Calculations of the Mueller scattering matrix for a DNA plectonemic helix

D. B. Shapiro
Lawrence Berkeley Laboratory, University of California, Berkeley, California 94720 and Graduate Group in Biophysics, University of California, Berkeley, California 94720

P. G. Hull
Department of Physics, Tennessee State University, Nashville, Tennessee 37209-1561

A. J. Lunt
Lawrence Berkeley Laboratory, University of California, Berkeley, California 94720

J. E. Hearst
Lawrence Berkeley Laboratory, University of California, Berkeley, California 94720 and Graduate Group in Biophysics, University of California, Berkeley, California 94720

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The polarization state of a beam of light is fully described by the four elements of the Stokes vector. How the Stokes vector is transformed upon scattering is described by the sixteen element Mueller scattering matrix. Polarized light scattering provides a useful tool to study macromolecular structure. In particular, measurements of Mueller matrix elements have been used to study chromosome structure; changes in these measured quantities can be used to monitor structure as a function of cell cycle. Such measurements done on helical structures can be compared to theoretical computations to determine the geometrical parameters describing the helix. The matrix elements that are most sensitive to the chiral parameters of the helix are largest when the wavelength of light is of the order of the pitch of the helix. Therefore, polarized light scattering measurements made on DNA plectonemic helices would provide the most information in the far ultraviolet (UV) and x-ray region. The Mueller matrix elements are calculated using the coupled dipole approximation in the orientation average at wavelengths in the visible, ultraviolet, and x-ray regions. Each base-pair of the idealized plectonemic helix is represented by a single dipole. A complex polarizability tensor is assigned to each dipole. Calculations are sensitive to the writhe and polarizability of the DNA molecule.

I. INTRODUCTION

A plectonemic DNA helix is an interwound closed DNA molecule. The interwinding is known as supercoiling and is measured by writhe. The DNA linking number can be expressed as the sum of writhe and twist. Because supercoiling affects many biological processes,1–3 much attention has been focused on the study of supercoiled DNA in the form of the plectonemic helix. Computational studies have been made in which energy considerations are used to predict the distribution of linking number between twist and writhe.4–5 Other determinations of the distribution of linking number have been done using Monte Carlo simulations.6–8 The confirmation of these models has relied on electron microscopy and dynamic light scattering.9,10 Polarized light scattering may provide a new nonintrusive method for studying DNA plectonemic helices, providing information other techniques cannot.

Polarized light scattering has become a useful tool in many scientific disciplines.11–14 Polarized light scattering yields more structural information than total scattering intensity measurements.15 In particular, it has been proposed that polarized light scattering may be used to study macromolecular structure. This application has been successful, in certain cases, using visible light. However, more structural information is obtainable when the wavelength of light used is comparable to the dimensions of the molecule being probed. With the increasing availability of synchrotron radiation, it is likely that polarized light scattering can be extended to the X-UV and x-ray region, and possibly provide new insights into macromolecular structure.

The polarization effects of scattering are fully described by the elements of the 4x4 Mueller scattering matrix. The Mueller matrix has been calculated for a variety of structures using electromagnetic theory. For the study of macromolecules a lot of attention has been paid to the S_{14} matrix element (also known as circular intensity differential scattering) because of its sensitivity to the chiral properties of the scattering particle. Diaspro and Nicollini calculated S_{14} for a representation of chromatin by assigning a triaxial polarizability to points along a helical contour.16 Each point was assumed to correspond to an ellipsoidal nucleosome. The internal field caused by interactions between nucleosomes was accounted for. Patterson et al. calculated S_{14} for a model superhelix.17 Their model consisted of a coiled coil. S_{14} was calculated by Patterson et al. using the first Born approximation where the induced internal field is ignored. Bustamante et al. examined the feasibility of measuring S_{14} from an ensemble of randomly oriented helices in the soft x-ray region using the first Born approximation.18 In this work we calcula-
late the Mueller matrix elements for a model of a DNA plectonemic helix using the coupled dipole approximation in the orientation average. The coupled dipole approximation accounts for internal interactions and retardation. We find that the intensity of scattered light from the DNA plectonemic helices used in our calculation would be larger for a given incident intensity in the soft x-ray region than for the same incident intensity in the UV and visible. Several matrix elements show sensitivity to the writhe of the DNA plectonemic helix.

II. MUELLER MATRIX FORMALISM

The Mueller scattering matrix describes how the polarization state of light is altered upon scattering. The polarization state of light is fully described by the elements of the Stokes vector, \( \{I, Q, U, V\} \). These elements are defined in terms of components of the electric field parallel and perpendicular to the scattering plane, the plane defined by the incident and scattered light.

\[
I = \langle E_I^* E_I + E_I E_I^* \rangle, \\
Q = \langle E_I^* E_J - E_J E_I^* \rangle, \\
U = \langle E_I^* E_J + E_J E_I^* \rangle, \\
V = \langle i(E_I E_J^* - E_J E_I^*) \rangle.
\]

where the electric field has been written,

\[
E = (E_{r1} + E_{l1}) e^{i(kr - io)},
\]

with the subscripts \( r \) and \( l \) referring to the parallel and perpendicular directions and \( \bar{O} \) denotes the time average. \( k \) and \( r \) are the propagation and position vectors. The element \( I \) corresponds to the total intensity of the light. \( Q \) describes the amount of linear polarization parallel or perpendicular to the scattering plane and \( U \) describes the amount of linear polarization along the diagonal (45° to the scattering plane). \( V \) describes the amount of right or left circularly polarized light.

The Mueller scattering matrix relates the Stokes vector of the incident light to that of the scattered light,

\[
\begin{pmatrix}
I_i \\
Q_i \\
U_i \\
V_i
\end{pmatrix} = \begin{pmatrix}
S_{11} & S_{12} & S_{13} & S_{14} \\
S_{21} & S_{22} & S_{23} & S_{24} \\
S_{31} & S_{32} & S_{33} & S_{34} \\
S_{41} & S_{42} & S_{43} & S_{44}
\end{pmatrix} \begin{pmatrix}
I_i \\
Q_i \\
U_i \\
V_i
\end{pmatrix},
\]

where the subscripts \( i \) and \( s \) refer to the incident and scattered light.

The Mueller matrix elements have been categorized as dipole elements or retardation elements, and helicity or non-helicity elements.\(^{19,20}\) The dipole elements, \( S_{11}, S_{22}, S_{33}, S_{44}, S_{12}, S_{13}, S_{14}, S_{23}, S_{24}, S_{22}, S_{33}, S_{34}, S_{44}, S_{12}, S_{13}, S_{14}, S_{23}, S_{24}, \) and their transposes, are nonzero even for small, poorly scattering particles. All the retardation elements (those that are not dipole elements), except for \( S_{14}, S_{24} \) and their transposes, become identically zero in the orientation average for small, weakly interacting particles. Calculations using the first Born approximation (which is valid only for weakly interacting particles) confirm that the retardation elements are zero in the orientation average.\(^{19}\) \( S_{14}, S_{24} \) and their transposes are small for weakly interacting particles in the orientation average and may be considered quasiretardation elements. The retardation elements are generally more sensitive to the induced electric field resulting from internal interactions in the particle. The off-diagonal block elements, \( S_{13}, S_{14}, S_{23}, S_{24}, \) and their transposes are helicity elements. They are zero in the orientation average unless the particles are chiral.

III. COUPLED-DIPOLE APPROXIMATION

The net electric field within a dielectric material in an applied field was calculated by Kirkwood.\(^{21}\) An application of Kirkwood's treatment of dipolar interactions within a dielectric material to the problem of light scattering, known as the coupled-dipole model, was originally developed by Purcell and Pennypacker\(^{22}\) and further developed by other authors.\(^{19,23-25}\) The scatterer is subdivided into discrete subunits. The polarizability is assigned to a point for each subunit by using Maxwell–Garnett theory.\(^{26}\) Analytical solutions to the coupled-dipole approximation applied to particles in the orientation average have been obtained.\(^{19,27}\) In this work we use a fortran code based on one written by Singhamz\(^{27}\) to calculate the scattered electric field from a single particle whose orientation is defined by three Euler angles. The orientation average is accomplished by integrating over the Euler angles using a Gaussian–Legendre method. This numerical method that we have adapted converges faster than a Romberg method used previously.\(^{28}\)

Using the coupled-dipole approximation, the scattered electric field, \( \mathbf{E}' \), is given by

\[
\mathbf{E}'(\mathbf{r}_d) = \frac{k^2}{r_d} e^{ik\mathbf{r}_d} \sum_{j=1}^{N} \mathbf{a}_j \cdot \mathbf{E}_j. 
\]
\[ E_i = E_i^0 e^{ikr_i} + \sum_{j \neq i}^N a_{ij} \hat{\alpha}_j E_j + b_{ij} (\hat{\alpha}_j E_j \cdot \hat{n}_{ij}) \hat{n}_{ij}, \]

where

\[
a_{ij} = \frac{e^{ikr_{ij}}}{r_{ij}} \left( k^2 - \frac{1}{r_{ij}^2} + \frac{ik}{r_{ij}} \right),
\]

\[
b_{ij} = \frac{e^{ikr_{ij}}}{r_{ij}} \left( -k^2 + \frac{3}{r_{ij}^2} \right),
\]

\[ E_i^0 \] is the incident electric field, \( \hat{n}_{ij} \) is a unit vector pointing from the \( i \)th to the \( j \)th subunit, and \( r_{ij} \) is the distance between the \( i \)th and \( j \)th dipoles. When radiation arising from other dipoles is ignored then only the first term in Eq. (5) need be included. This is equivalent to the first Born approximation applied to a set of point polarizable groups.

The coupled dipole approximation assumes that each subunit acts as a single radiating dipole. This assumption is invalid when the wavelength of light approaches the size of the subunit. It has been suggested that the wavelength should be at least 10 times the size of the subunit for accurate calculations.\(^4\) Another limitation of the coupled dipole approximation is that the calculation involves the inversion of a large \((3N \times 3N)\) matrix. In addition, there is a double sum over the number of subunits included in the calculation. Thus, these calculations become more computer intensive as the number of subunits increases. Our calculations were conducted using a CRAY supercomputer.

### IV. THE POLARIZABILITY

The polarizability tensor is defined by the directions of the principle axes and the strengths along these axes,

\[
\bar{\alpha} = \alpha_{tt} \hat{n} \hat{n} + \alpha_{nn} \hat{\mathbf{p}} \hat{\mathbf{p}} + \alpha_{pp} \hat{\mathbf{m}} \hat{\mathbf{m}}, \tag{6}
\]

and

\[ \alpha_j = \alpha_j^t + i \alpha_j^n, j = tt, nn, pp, \tag{7} \]

where \( t, n, p \) are in the directions of the principle axes in the chosen coordinate system. The real and imaginary parts of the magnitude of the polarizability can be obtained directly from absorption measurements using the following relations:\(^29,30\)

\[
\alpha_j^t = A(\nu) 2 \frac{C_1}{\pi} \int_0^\infty \frac{x \xi(x) dx}{\sqrt{x^2 - \nu^2}}, \tag{8}
\]

\[
\alpha_j^n = A(\nu) \frac{-C_1 \xi(\nu)}{\nu}, \tag{9}
\]

where \( \nu \) is the frequency of light, \( P \) means the principle part, \( x \) is a variable of integration, \( \xi \) is the measured extinction, \( C_1 = 6909c/8 n^2 N_0 \), with \( c \) representing the speed of light, and \( N_0 \) is Avogadro's number, and \( A(\nu) \) is given by

\[
A(\nu) = (n_s^2 + 2)^2 \frac{9n_s^2}{9n_s^2}, \tag{10}
\]

where \( n_s \) is the index of refraction of the solvent.

A base pair is represented by a single point polarizable group. The polarizability was calculated parallel and perpendicular to the base pair plane using the average absorption of each nucleotide. The absorption above 2000 \( \text{Å} \) was assumed to be completely in the base-pair plane. An isotropic absorption was assigned at 1200 \( \text{Å} \) to represent continuum transitions in the vacuum UV. These assignments are identical to those used previously.\(^31\) In the x-ray region, the polarizability was assigned to each dipole element using absorption data for benzene.\(^32\)

### V. THE MODEL PLECTONEMIC HELIX

A three-dimensional contour is defined to approximate a DNA plectonemic helix. A code written in MATHEMATICA, given in the Appendix, generates this contour. The plectonemic helix is composed of two interwound simple helices attached by a loop on each end. The simple helices are 180° out of phase.

Each base pair is represented by ellipsoidal polarizable groups. The principle axes of the polarizability of the ellipsoids are defined with respect to the tangent, normal, and binormal of the curve representing the plectonemic helix. Figure 1 shows the two and four turn helices used in this work. Each had 462 base-pairs. The dimensions of the two turn helix are 700 \( \text{Å} \) long, and helix radius 25 \( \text{Å} \). Helix radius refers to the radius of the helical region of the plec-
tonemic model. The four turn helix is 626 Å long with a helix radius of 22 Å. Each dipole is about 3.42±0.02 Å apart.

VI. RESULTS

To determine structural information that may be obtainable from polarized light scattering measurements we have made calculations of Mueller matrix elements as a function of wavelength, writhe (or pitch), and the strength, direction and anisotropy of the polarizabilities. We do not report all the matrix elements here although our fortran code generates all of them. We have chosen to report the elements, \( S_{11}, S_{12}, S_{13}, S_{34}, \) and \( S_{14} \) because these represent dipole elements, retardation elements, and helicity elements. All the matrix elements are normalized by the total intensity element \( S_{11} \) except for \( S_{11} \) itself. We have made calculations of DNA in water. We attempted to calculate Mueller matrix elements at wavelengths where measurements are possible; where there is a sufficient transmission through water. It would have been desirable to calculate the Mueller matrix elements at the carbon K-edge (the binding energy of the 1s electron, \( \lambda = 43.6 \) Å) but this wavelength is too small compared to the width of the DNA base-pair, so use of the coupled-dipole approximation cannot be justified. Instead we have used the polarizability of the base pair (taken as that of benzene) at the carbon edge (\( \lambda = 43 \) Å) for calculations of the Mueller matrix elements at 200 Å.

Figure 2 shows calculated Mueller matrix elements at different wavelengths, 6330, 2600, and 200 Å for the two turn helix. The strengths of the polarizabilities in Å\(^3\), obtained using absorption data and Kramer-Kronig relations, are as follows:

<table>
<thead>
<tr>
<th>Wavelength</th>
<th>( \alpha_{\text{H}} )</th>
<th>( \alpha_{\text{nn}} )</th>
<th>( \alpha_{\text{pp}} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>6330 Å</td>
<td>12.8</td>
<td>44</td>
<td>44</td>
</tr>
<tr>
<td>2600 Å</td>
<td>15.2+0.88i</td>
<td>48.8+36.8i</td>
<td>48.8+36.8i</td>
</tr>
<tr>
<td>200 Å</td>
<td>0.72+0.4i</td>
<td>0.72+0.4i</td>
<td>0.72+0.4i</td>
</tr>
</tbody>
</table>

\( S_{11} \) (plotted on a log scale in Fig. 2) shows that our model plectonemic helices have the largest intensity of scattering for a given incident intensity at 200 Å. The other matrix elements in Fig. 2 are plotted as a relative intensity; they are normalized by \( S_{11} \). The \( S_{13} \) and \( S_{34} \) elements are zero when the wavelength is 6330 Å on the scales used in Fig. 2. \( S_{34} \) and \( S_{13} \) are largest at 200 Å.

In Fig. 3, the Mueller matrix elements calculated for a two and four turn plectonemic helix are shown. The structure of the total intensity element \( S_{11} \) is not very sensitive to a change in the writhe of the helices (Fig. 3). \( S_{14} \) shows the most change with respect to the number and position of nodes, maxima, and minima for the calculation conducted at 200 Å.

The excitations at the carbon edge involve the core carbon electrons. The polarizability is thus mainly isotropic. However, there are resonant excitations to the \( \pi \) and \( \sigma \) molecular orbitals. These resonances result in an anisotropy in the polarizability parallel (\( \pi \)) or perpendicular (\( \sigma \)) to the DNA 20 Å strand. We have investigated the sensitivity of the Mueller matrix to an anisotropy in the polarizability at 200 Å. To illustrate this sensitivity, several matrix elements are shown in Fig. 4, for the two turn helix, where the following polarizability strengths in Å\(^3\) were used:

<table>
<thead>
<tr>
<th>Curve</th>
<th>( \alpha_{\text{H}} )</th>
<th>( \alpha_{\text{nn}} )</th>
<th>( \alpha_{\text{pp}} )</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.8+0.48i</td>
<td>0.64+0.32i</td>
<td>0.64+0.32i</td>
</tr>
<tr>
<td></td>
<td>0.64+0.32i</td>
<td>0.8+0.48i</td>
<td>0.8+0.48i</td>
</tr>
</tbody>
</table>

\( S_{13} \) and \( S_{12} \) did not show much sensitivity to anisotropy in the polarizability. Of those shown in Fig. 4, \( S_{11} \) is least sensitive to polarizability anisotropy.
VII. DISCUSSION

Measuring several Mueller matrix elements imposes more constraints on modeling efforts than any single Mueller matrix element and thus provides a more accurate determination of structure. For example, different structures may produce similar $S_{34}$ but have entirely different $S_{14}$. The most commonly measured element, $S_{11}$, is least sensitive to structure. Thus, if light scattering is to be used to uncover new information regarding the structure of plectonemic helices, the investigation should involve several Mueller matrix elements.

The model used here to approximate DNA plectonemic helices differs in many respects from real samples obtainable in the laboratory. Whereas DNA molecules in water undergo various internal motions, our model is rigid. A sample prepared in the lab would have a distribution of writhe for a given linking number. A better model would account for internal motions and inhomogeneity of the sample. These internal motions and inhomogeneity could diminish the magnitude and angular distribution of the measured Mueller matrix elements. We have investigated scattering at the carbon edge using polarizabilities calculated from benzene absorption data at 43 Å (at the C-edge) for calculations conducted at 200 Å. The calculations could be performed at 43 Å if more than one point polarizable group is used to represent a single base-pair. The addition of point polarizable groups, however, increases the time and memory requirements of the computer. We have found this calculation to be too computationally intensive for the computer facilities available to us. Nevertheless, if more resources were available, it should be possible to do the calculation at 43 Å.

Despite the model's limitations several useful observations can be made. Using our calculated polarizabilities, the wavelength dependence of the total intensity element, $S_{11}$,
The Mueller matrix elements calculated for model plectonic helices were sensitive to writhe in the visible, ultraviolet, and x-ray regions. The sensitivity of to writhe at 6330 Å is consistent with experiments conducted by Nicollini et al. on supercoiled DNA. The sensitivity to polarizability anisotropy in the carbon edge could aid in structural determination by carrying out near edge polarized light scattering spectroscopy.

With recent advances in x-ray optical elements, all of the Mueller matrix elements could be measurable in the soft x-ray region using synchrotron radiation. Damage due to radiation would be severe but might be overcome by using a flow system. The elements $S_{11}$, $S_{12}$, and $S_{13}$ could be measured using two detectors that rotate about the sample along two perpendicular scattering planes. Light emitted from a synchrotron is highly polarized in the plane of the accelerator ring. In order to measure $S_{12}$ the two scattering planes should be parallel and perpendicular to the plane of the accelerator. Relative to the scattering plane that is perpendicular to the ring, the incident light is vertically polarized, and relative to the scattering plane that is parallel to the ring, the incident light is horizontally polarized. The intensities of the scattered light detected at the two perpendicular planes would be proportional to $S_{11} + S_{12}$ and $S_{11} - S_{12}$. Thus $S_{12}$ is the difference of these measured intensities and $S_{11}$ would be their sum. $S_{13}$ can be measured in the same way with the two mutually perpendicular scattering planes being rotated 45°. A chopper that alternately blocks light to one of the two detectors would allow for a modulation of the difference signal between the two detectors. New developments in the use of multilayer devices could be used to measure the other elements in the second and third columns of the Mueller matrix. Thus, for example, if vertical polarizers were installed in front of the detectors that move in the planes parallel and perpendicular to the ring, the detected intensities would be $S_{11} + S_{12} + S_{22}$ and $S_{11} - S_{12} - S_{22}$. The difference between these measured intensities and the subtraction of the previously measured element gives $S_{22}$. Using diagonal polarizers and a combination of polarizers and 1/4 wave plates allows the measurement of the third and fourth row elements. In order to measure the elements in the fourth column, the incident light must be varied from right to left circularly polarized. One method of doing this would be to use right and left elliptically polarized light from above and below the ring plane. This would allow for measurement of the fourth row Mueller matrix elements in the same way that they are measured conventionally.

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APPENDIX

The contour is defined in terms of the parameter $u$. $n$ is the number of turns for a single, simple helix. $d$ defines the size of the loop. The following equations define the contour of the two turn plectonemic helix. The four turn helix is defined with $n = 4$ and $d = 0.8$. The Display command creates an image file, "3dg," that can be plotted. This Mathematica code, shown below, is readily translatable to other computer languages.

```mathematica
<<DNAaxe.coord.m
<<thicken.twist.m
del = 4
nbases = 462
alpha = N(2 Pi nbases/(10.5*(2+2d)))
n = 2
a3 = 14.00

d = 1.6

DNA1[u_] := thicken[DNAaxe1[u, a, alpha], Hue[u/(2Pi)]]
DNA2[u_] := thicken[DNAaxe2[u, a, alpha], Hue[u/(2Pi)]]
DNA3[u_] := thicken[DNAaxe3[u, a, alpha], Hue[u/(2Pi)]]
DNA4[u_] := thicken[DNAaxe4[u, a, alpha], Hue[u/(2Pi)]]
DNA5[u_] := thicken[DNAaxe5[u, a, alpha], Hue[u/(2Pi)]]
DNA6[u_] := thicken[DNAaxe6[u, a, alpha], Hue[u/(2Pi)]]
DNA7[u_] := thicken[DNAaxe7[u, a, alpha], Hue[u/(2Pi)]]
DNA8[u_] := thicken[DNAaxe8[u, a, alpha], Hue[u/(2Pi)]]

DNA1[u_] := Append[DNA1[u], Hue[u/(2Pi)]]
DNA2[u_] := Append[DNA2[u], Hue[u/(2Pi)]]
DNA3[u_] := Append[DNA3[u], Hue[u/(2Pi)]]
DNA4[u_] := Append[DNA4[u], Hue[u/(2Pi)]]
DNA5[u_] := Append[DNA5[u], Hue[u/(2Pi)]]
DNA6[u_] := Append[DNA6[u], Hue[u/(2Pi)]]
DNA7[u_] := Append[DNA7[u], Hue[u/(2Pi)]]
DNA8[u_] := Append[DNA8[u], Hue[u/(2Pi)]]

SetOptions[ParametricPlot3D, Boxed -> False, Axes -> False]
SetOptions[ParametricPlot3D, DisplayFunction -> Identity, Lighting -> False]

dessin = ParametricPlot3D[DNA[u], {t, -1, 1 + 2d}, {u, 0, 2Pi}, PlotPoints -> {nbases, 10}]

dessin = ParametricPlot3D[DNA1[u], {t, -1, 1 + 2d}, {u, 0, 2Pi}, PlotPoints -> {nbases, 10}]

dessin = ParametricPlot3D[DNA2[u], {t, -1, 1 + 2d}, {u, 0, 2Pi}, PlotPoints -> {nbases, 10}]

dessin = ParametricPlot3D[DNA3[u], {t, -1, 1 + 2d}, {u, 0, 2Pi}, PlotPoints -> {nbases, 10}]

dessin = ParametricPlot3D[DNA4[u], {t, -1, 1 + 2d}, {u, 0, 2Pi}, PlotPoints -> {nbases, 10}]

DNAaxe1[u_] := {a*Sin[(n*Pi*(u + 1 - 1/(2*n)))/a], Sin[n*Pi*(u - d)], f3[u]}
DNAaxe2[u_] := {Cos[n*Pi*(u - d)], Sin[n*Pi*(u - d)], f3[u]}
DNAaxe3[u_] := {a*Sin[(n*Pi*(u - 1 - d + 1/(2*n)))/a], b*Sin[n*Pi*(u - d)], f3[u]}
DNAaxe4[u_] := {a*Sin[(n*Pi*(u - 1 - d + 1/(2*n)))/a], b*Sin[n*Pi*(u - d)], f3[u]}

b := (n*Pi)/2