</td>
</tr>
<tr>
<td>5: FECD OF AN M-HELICENE DIMER</td>
<td>22</td>
</tr>
<tr>
<td>6: FECD OF A SINGLE ACHIRAL MOLECULE</td>
<td>27</td>
</tr>
<tr>
<td>7: POLARIZATION RESOLVED SPECTRA OF SINGLE PDI2 MOLECULES</td>
<td>31</td>
</tr>
<tr>
<td>DISCUSSION AND FUTURE WORK</td>
<td>36</td>
</tr>
<tr>
<td>APPENDICES</td>
<td></td>
</tr>
<tr>
<td>1: APPENDIX 1: VARIABLE RETARDER ALIGNMENT</td>
<td>38</td>
</tr>
<tr>
<td>2: APPENDIX 2: DISENTANGLING THE ROLE OF LINEAR TRANSITION DIPOLE IN BAND-EDGE EMISSION FROM SINGLE CDSE/ZNS QUANTUM DOTS: COMBINED LINEAR ANISOTROPY AND DEFOCUSED EMISSION PATTERN IMAGING</td>
<td>See supplemental</td>
</tr>
<tr>
<td>3: APPENDIX 3: DEFOCUSED EMISSION PATTERNS FROM CHIRAL FLUOROPHORES: APPLICATION TO CHIRAL AXIS ORIENTATION DETERMINATION</td>
<td>See supplemental</td>
</tr>
<tr>
<td>4: APPENDIX 4: CHIROPTICAL DISSYMMETRIES IN FLUORESCENCE EXCITATION FROM SINGLE MOLECULES OF (M-2) HELICENE DIMERS</td>
<td>See supplemental</td>
</tr>
<tr>
<td>REFERENCES</td>
<td>42</td>
</tr>
</tbody>
</table>
LIST OF FIGURES

<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Physical representation of the (translational) electric dipole moment projecting onto the (rotational) magnetic dipole moment to create a net helical transition moment.</td>
<td>6</td>
</tr>
<tr>
<td>2</td>
<td>(a) Molecular axis in the lab frame showing the solid angle collected or illuminated by the laser passing through the objective. (b and c) Image of the rotatory strength tensor for the bridged triarylamine helicene molecule superimposed onto the molecular frame of the helicene molecule.</td>
<td>8</td>
</tr>
<tr>
<td>3</td>
<td>An example of an FECD trajectory – of a single Dil-C18 molecule. As the excitation polarization is switched from right to left circularly polarized light (RCP and LCP) the fluorescence intensity increases and continues to correlate with excitation polarization. The mean intensity of each half-cycle is used to obtain the anisotropy parameter.</td>
<td>9</td>
</tr>
<tr>
<td>4</td>
<td>(Left) Distribution of anisotropy values of the M2 (red) and P2 (blue) enantiomeric forms of individual helicene molecules. (Right) The P2 distribution fit to 3 Gaussian functions. The three Gaussians were hypothesized to correspond to three preferred molecular orientations. Reprinted from reference 31.</td>
<td>9</td>
</tr>
<tr>
<td>5</td>
<td>Schematic of the FECD microscope setup. (Blue) excitation light is circularly polarized by the linear polarizer and quarter waveplate and (red) emitted light is collected. The quarter waveplate is rotated to generate left or right circularly polarized light. Experiments prior to 2009 lacked the variable retarder and did not correct for the reflection off of the dichroism mirror.</td>
<td>10</td>
</tr>
<tr>
<td>6</td>
<td>Ground to excited state electronic transitions for a CdSe-ZnS quantum dot. The circular, X±iY, transitions are optically allowed while the Z transitions are nominally optically forbidden.</td>
<td>12</td>
</tr>
<tr>
<td>7</td>
<td>(a) DEP of CdSe quantum dot. Defocused to a depth of ~800nm. (b) Best fit contour plot of simulated 2D-DD. (c) Best fit contour plot of simulated 2D-DD with a z dipole component with a 0.42 relative dipole strength. (d) Polarization modulation trajectory of the CdSe quantum dot (red), fit to Cos2(θ) (black), expected modulation depth from 2D-DD model (blue), and the expected fit adjusted with a relative z dipole component of 0.42 relative to the x any dipoles (green).</td>
<td>16</td>
</tr>
<tr>
<td>8</td>
<td>(a) Simulated emission patterns and (b) dipole (red) and chiral axis (blue) of a chiral emitter at varying polar angles (30° increments) with the chiral axis along the optical axis. (c)Simulated emission patterns and (d) dipole (red) and chiral axis (blue) of a chiral emitter at varying azimuthal angles (30° increments) as the chiral axis turns away from the optical axis. From reference 70.</td>
<td>20</td>
</tr>
</tbody>
</table>
9: (Top) Structure of the M-helicene dimer (MH2). (Bottom) CD spectrum of the M-helicene monomer and MH2 with a peak anisotropy at 445nm.

10: FECD trajectories of the PDI dimer collected at 457nm excitation. Trajectories start under LCP light and switch to RCP light after 10 frames. Each frame has a 2 second exposure.

11: Histogram of circular anisotropy parameters of Fluorospheres (green), PDI (black dashed), and MH2 (red). Fluorospheres \( g = -0.002 \), FWHM=0.04; PDI \( g = 0.0097 \), FWHM=0.26; MH2 \( g = -0.09 \), FWHM=0.52

12: Proposed dimer, trimer and hexamer molecules for future single molecule chiroptical spectroscopy experiments.

13: Rotatory strength tensor superimposed on the molecular frame of a DiI-C18 molecule. Red/blue regions indicate a preferential absorption for right/left circularly polarized light. Figure provided by unpublished work from Dr. Bart Kahr at NYU.

14: Various orientations for DiI-C18 along the optical axis. Each DiI molecule has the rotatory strength tensor superimposed onto the molecule.

15: (Top Left) FECD trajectory of DiI showing low anisotropy values. (Bottom Left) The DEP of the corresponding trajectory along with a matching simulated DEP (Axial tilt of 0°). (Top Right) FECD trajectory of DiI-C18 showing high anisotropy values. (Bottom Right) The DEP of the corresponding trajectory along with a matching simulated DEP (Axial tilt 30°).

16: Defocused emission patterns of DiI (left) with the corresponding anisotropy values (right). Those patterns with the largest corresponding anisotropy values (blue – positive anisotropy, red – negative anisotropy) are aligned along the x or y axis.

17: (Left) Electronic transitions in the PDI dimer. Blue(red) lines correspond to x-transitions(y-transitions). (Right)Simulated emission spectra of the unpolarized (black), X(blue), and Y(red) transitions. Reprinted from reference 79.

18: Structure of the PDI dimer with the dipole moments and x and y directions illustrated. Reprinted from reference 79.

19: Absorption spectrum of the PDI dimer in chloroform. Note the shoulder near the peak at 525nm (19000 cm\(^{-1}\)) which is indicative of the Davydov splitting.

20: (a,b) Emission spectra from single PDI2 molecules, excited with s-(red) and p-(blue) polarized light. (c) Emission spectra of a single PDI monomer excited with s-(red) and p-(blue) polarized light.
INTRODUCTION

Optical activity – optical rotation and circular dichroism – is a manifestation of molecular chirality and plays an important role in physics, chemistry and biology. The origin of optical activity is well understood\textsuperscript{1-7} and it is a useful tool for analyzing chemical structure in protein,\textsuperscript{8-11} polymer,\textsuperscript{12} and organometallic\textsuperscript{13} systems. While we understand and make use of optical activity in bulk materials there is much we don’t know about the contribution of an individual molecule to the ensemble properties; single molecule chiroptical measurements are inherently difficult because both the signal of ORD and CD scale linearly with concentration.

Is the single molecule chiroptical response representative of the ensemble or are there structural or environmental heterogeneities which make the chiroptical response of a single molecule differ from that of the ensemble? For example, Vaccaro\textsuperscript{14-19} has shown with cavity ring down polarimetry (CRDP) experiments that the surrounding solvent environment can alter, and even invert the sign of the optical rotation. Simulations by Kongsted\textsuperscript{20-23} support this observation, suggesting the optical activity of molecules can be drastically altered by perturbing the electronic structure with the surrounding solvent. Further, we know from theory\textsuperscript{24} that molecular orientation is intimately tied to optical activity and in order to study the local environmental effects on the chiroptical response of single molecules, we will need to decouple the effect of molecular orientation. How does molecular orientation affect the observed chiroptical response of a single molecule? The role of orientation and structure as it relates to the optical activity of bulk materials is well studied. For instance, Kahr\textsuperscript{25,26} has shown that achiral molecules (even water) with well defined orientations can show a chiroptical response and studies of domains in
annealed polyfluorene films by Meskers\textsuperscript{27-29} have shown large anisotropy values due to structured domains.

In his paper, “Circular Dichroism Tensor of a Triarylmethyl Propeller in Sodium Chlorate Crystals,” Professor Bart Kahr states:\textsuperscript{30}

“We know virtually nothing by experiment about the orientational dependence of the optical rotation and circular dichroism in molecules.”

Using single molecule fluorescence spectroscopy to remove the effects of ensemble and rotational averaging, my thesis work seeks to address these questions and Dr. Kahr’s concern.

The ground work for single molecule chiroptical measurements was established in a paper by Hassey et al.\textsuperscript{31} in which distributions of circular anisotropy values of single bridged triarylamine helicene molecules were probed using fluorescence excitation circular dichroism (FECD). In FECD the fluorescence intensity from a single molecule is used as a reporter of absorption cross section of left circularly polarized (LCP) or right circularly polarized (RCP) light. In the measurement, LCP and RCP light alternately excite a molecule and the collected fluorescence intensity is recorded, differences in fluorescence intensity during the LCP and RCP excitation reveal relative differences in the absorption cross sections for LCP and RCP. In this paper a broad distribution of circular anisotropy values (between -1.5 and 1.5) suggest that the molecular orientation plays a significant role in the observed anisotropy value of a single molecule. The first part of my thesis research was to expand upon this paper and correlate molecular orientation information with circular anisotropy values from single chiral molecules.
This thesis is structured in seven chapters. (Chapter 1) provides and explanation of the requisite theory of optical activity for understanding this thesis. Chapter 2 provides a summary of the research of a previous group member, Ruthanne Hassey-Paradise, who probed the chiroptical properties of individual bridged triarylamine helicenes – research which provided the starting point for this thesis project. Chapter 3 and 4 of this thesis focuses on obtaining orientation information from defocused emission patterns from chiral chromophores. Building on the work of Enderlein, who helped formalize the theory for extracting orientation information from linear and 2D dipole sources, we expanded the use of defocused emission patterns for extracting molecular orientation to chiral chromophores. We used a coupled oscillator to mimic the magnetic dipole character of a chiral emitter and generated a library of emission patterns which could be compared with experimentally obtained emission patterns and assign Euler angles to the chiral axis of the molecule. Simulated emission patterns demonstrate a lack of bilateral symmetry that is distinct for chiral emitters and matches, qualitatively, observed emission patterns from chiral chromophores. Chapter 5 of this thesis is concerned with improving the resolving power of the fluorescence excitation circular dichroism (FECD) measurement. The primary difficulty with this measurement was that several factors may contribute to a false signal, these factors include: photobleaching, fluorescence intermittency, and linear polarization artifacts. A refined measurement of single molecule circular anisotropy values was recently made using helicene dimer molecules (discussed in chapter 5). This measurement showed that previous circular anisotropy distributions may have been broadened by these artifacts but a net asymmetric form in the distribution is maintained.
In the final, ongoing set of experiments (Chapter 7), the correlation between circular anisotropy values from chiral perylene dimer molecules and molecular orientation values derived from defocused emission patterns was investigated. The switch to the perylene dimer solves a problem that prohibited this experiment with the helicenes; the perylene has a much higher signal budget than the helicenes, which enables both the orientation and anisotropy data to be obtained. The perylene dimer has shown evidence of a Davydov splitting that, at the single molecule level, may lead to polarization dependent spectral character. Another set of experiments looks at the chiroptical response of an achiral molecule (DiI-C18) with fixed orientation (Chapter 6). The Appendix section contains the papers discussed in Chapter 3-5; as well as explanations and details on some of the experimental methods used in this thesis – hopefully this will be useful for any future students who wish to expand upon my thesis project.
CHAPTER 1

INTRODUCTION TO OPTICAL ACTIVITY
Optical activity refers to two distinct, but related phenomena: optical rotatory dispersion (ORD) in which the plane of linearly interacting non-resonantly polarized light is rotated as it passes through an optically active material, and circular dichroism (CD) in which a material differentially absorbs left and right circularly polarized light. Both ORD and CD are related to the index of refraction of a material:

\[ \tilde{n} = n + in' \]

Where \( n \) is the refractive index and \( n' \) is the absorptive index. The real part of the complex refractive index pertains to ORD. This rotation of linearly polarized light arises from the different indices of refraction for left and right circularly polarized light which changes the phases of the left and right circularly polarized contributions. Optical rotation is expressed as a difference between indices of refraction:

\[ \phi = \frac{180l(n_L - n_R)}{\lambda} \]

Where \( \phi \), the optical rotation in degrees, is related to the index of refraction of left circularly polarized light \( (n_L) \), the index of refraction of right circularly polarized light \( (n_R) \), the path length \( (l) \), and the wavelength \( (\lambda) \).

Circular dichroism is related to the imaginary part of the index of refraction. The imaginary absorptive index reproduces the exponential decay of the Beer-Lambert law. Circular dichroism, expressed as the difference between absorptivities:

\[ \Delta \varepsilon = \varepsilon_L - \varepsilon_R \]
where the circular dichroism, $\Delta \varepsilon$, is the difference between the extinction coefficient for left circularly polarized light ($\varepsilon_L$) and the extinction coefficient for right circularly polarized light ($\varepsilon_R$). The circular dichroism is commonly reported in molar ellipticity: $^{35}$

$$\left[ \theta \right] = 3300 \Delta \varepsilon$$

where $\theta$ is the ellipticity and the brackets indicate a per mole quantity. Because of this relation to the complex form of the index of refraction, circular dichroism and optical rotation are related to each other through the Kramers-Kronig transform: $^{36}$

$$\left[ \theta (\lambda) \right] = -\frac{2\lambda}{\pi} \int_0^\infty \frac{[\phi(\lambda')]}{\lambda^2 - \lambda'^2} d\lambda'$$

The magnitudes of ORD and CD are related to the rotatory strength tensor:

$$R_{f\rightarrow i} = -\frac{1}{3} \omega_{f\rightarrow i} [\text{Re}(\mu \times \bar{\theta}) - \text{Im}(\mu \cdot \bar{m})]$$

Where both the product with the electric quadrupole ($\bar{\Theta}$) and magnetic dipole ($\bar{m}$) moments contribute to the rotational strength of the molecule. In solution, the quadrupole component is typically rotationally averaged to zero in which case the rotatory strength tensor reduces to the Rosenfeld equation:

$$R_{f\rightarrow i} = \omega_{f\rightarrow i} \text{Im}(\mu \cdot \bar{m})$$

Figure 1 shows that the time-dependent electric dipole moment operator manifests as an infinitesimal generator of translations of electric charge while the magnetic dipole moment operator manifests as an infinitesimal generator of circulations of electric charge. The projection of one onto the other creates a specific helical sense; both helical directions are allowed.

![Figure 1: Physical representation of the (translational) electric dipole moment projecting onto the (rotational) magnetic dipole moment to create a net helical transition moment](image)
but for chiral molecules one of these directions is preferred. To better understand the role orientation plays in a molecule’s chiroptical response it is helpful to visualize the rotatory strength tensor projected along the optic axis:\(^{37}\)

\[
\Gamma(\theta, \phi) = \hat{n} \cdot \vec{R} \cdot \hat{n} = \sin^2 \theta \left[ R_{xx} \cos^2 \phi + R_{yy} \sin^2 \phi + \frac{1}{2} (R_{xy} + R_{yx}) \sin 2\phi \right] \\
+ \sin 2\theta \left[ \frac{1}{2} (R_{xz} + R_{zx}) \cos \phi + \frac{1}{2} (R_{yz} + R_{zy}) \sin \phi \right] \cos^2 \theta \frac{1}{2} R_{zz}
\]

Figure 2 shows a visualization of the rotatory strength tensor where a surface plot of \(\Gamma(\theta, \phi)\) is superimposed on the molecular frame of a bridged triarylamine helicene. This illustrates the idea that the observed dissymmetry will depend on the specific orientation of the molecule in the lab frame, as well as the solid angle (As shown in Figure 2(a)) over which the light is absorbed. The shape of the surface plot in Figure 2 clearly shows that orientation will play a critical role in probing the chiroptical response of a single molecule. The measured dissymmetry for a molecule with fixed orientation will depend sensitively on the orientation of \(\Gamma(\theta, \phi)\) with respect to the optical axis.
Figure 2: (a) Molecular axis in the lab frame showing the solid angle collected or illuminated by the laser passing through the objective. (b and c) Image of the rotatory strength tensor for the bridged triarylamine helicene molecule superimposed onto the molecular frame of the helicene molecule.
CHAPTER 2

SUMMARY OF PRIOR WORK

The work in this thesis as motivated by pioneering studies of single molecule chiroptical properties. In these initial studies by Dr. Ruthanne Hassey Paradise, the chiroptical response of single bridged triarylamine helicenes was probed via fluorescence excitation circular dichroism (FEC).\textsuperscript{31,38} This is a modified version of fluorescence detected circular dichroism (FDC).\textsuperscript{39-42} In FEC, the excitation polarization is switched between left and right circularly polarized light and fluorescence intensity (see Figure 3) is used as a probe of the relative absorption cross section for left and right circularly polarized light.

![Figure 3: An example of an FEC trajectory -- of a single DiI-C18 molecule. As the excitation polarization is switched from right to left circularly polarized light (RCP and LCP) the fluorescence intensity increases and continues to correlate with excitation polarization. The mean intensity of each half-cycle is used to obtain the anisotropy parameter.](image)

Figure 4: (Left) Distribution of anisotropy values of the M2 (red) and P2 (blue) enantiomeric forms of individual helicene molecules. (Right) The P2 distribution fit to 3 Gaussian functions, hypothesized to correspond to three preferred molecular orientations. Reprinted from reference 31.
The mean intensity of the fluorescence during LCP and RCP excitation are used to determine an anisotropy parameter $g$, defined as $g = \frac{2(I_{LCP} - I_{RCP})}{I_{LCP} + I_{RCP}}$. It was observed that for both enantiomeric forms of the molecule, a wide range of anisotropy parameters spanning both positive and negative values could be observed. Further, it was speculated that the range of anisotropy parameters was due to local environmental effects and molecular orientation. Notably, there was a broad range of anisotropy parameters, suggestive of local heterogeneities in local environment and molecular orientation. The three Gaussian fit suggests three dominant molecular orientations.

In 2009, a comment by Dr. Adam Cohen was published regarding this paper. His concern was that by reflecting the excitation polarization off of the dichroic filter (see Figure 5 for the context of our FECD setup), the polarization purity of the circularly polarized light was significantly reduced. This occurs because dichroic mirrors do not reflect the s and p polarized components of light in equal amounts. This leads to a phase aberration in the initially circularly polarized excitation, transforming it into elliptically polarized light. Cohen could not replicate the anisotropy parameter distribution in from reference 31 when he used a variable retarder to correct for the effect of the dichroism mirror.

Figure 5: Schematic of the FECD microscope setup. (Blue) excitation light is circularly polarized by the linear polarizer and quarter waveplate and (red) emitted light is collected. The quarter waveplate is rotated to generate left or right circularly polarized light. Experiments prior to 2009 lacked the variable retarder and did not correct for the reflection off of the dichroism mirror.
dichroic. Instead of two mirror imaged distributions, his study found two, narrower, overlapping distributions. Cohen insisted that our results were actually due to linear dichroism artifacts.

However, it is overreaching to dismiss these results for several reasons. The presence of linear polarization artifacts does not account for the net negative (and positive) anisotropy values in mirror image anisotropy distributions. As Chapter 5 shows, a proper FECD signal can still be measured even if the distribution is broadened by linear polarization artifacts. Another key difference is that Cohen did not screen for FECD trajectories with high photostability – a necessity for obtaining stable anisotropy values are trajectories that survive for multiple polarization half cycles.

Still, the core critique, that the dichroic mirror needs to be accounted for and merits closer attention. As Figure 5 shows, our current FECD setup uses a variable retarder to counter the effect of the dichroic and the experiments in chapter 5 and 6 are better for it. As will be discussed further in Chapter 5, the presence of linear polarization artifacts is an annoyance that, to some degree, must be tolerated with these experiments. The method for reducing the magnitude of linear polarization artifacts using a variable retarder is discussed in Appendix 1.
In order to probe the relationship between the orientation and chiroptical response of single molecules, we needed a way of extracting orientation information from fluorescence images. Defocused emission pattern (DEP) imaging\textsuperscript{46-52} – a method using optical interference to establish molecular orientation which will be explained in detail in Chapter 4 – provides a method for obtaining the molecular orientation in both chiral and achiral systems. DEP imaging has been used extensively to probe the orientation of linear dipole systems such as DiI-C18.\textsuperscript{48,53,54}

DEP imaging has been applied to more complex systems, such as the 2D degenerate dipole (2D-DD) system found in CdSe-ZnS quantum dots.\textsuperscript{55} In CdSe-ZnS quantum dots the 2D degenerate dipole arises from the allowed optical transitions.\textsuperscript{56} As shown in Figure 6, excitations from the ground to excited state can take the form of Z or $X \pm iY$ polarized transitions.\textsuperscript{57} Equal contributions from the optically allowed $X \pm iY$ transitions are responsible for the 2D-DD character of the quantum dot. Conservation of angular

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure6.png}
\caption{Ground to excited state electronic transitions for a CdSe-ZnS quantum dot. The circular, $X \pm iY$, transitions are optically allowed while the $Z$ transitions are nominally optically forbidden.}
\end{figure}
momentum permits only $\pm \frac{3}{2} \rightarrow \pm \frac{1}{2}$ and $\pm \frac{1}{2} \rightarrow \mp \frac{1}{2}$ transitions. These circularly polarized transitions make the quantum dots a useful prototype system of chiral molecules. While the quantum dot has an equal preference for absorbing LCP and RCP light, a chiral system would have a dissymmetric preference for absorbing LCP or RCP light.

The problem of simulating 2D emission dipoles and extracting Euler angles from experimentally obtained emission patterns has been solved previously by the work of Enderlein.\textsuperscript{33,58} Although the procedure for matching 2D emission patterns is well documented, we did need to implement this functionality for ourselves in order to apply this methodology to chiral systems.

Another popular method for establishing molecular orientation has been polarization resolved absorption and emission measurements of fluorescence. Such polarization experiments have been reported by Bawendi\textsuperscript{59,60} in CdSe-ZnS quantum dots studies as well as for other systems.\textsuperscript{61,62} This method works by varying the orientation of linear polarization of the excitation light relative to the molecular transition dipole. In the case of a 1D linear dipole where the excitation polarization is modulated, the observed fluorescence intensity will follow a $\cos^2(\theta)$ distribution, where a minimum corresponds to an excitation polarization orthogonal to the dipole and a maximum corresponds to an excitation polarization parallel to the dipole. This linear dichroism, expressed as modulation depth, $M = (I_{\max} - I_{\min})/(I_{\max} + I_{\min})$ provides information about the orientation of the transition dipole relative to the known excitation polarization orientation. Similarly, this method can be applied to emission measurements and 2D degenerate dipole systems to determine the orientation of the ‘dark’ axis.\textsuperscript{63}
We tested our code using CdSe-ZnS quantum dots, a 2D emitter which produces a high signal (for an overview of the code used, refer to Chapter 4). It was commonly stated in literature\textsuperscript{51,52,59} that CdSe-ZnS quantum dots behaved, in emission, as a 2D dipole. In order to confirm that our orientation assignments were correct, we correlated these measurements with polarization modulation measurements of the emitted light from the quantum dots. We found that the emission dipole structure of CdSe-ZnS quantum dots is more complex than the common 2D degenerate dipole (2D-DD) model would assume.

The experimental details can be found in (Appendix 2)\textsuperscript{64}, however, I will provide an overview of the experiment here. CdSe-ZnS quantum dots, on bare glass, were excited with 457nm excitation. The emission was collected and the linear polarization anisotropy was resolved by rotating a linear analyzer in the emission path. By sweeping through the polarization angles, we produced a sinusoidal modulating fluorescence trajectory which provided the modulation depth for a given quantum dot. After obtaining the modulated fluorescence trajectory, the image was defocused (~800nm) and set to a long exposure time to collect emission patterns of the quantum dots.

The Euler angles for each quantum dot were extracted by matching the defocused emission pattern to a library of simulated emission patterns and picking the best fit via a least squares fitting method. With the Euler angles known, we predicted the modulated trajectory we should see for each quantum dot assuming a 2D-DD model. In many cases the modulated trajectory matched that trajectory predicted by the Euler angles. However, in several cases the measured modulation depth was smaller than was predicted by the
Euler angles, suggesting a flaw in the assumption that the quantum dots followed a strict 2D-DD model.

As a solution to this inconsistency we incorporated a z dipole component, in addition to the 2D-DD model, which softened the predicted modulation depths. The origin of this z dipole component involves nominally, optically forbidden transitions in the quantum dot electronic structure. While the z polarized transition is optically forbidden, energy transfer via phonon interactions and structural heterogeneities in the quantum dots permit emission from this pathway. In the case of the 2D-DD model the modulation depth decreases sharply as the crystal axis of the quantum dot becomes orthogonal to the optical axis. The presence of this allowed z polarized transition has the effect of softening the modulation depth observed from the quantum dot.

We used the sequential measurements of the emission patterns and the modulation depth to estimate the relative dipole strength of the z polarized emission dipole. Comparing the emission pattern from the 2D-DD model, Figure 7b, and the 2D-DD with a Z-dipole model, Figure 7c, revealed comparable emission patterns. While the z-dipole component does not greatly affect the observed emission pattern, it does significantly impact the polarization of the emitted fluorescence. Extracting the Euler angles from the emission pattern gives us the expected modulation depth trace (blue dashed line). By varying the z-component of our model, we adjusted the expected trace to match the observed trajectory. This procedure gave us a value for the magnitude of the z-dipole relative to the magnitude of the X and Y axis dipoles. In the case of this trajectory a magnitude of 0.42 was assigned to the z-dipole. In some cases a phase shift is visible between the estimated and observed trajectory. The phase shift could be due to a rotation
of the quantum dot since these measurements were taken sequentially, not simultaneously. Other possible origins of this phase shift are discussed in Appendix 2.

Using defocused emission pattern imaging and emission polarization modulation we have examined an assumption about the nature of the CdSe-ZnS quantum dot emission dipole structure. Further we have established our ability to assign orientation values to emission patterns via pattern matching with simulations - an important step towards extracting molecular orientation from chiral molecules.
CHAPTER 4

ORIENTATION IMAGING OF CHIRAL MOLECULES BY DEFOCUSED EMISSION PATTERN IMAGING

In reference 31, asymmetric emission patterns from chiral helicene molecules were observed, albeit with low signal to noise. This distinct asymmetric pattern must be replicable in order to extract orientation values from chiral molecules. As a starting point, we expanded on the work of Enderlein and others using the Kirkwood model for a chiral emitter we modified the existing code to add two coherently coupled dipoles and produce an interference pattern simulating that of a chiral molecule.

By defocusing the sample, the distance traveled through the collection optics is different for the interior angles and the exterior angles; this produces an interference pattern on the detector. The form of the interference patterns depends primarily on the defocused depth, the type of emitter, and the emitter orientation. By comparing experimental emission patterns against a library of simulated emission patterns it is possible to assign orientation values to the individual molecules.

The code used to generate the library of emission patterns is based on a 2003 paper by Enderlein et al and adapted to an Igor Pro code base by a post-doctoral researcher in our group, Dr. Kevin McCarthy. First, the s and p polarized components of the electric field vectors are obtained for a dipole perpendicular and parallel to the interface, the parallel components contain the angular dependence. These electric and magnetic field amplitude components are found in reference 33 by integrating the electric and magnetic fields of the dipole over the angular space (one polar angle, and an azimuthal angle limited by the numerical aperture of the objective). As shown in
reference 33, the solutions to these integrations for individual parallel and perpendicular components are sums of Bessel functions which depend on the orientation of the source dipole, indices of refraction, and numerical aperture of the objective. These functions are reproduced in the Igro Pro code to obtain the amplitude for the parallel and perpendicular components of the electric and magnetic field from the source dipole. From these amplitudes, the z component of the Poynting vector is calculated and this gives the position dependent intensity distribution, i.e. the emission pattern.

Although Enderlein solves this for a single dipole emitter, it is possible to expand this procedure to 2D dipoles without much modification. A 2D dipole is approximately equivalent to two coherently added (effectively adding the intensity profiles, not the electric fields) orthogonal single dipoles. By running the procedure twice, once for a dipole along the x direction and another along the y direction and adding the electric fields before calculating the Poynting vector, a 2D interference pattern is obtained.

Simulations of chiral emission patterns\textsuperscript{32} were based on the Kirkwood model of optical activity\textsuperscript{68}, in which (as shown in Figure 8) two non-coplanar, non-orthogonal achiral emitters are coupled to generate a chiroptical response. In this model, an initial electric field generates an electric dipole in a molecule; this electric field then generates an electric dipole (along a different direction) in a neighboring molecule. The interference between initial wave and the induced waves results in a rotation of the electric field.\textsuperscript{25,26,69}

This model can be adapted to generate emission patterns by running the simulation for two dipoles and adding the electric fields before calculating the Poynting vector (coherent addition). One difference to note, because it was not trivial to modify the code
to incorporate the distance between the coupled dipoles, this distance is mimicked by adding a phase factor into the electric field of the second dipole as shown below:

\[ E = E_1^s \cos \theta_1 + E_1^p \sin \theta_1 + e^{-i\xi} (E_2^s \cos \theta_2 + E_2^p \sin \theta_2) \]

\[ B = B_1^s \cos \theta_1 + B_1^p \sin \theta_1 + e^{-i\xi} (B_2^s \cos \theta_2 + B_2^p \sin \theta_2) \]

Where \( E \) and \( B \) are the the electric and magnetic field vectors, \( s \) and \( p \) refer to the \( s \) and \( p \) refer to the polarization states parallel to the glass interface, the number specifies a particular dipole and \( \xi \), a phase shift, is applied to dipole 2. Figure 8, shows the simulated emission patterns for a selection of chiral axis orientations. For orientations in which the chiral axis is parallel to the optical axis, the emission patterns show a lack of bilateral symmetry, recreating qualitatively the lack of bilateral symmetry observed earlier. We can also see that as the chiral axis becomes perpendicular to the optical axis the bilateral symmetry is restored, to a point where the patterns look qualitatively similar to symmetric \( z \)-oriented emission patterns in 2D and 1D dipole systems.
As discussed in the paper, there are two major difficulties with extracting orientation information from chiral molecules. The helicenes used have low photostability corresponding to a small number of detected photons, whereas obtaining defocused emission patterns requires high signal to noise and long exposure times. A further complication is that there is a large parameter space. In addition to the two Euler angles of the chiral axis, the phase between dipoles and relative magnitude of the dipoles are adjustable parameters. Although each set of parameters generates a unique image, this large parameter space could make matching computationally difficult for molecules, such as helicenes, with a molecular structure that does not obviously match the Kirkwood model. In this case, the electric dipole strengths and angle between dipoles is an adjustable parameter and it would be necessary to calibrate the DEP with a molecule.
whose orientation is approximately known. Despite this difficulty it is possible make qualitative matches and estimate the orientation of the chiral axis, provided that the molecules fluoresce long enough to obtain an emission pattern.
Given the signal to noise constraints with extracting orientation values from helicenes, we wanted to investigate the chiroptical response of a molecule with restricted orientations. With a restricted orientation, it would be easier to investigate local environmental effects on a single molecule’s chiroptical response. A good candidate for this was an M-helicene dimer (MH2)\textsuperscript{70} derived from the bridged triarylamine helicenes.\textsuperscript{71} This molecule has a stronger chiroptical response than the monomer – essential for an FECD measurement – and it was hoped that the long molecular frame would restrict the orientation of the molecule when resting on glass.

A thorough description of the experimental procedure can be found in Appendix 2. Briefly, the excitation polarization of a standard inverted microscope configuration was alternated between left (LCP) and right circularly polarized (RCP) light. For this particular experiment a variable retarder corrected for the distortion of the circular polarization produced by the dichroic mirror. The FECD trajectories of hundreds of M-helicene dimer (MH2) molecules were obtained and for each molecule the local background was subtracted. The individual FECD trajectories show, in Figure 10, an
interesting behavior – there are discrete photobleaching events attributable to the photobleaching of one sub-unit. After these events the sign and sometimes magnitude of the anisotropy parameter is maintained. This observation of a change in anisotropy value after sub-unit photobleaching illuminates our query about the role of local molecular environment in a single molecule chiroptical response.

Figure 10: FESC trajectories of the PDI dimer collected at 457nm excitation. Trajectories start under LCP light and switch to RCP light after 10 frames. Each frame has a 2 second exposure.
Figure 11 shows a distribution of anisotropy parameters for MH2 and two control molecules. There were two control samples used to establish that we are indeed measuring a chiroptical response and not linear polarization artifacts. The fluorosphere standard is insensitive to excitation polarization; any width in the distribution of anisotropy values is due to variation in laser intensity (likely due to movement of the optics). This sample had a mean -0.0002 anisotropy with a standard deviation of ~0.02. The PDI standard is a linear, centrosymmetric molecule and does not have a preference for left or right circularly polarized light. Any width in this distribution is due to a residual ellipticity in the circularly polarized excitation. The PDI distribution had a mean anisotropy of -0.01 with a standard deviation of 0.125 from 200 molecules. The MH2 distribution showed a net negative anisotropy with larger anisotropy values correlated to lower count rates. This is consistent with the sign observed by the CD spectrum (Figure 9) with a peak at -0.12.
This peak is close to one standard deviation of the PDI distribution and the mean anisotropy was -0.09 with a standard deviation of ~0.22 from 400 molecules. The large distribution was attributed to shot noise, intensity fluctuations, and a lower signal than the PDI. The standard deviation of the mean of the PDI is 0.01 (with a mean of -0.01) for 200 molecules giving a 95% confidence interval (two standard deviations) from 0.008 to -0.028 and the MH2 had a standard deviation of the mean of 0.011 (with a mean of -0.09) for 400 molecules giving a 95% confidence interval from -0.068 to -0.11. This measurement confirms that we observed a net chiroptical response. The low signal and poor photostability of this molecule are issues that need to be addressed in future experiments.

Future studies of helicenes will focus on new dimer, trimer, and even hexamer forms (see Figure 12) of this molecule. The chemistry scheme for synthesizing these
dimers may extend itself to these new helicene configurations – at least to an M-P helicene conformation. As was suggested by some of the partially photobleached MH2 FECD trajectories, local helicenes may add to chiroptical response of the dimer, trimer, or hexamer species. An FECD survey of M-P dimer (a dimer with a left and right handed helicene), M trimer, and M hexamer molecules would help reveal the extent to which the chiroptical response of the helicene can be added to or amplified by neighboring helicenes.

Figure 12: Proposed dimer, trimer and hexamer molecules for future single molecule chiroptical spectroscopy experiments.
CHAPTER 6

FECD OF A SINGLE ACHIRAL MOLECULE

During preliminary experiments with MH2, DiI-C18 (1,1’-dioctadecyl-3,3,3’,3’-tetramethylindocarbocyanine perchlorate) was used as a standard, due to it being a linear, achiral molecule. However, in private communications, Dr. Bart Kahr pointed out that DiI-C18 was not a good control, because it belongs to the C$_{2v}$ point group, and for certain molecular orientations could in fact show a preferential absorption for left or right circularly polarized light.

In his paper, “Optical rotatory power of water” Dr. Kahr showed that achiral molecules can have a chiroptical response for certain fixed orientations. Figure 13 shows that between the mirror planes of symmetry DiI-C18 has a non-zero chiroptical response.

In an achiral molecule the magnetic and electric dipole moments are orthogonal to each other; however, a chiroptical response is still possible due to projections of the electric and magnetic dipole moments onto the wave vectors of absorbed photons. This can occur for all molecules belonging to the C$_{2v}$, D$_{2d}$, S$_4$, or C$_s$ point groups. Molecules that are centrosymmetric will not show this response.

![Figure 13: Rotatory strength tensor superimposed on the molecular frame of a DiI-C18 molecule. Red/blue regions indicate a preferential absorption for right/left circularly polarized light. Figure provided by unpublished work from Dr. Bart Kahr at NYU.](image)
Belonging to the same $C_{2v}$ point group, DiI-C18 has rotatory strength tensor with a similar shape to that of water. Shown in Figure 13 is the rotatory strength tensor superimposed on the DiI-C18 structure. DiI-C18 is a cyanine dye that has been a model fluorescent probe in the realm of single molecule measurements\textsuperscript{47,73-76} due to its high photostability and high signal budget. DiI-C18 has a peak absorption near 514nm which makes it compatible with PMMA. When embedded in a PMMA polymer layer, DiI-C18 can fluoresce for a period of 5-30 minutes before photobleaching. This high photostability, in addition to easily obtainable defocused emission patterns makes DiI-C18 a promising candidate for correlating molecular orientation with chiroptical response.

Figure 14 shows the DiI-C18 molecules and the rotatory strength tensors with respect to the optical axis of the microscope. For orientations where the mirror planes of the molecule are parallel or orthogonal to the optical axis, the observed anisotropy will be close to zero. For all other orientations the anisotropy will be non-zero with a maximum positive or negative anisotropy occurring when the mirror plane of the molecule is 45° from the optical axis.

The FECD trajectories in Figure 15 were collected using the 514nm line of an Argon ion laser. In order to avoid reflection off of the dichroic mirror, a top down microscope configuration was used in lieu of the standard inverted setup. A 514nm notch filter (SuperNotch filter from Kaiser Optical) was used to block the laser light from the collection optics. The FECD trajectories were collected on a PhotonMax CCD camera.
with an exposure time of 2 seconds per frame. The excitation polarization was switched between left and right circularly polarized light with a half cycle dwell time of 15 frames. Current results (Figure 14) comparing emission patterns and FECD trajectories suggest a compelling correlation between molecular orientation and circular anisotropy.

![Figure 15](image)

**Figure 15**: (Top Left) FECD trajectory of DiI showing low anisotropy values. (Bottom Left) The DEP of the corresponding trajectory along with a matching simulated DEP (Axial tilt of 0°). (Top Right) FECD trajectory of DiI-C18 showing high anisotropy values. (Bottom Right) The DEP of the corresponding trajectory along with a matching simulated DEP (Axial tilt 30°).

Molecules that are in plane show the smallest anisotropy, whereas molecules with about a ~30° out of plane tilt show the largest anisotropy values. The anisotropies are large when compared to those expected from simulations (off by near 2 orders of magnitude); this is likely due to a residual ellipticity in the excitation polarization. As seen in Figure 16 (next page), a survey of molecules, with a large anisotropy (|g| ~ 0.3) shows that those molecules with a positive anisotropy parameter have their dipole axis oriented along the y-axis (and those with negative anisotropy parameters are aligned along the x-axis). Using emission patterns to extract the DiI-C18 molecular orientation and knowing the lab frame where this linear polarization artifact will be the most severe, will enable us to select molecules where the linear polarization artifacts are minimized or separate the artifact from the circular anisotropic signal.
Figure 16: Defocused emission patterns of DiI (left) with the corresponding anisotropy values (right). Those patterns with the largest corresponding anisotropy values (blue – positive anisotropy, red – negative anisotropy) are aligned along the x or y axis.
CHAPTER 7

POLARIZATION RESOLVED SPECTRA OF SINGLE PDI2 MOLECULES

One of the major experimental hurdles with FECD measurements of single molecules has been the poor photostability of the helicene monomer and dimer. These molecules typically bleach within a minute after being exposed to about 1µW/µm² of 457nm laser light. The absorption at 457nm prohibits use of PMMA to reduce photobleaching and fluorescence intermittency because PMMA will fluoresce at this wavelength. The helicenes are also not as bright as other organic molecules (such as DiI-C18); this reduces the overall signal to noise of the experiment and demands longer camera exposure times. For these reasons, it was necessary to explore other molecular systems that may have strong chiroptical responses with greater photostability.

In the previous chapter, PDI was used as a standard for the FECD measurements with the helicene dimer and was found to have excellent photostability (PDI can last for 10-20 minutes in PMMA). We became interested in a chiral dimer made up of two PDI sub-units. In the literature, PDI2 has been shown to have a strong circular anisotropy in the 514nm-532nm wavelength range, making this an excellent target for FECD/emission pattern experiments, although our own measurements showed a red-shifted, ~560nm, CD absorption peak. Recent FECD trajectories of PDI2 have showed very small anisotropy values, this may be due to the orientation of the molecule in the PMMA, or due to a mismatch between the excitation wavelength (514nm or 532nm) and the peak of the CD spectrum. PDI2 may still prove a useful target for FECD measurements but further experiments in this direction need to be done.
PDI2 has also demonstrated interesting spectral properties due to the polarization dependent nature of its absorption spectrum. In a recently published paper, Spano et al. simulated absorption and emission spectra of the PDI dimer for two orthogonal polarization states. The dimer was expected to show photophysical properties analogous to H and J aggregates in conjugated polymers, due to two Davyдов components in each vibronic state.

Figure 17: (Left) Electronic transitions in the PDI dimer. Blue(red) lines correspond to x-transitions(y-transitions). (Right) Simulated emission spectra of the unpolarized (black), X(blue), and Y(red) transitions. Reprinted from reference 79.

Figure 18: Structure of the PDI dimer with the dipole moments and x and y directions illustrated. Reprinted from reference 79.

Figure 17 shows the electronic structure and the absorption spectrum of PDI2 in which the two PDI sub-units are fixed 86° apart from each other. The C2 symmetry of PDI2 leads to a Davyдов splitting of the excited states, as shown in the electronic structure diagram in Figure 16. The symmetric (+) and antisymmetric (-) states are polarized in the direction of $\mu_1+\mu_2$ and $\mu_1-\mu_2$ respectively. Figure 17 shows the calculated
absorption spectrum for x- and y-polarized transitions (x and y correspond to the molecular axis as shown in Figure 18), along with the unpolarized absorption spectrum. This unpolarized absorption spectrum, which we have confirmed through solution phase UV-Vis measurements (see Figure 19), manifests this Davydvov splitting in broadened peaks and a shoulder in the 0-0 transition. Though obscured in bulk solution phase measurements due to orientation averaging, we may be able to probe these X and Y transitions independently using single molecule spectral measurements.

A recent experiment looking at single PDI dimer molecules with s and p polarized light suggests this is possible. Figure 20 (a and b) shows the emission spectra of two PDI2 molecules observed under s-polarized (red) and p- polarized (blue) linear excitation. Figure 19(c) shows the emission spectrum of the PDI monomer under s- and p-polarized excitation. In Figure 19(a) two distinct emission spectra can be observed with a splitting between peaks of ~390cm⁻¹, close to Spano’s prediction of ~403cm⁻¹. No such splitting is observed for the PDI monomer where only an intensity difference is observed.
Future experiments will quantitatively establish the orientation of the PDI2 molecule to ascertain that we are indeed observing the Davydo\textquoteright\textregistered splitting. It would also be interesting to perform these polarization resolved spectral measurements on PDI dimer species with linkers besides binaphthalene. Linkers with less than or greater than a 90° angle between sub-units or even PDI monomers stacked end to end would be expected to show very different ratios between the upper and lower Davydo\textquoteright\textregistered components. Perhaps a progression of these dimers with different linker angles could be developed to probe the polarization and spectral properties of the coupled oscillator system – a goal which could converge nicely our chiral DEP simulations based on a Kirkwood (i.e. coupled oscillator)
model. The tools exist to quantitatively analyze PDI2 - and these other hypothetical dimers - in terms of its orientation, polarization, and spectral properties.
DISCUSSION AND FUTURE WORK

In this thesis, I have discussed the experimental difficulties in measuring the orientation and chiroptical response of single molecules. With regards to orientation, we have established a method for determining orientation of single chiral molecules based on defocused emission pattern imaging. More photostable chiral molecules, such as the PDI dimer, will quantitatively improve orientation determination from imaging of chiral emission patterns due to higher signal to noise. The PDI dimer is closer in form to the Kirkwood model on which our emission pattern code is based – making matches easier by restricting the angle between dipoles and making the two dipoles equal in magnitude.

We have observed the chiroptical response of single helicene dimer molecules and found that while the statistics of individual helicene dimer molecules is representative of bulk properties, any individual molecule can have a chiroptical response very different from the mean value. Many of the single molecules showed a dynamic chiroptical response, likely due to partial photobleaching of the dimer sub-unit. Future experiments will investigate the chiroptical response of helicene m-p dimers, m-trimers and m-hexamers in order to probe the effect of multiple chiral groups on a molecule’s chiroptical response.

Further improvements to the FECD measurements have been made using a variable retarder to correct for polarization distortion due to the dichroic mirror and we currently have the capability to perform FECD measurements in a top down microscope configuration which avoids the dichroic mirror altogether. Future improvements to this setup will focus on using strain free objectives (to further
reduce polarization distortion) as well as removal of oxygen from the sample to improve molecule photostability (and hence signal to noise).

There are promising first results looking at the chiroptical properties of single achiral molecules where the high photostability of the DiI-C18 provides the opportunity to measure both the chiroptical and the molecular orientation. While there seems to be a residual ellipticity, it is shown in Chapter 6 that it is possible to filter out the molecules most affected by this distortion. Finally, a survey of the chiral PDI dimer reveals polarization dependent H and J character which may be probed by single molecule spectroscopy. Measurements are currently underway to determine if this Davydov splitting is consistent among the individual molecules. Using defocused emission patterns we will be able to excite, linearly polarized light along the x and y directions of the molecular frame in order to resolve the individual Davydov components.
APPENDIX 1

VARIABLE RETARDER ALIGNMENT

In an effort to reduce linear polarization artifacts, three different microscope configurations were used, each with benefits and caveats. The first two use a liquid crystal variable retarder (LCVR) to either correct or circumvent the effect of the dichroic on circularly polarized excitation. A setup for performing FECD experiments and instructions for alignment in which circularly polarized light is generated after the dichroic is described. Two configurations of optics will be needed for this: one to align the quarter waveplate relative to the linear polarizer and one to align the LCVR to the linear polarizer. The body of the TE-300 microscope has been modified (by using a lens tube (SM1 thread) to objective (M25 thread) adapter). A quarter waveplate ($\lambda/4$ #1) has been placed in the lens tube above the dichroic and before the objective. Because rotating mounts could not be used the quarter waveplate is placed in the lens tube with an arbitrary orientation – the linear polarizer will have to be aligned to this waveplate.

With the quarter waveplate in the microscope, place the linear polarizer (lin #1) before the entrance to the microscope. Remove the objective and place a mirror above the quarter waveplate, place a 50/50 beam splitter before the linear polarizer. Using this configuration, rotate the linear polarizer until the retroreflected beam is at a maximum (indicating that the transmission axis of the linear polarizer and the fast or slow axis of the waveplate are aligned) and record this number (for determining the purity of polarization). Rotate the linear polarizer until the retroreflected beam is at a minimum intensity (this indicates circularly polarized light is being generated), record the intensity.
of the beam and the orientation of the transmission axis of the linear polarizer relative the fast axis of the quarter waveplate. The waveplate is now aligned with the linear polarizer.

Next, turn the objective turret and move the quarter waveplate out of the beam path. Place another quarter waveplate ($\lambda/4$ #2). Align this waveplate to the linear polarizer as described above (do not touch the linear polarizer, adjust the waveplate). Make sure waveplate #1 and #2 have their fast axes aligned (this will create a zero waveplate for the next step).

Place $\lambda/4$ #1 and #2 in the beam path. Place another linear polarizer above the #2 waveplate and align it to be orthogonal with linear polarizer #1. Place the LCVR between the linear polarizers (and the zero waveplate made by the two quarter waveplates). Set the LCVR to act like a half waveplate and rotate the LCVR to maximize light through the beam path. The LCVR and quarter waveplate #1 are now aligned with linear polarizer #1. Remove linear polarizer #2 and quarter waveplate #2.

Place a mirror above waveplate #1 and repeat the retroreflection check, this time with the LCVR in the beam path. Make sure the orientation of the LCVR gives a minimum for the retroreflected beam. Everything is now aligned. Note that the LCVR cannot act like a true zero waveplate, though it gets quite close, the minimum intensity will not match that of the LCVR acting as a half waveplate.

The benefit of this configuration is that circularly polarized light is not encountering the dichroic and thus avoids the problem of reducing polarization purity. The other benefit is that if the LCVR is used as a polarization switch, it can be coupled to the trigger signal from the camera (using the program “FECD Automate”) and the
FECD polarization switching can occur automatically based on a specified number of dwell frames. One cautionary note when using the LCVR in this fashion, the LCVR cannot serve as a perfect zero waveplate; the retardance approaches zero as the applied voltage increases. Thus using the LCVR as a zero and half waveplate switch may not be ideal for maintaining circular polarization purity. The success of this setup depends on the s and p polarization being reflected in equivalent intensities, this varies from dichroic to dichroic, this worked for the 457nm dichroic but not the for the 514nm dichroic.

The other option for using the LCVR is to use it to correct for the retardance of the dichroic. The easiest way to do this is to first align the LCVR as discussed above, then remove it from the excitation path. Setup, using the retroreflection method, circularly polarized light in the excitation path. Place a detector (the photodiode works best because the signal history can be recorded using labview) above the objective and place a linear polarizer between the objective and the photodiode. With the first linear polarizer and quarter waveplate send circularly polarized light into microscope, rotate the second linear analyzer and record the modulation depth that is registered by the detector. For circularly polarized light, the modulation depth should be close to 0. Without correcting for the dichroic this modulation depth will likely be in the 0.10-0.25 range – the value will depend on the wavelength and dichroic used. It would be ideal to leave the objective in place, as high NA objectives are often strained and can further reduce polarization purity. However, placing a diverging beam on the detector greatly increases the noise of the detector signal making it difficult to obtain the modulation depth, remove the objective if needed.
After measuring the modulation depth of the circularly polarized light reflecting off of the dichroic, insert the LCVR between the first linear polarizer and the quarter waveplate. Adjust the LCVR voltage until the modulation depth is on the order of ~0.01. A different voltage will likely be needed for correcting both left and right circularly polarized light. One final caveat, the operating wavelength for this LCVR is between 450nm-600nm, the LCVR will not function properly using 405nm excitation.
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