Mueller matrix polarimetry and polar decomposition of articular cartilage imaged in reflectance

Ruby N. Huynh,1 George Nehmetallah,2 and Christopher B. Raub1,*

1Department of Biomedical Engineering, The Catholic University of America, 620 Michigan Avenue NE, Washington, DC 20064, USA
2Department of Electrical Engineering and Computer Science, The Catholic University of America, 620 Michigan Avenue NE, Washington, DC 20064, USA
*raubc@cua.edu

Abstract: Articular cartilage birefringence relates to zonal architecture primarily of type II collagen, which has been assessed extensively in transmission, through thin tissue sections, to evaluate cartilage repair and degeneration. Mueller matrix imaging of articular cartilage in reflection is of potential utility for non-destructive imaging in clinical and research applications. Therefore, such an imaging system was constructed to measure laser reflectance signals, calibrated, and tested with optical standards. Polar decomposition was chosen as a method to extract fundamental optical parameters from the experimental Mueller matrices, with performance confirmed by simulations. Adult bovine articular cartilage from the patellofemoral groove was found to have ~0.93 radians retardance, low diattenuation of ~0.2, and moderately high depolarization of 0.66. Simulations showed that variation in depolarization drives inaccuracy of depolarization and retardance maps derived by polar decomposition. These results create a basis for further investigation of the clinical utility of polarized signals from knee tissue and suggest potential approaches for improving the accuracy of polar decomposition maps.

© 2021 Optical Society of America under the terms of the OSA Open Access Publishing Agreement

1. Introduction

Polarization-based imaging techniques have great utility for evaluating tissue microstructure from measured optical parameters. Birefringence, diattenuation, and depolarization in tissues vary over the microscale, permitting contrast-enhanced imaging [1, 2]. Cells and macromolecules influence the bulk polarization properties of tissues, so polarization image contrast can be interpreted as relating to cell-based tissue remodeling [3] and macromolecular organization [4]. For example, Mueller matrix imaging determined tumor borders in unstained thin sections [5] and thick biopsies [6], glucose levels in bulk tissues [7], and macromolecular alignment within tissues, even in the presence of multiple scattering [8]. Several comprehensive reviews and books were published in the last decade discussing polarized light applications for biological studies [9].

Mueller matrix polarimetry measures the sixteen element Mueller matrix of a specimen, completely describing its polarization-transforming properties and allowing calculation of more fundamental parameters. The elements of this square matrix characterize the specimen experimentally but are not reproducible and consistent across many laboratories and instruments. For example, the element values may depend on orientation of an anisotropic specimen with respect to polarizing elements in the instrument. More fundamental parameters are derived from experimental Mueller matrices using several techniques, notably polar [10] and differential decomposition [8]. Polar decomposition divides the matrix into three submatrices to produce parameters of retardance, depolarization, diattenuation, and azimuth. The order of these submatrices is mathematically feasible and does not necessarily reflect the order of optical effects.
along the light path in the specimen. Further, polar decomposition can produce inaccurate parameter values due to propagation of measurement uncertainties or evaluation of singular matrices. The relative contribution of natural signal variation versus computational error can be difficult to determine in microstructurally complex tissues.

Mueller matrix imaging polarimetry produces maps of the Mueller matrix elements in an imaged field-of-view. Transillumination and reflection configurations are well-suited for thin/transparent and thick/opaque biological specimens, respectively. Recent advances in Mueller matrix imaging polarimetry include fast and single snapshot acquisition [11], multi-wavelength imaging [12, 13], depth resolution through optical coherence tomography [14] or OCT correlated to polarimetry [15], and super-resolution through structured illumination [16]. Multi-modal instruments consisting of a Mueller matrix imaging system with confocal reflectance and second harmonic signal imaging capabilities were recently described and used to measure polarization properties of extracellular matrix in collagen-rich tissues [17, 18].

Articular cartilage is a thick, semi-opaque tissue containing a type II collagen network microstructure that is altered by degeneration and osteoarthritis. Specifically, alterations such as surface roughening, fibrillation, and disorganization of the parallel-aligned collagen fibrils of the superficial zone of articular cartilage occur with and may even be preceded by altered surface and sub-surface polarization properties [19, 20]. Supporting this hypothesis, recent studies of polarized reflectance from bovine articular cartilage demonstrate a loss of texture with sandpaper roughening of the articular surface and/or removal of the superficial zone, and a higher reflectance contrast parameter indicating a greater relative contribution of superficial scattering and/or more depolarized light to the reflectance signal [1]. Mueller matrix-derived parameters could potentially serve as indicators for cartilage microstructural alterations relevant to mechanical function and pathology.

In order to determine the feasibility of collecting the full polarized reflectance properties of articular cartilage for potential use in orthopedic studies, we developed a laser-based Mueller matrix imaging polarimeter in reflection, demonstrated mitigation of laser speckle through an electrically actuated speckle reducer, calibrated the instrument and tested the accuracy of polar decomposition against depolarization, retardance, and diattenuation standards, and in simulations. We hypothesized that the polar decomposition maps would reveal additional information beyond polarimetric contrast maps reported previously using a different instrument [1, 21], related to collagen network microstructure in the superficial zone. Findings indicated that laser-based Mueller matrix imaging polarimetry produces accurate (<4%) and unbiased maps of articular cartilage with low speckle and visible birefringence texture similar to conventional polarized reflectance contrast imaging. The Mueller matrix decomposition technique was found to be less accurate for retardance with a depolarization index averaging >0.6 and a coefficient of variation of >8.3% across the image. Furthermore, a simulated field of view with uniform retardance but a range of random normal depolarization and diattenuation indices demonstrated accurate reconstruction of optical maps for coefficients of variation of <6-7%. Beyond that, more pixels produced erroneous retardance values associated both with and without singular value decomposition. Some regions retained accurate retardance calculations even with high variation in depolarization and diattenuation. Two novel features of this work, discussed further below, are: 1) use of laser illumination for Mueller matrix imaging, which will allow adding modalities of laser speckle contrast imaging and digital holographic microscopy to future configurations of the instrument, and 2) a simulation of Mueller matrix polar decomposition that tracks pixel calculations across image space to clearly visualize errors in computed parameters. These findings demonstrate feasibility of collecting Mueller matrix images and polar decomposition parameters from articular cartilage, with potential use in explant/graft studies, and with further validation, in various clinical situations.
2. Materials and methods

2.1. Experimental setup

Figure 1 presents the experimental setup. The illumination source, a He-Ne laser (633 nm), passes through a spatial filter, lens and the polarization state generator (PSG) consisting of a rotating polarizer (P1, Thorlabs, LPVIS100-MP2, $>10^5$;1 extinction ratio at 633 nm) and zero-order quartz quarter-wave plate (R1, Thorlabs, WPQSM05-633), both designed for 633 nm wavelength light. The photons backscattered from an oblique incident beam ($\theta = 60^\circ$) were collected by a 10X infinity-corrected objective (NA=0.3, Nikon) followed by a tube lens (TTL200, Thorlab) and the polarization state analyzer (PSA). The PSA comprised of a rotating quarter-wave plate (R2, Thorlabs) and polarizer (P2, Thorlabs). A monochrome TE-cooled CCD camera (Lumera, Inc. Infinity 3-1, 1.4 Megapixels, 70 decibels dynamic range, 8 e- read noise, <0.15 e-/s dark current noise at room temperature, 8-bit data acquisition) recorded digital images of the polarized reflectance over $1392 \times 1040$ pixels corresponding to $733 \times 547 \mu m$. A laser speckle reducer (Optotune, 6° divergence, 10 mm clear aperture, Edmund Optics) was placed after the PSG to remove speckle. The speckle reducer rotates at 300 Hz, creating an averaging effect when signal is acquired over more than one speckle reducer rotation period.

![Fig. 1. Schematic of the Mueller matrix experimental setup for reflectance imaging. Abbreviations are indicated in the legend.](image)

Instrument calibration and alignment involved identifying extinction, fast and slow axes through elements of the PSG and PSA by rotating each optical element separately to achieve maximum extinction of linearly polarized light (crossed polarizers only) and circularly polarized light (right and left circular polarization from the PSG extinguished by left and right polarization configurations of the PSA) using a power meter. Polarizers were also rotated in 15° increments and transmitted power recorded versus rotation angle.

In order to obtain the Muller matrix of samples, 36 intensity images from 6 configurations of the PSG and PSA were used as previously described (see also Table 1) [22]. Image averaging over 64 frames reduced noise from the sensor, which was cooled to 0 °C. The noise pixel histograms were of irregular distribution shape indicating a likely mixture of Poisson noise, readout noise and residual variation from speckle. Acquisition of the full dataset took 20 minutes and the recorded images were imported into MATLAB. All calculations were performed through a custom-written script (v2020b, The Mathworks, Natick, MA).
Table 1. 36 polarization configurations of the PSG and PSA

| PSG (input) | H | V | P | M | R | L |
|-------------|---|---|---|---|---|---|
| H           | HH| VH| PH| MH| RH| LH |
| V           | HV| VV| PV| MV| RV| LV |
| P           | HP| VP| PP| MP| RP| LP |
| M           | HM| VM| PM| MM| RM| LM |
| R           | HR| VR| PR| MR| RR| LR |
| L           | HL| VL| PL| ML| RL| LL |

*H: horizontal linear; V: vertical linear; P: -45° linear; M: +45° linear; R: right circular; L: left circular

2.2. Sample preparation

Standard controls were a Spectralon diffuse reflectance standard (SDRS, Labsphere), zero-order quarter waveplate, and linear polarizer, imaged separately and in combination. A mirror was placed just after the waveplate and polarizer to reflect transmitted light to the camera; otherwise, the mirror was replaced by the SDRS. The Spectralon reflectance standard is known to be a strong depolarizer, weak diattenuator and weak linear retarder [23]. Therefore it was used to introduce large depolarization into imaged controls also containing a polarizer and quarter waveplate, which do not possess strong depolarization.

Cartilage samples were harvested as previously described [1]. Briefly, bovine cartilage explants were harvested by puncturing femoral condyle articular cartilage with a 3 mm diameter dermal punch then undercutting with a scalpel. The explants later were fixed in neutral buffered formalin and cut in half longitudinally to retain the articular cartilage surface and superficial half of the explant. During image acquisition, explants were optically coupled to the glass coverslip by phosphate-buffered saline while the coverslip was placed at an angle to direct specular reflectance away from the light collection path. To evaluate the robustness of polar decomposition results to the quality of input images, the 36 acquired raw images were also processed with a median filter (radius=6 pixels) and independently, added Gaussian pixel noise with standard deviation 5% of image mean (CV=5%) was added to all raw images after normalization by M11. The median filter was chosen to preserve edge contrast for the observed lattice structures with typical size ~20-30 µm or 38-57 pixels, with a radius of 6 pixels ensuring randomly-distributed noise reduction without removal of important image features. Polar decomposition results were compared between the unaltered and processed raw image sets.

2.3. Simulation

Three simulation studies were performed. In the first study, a specimen was simulated as a matrix train of an ideal linear retarder and a potentially non-ideal depolarizer. The retardance was kept fixed at R=1.57 while the depolarization index, ∆, was varied from 0 to 0.99. Simulated images were 40 × 40 pixels, and random normal distributed pixel variation in ∆ was set at standard deviations of 0, 0.05, and 0.2, across the depolarization map, regardless of average depolarization index. Then, Mueller matrices were simulated and processed with polar decomposition to compare the derived depolarization and retardance parameters to their inputs. In the second study, the more general case of a potentially non-ideal depolarizer, a linear retarder, and a potentially non-ideal diattenuator with attenuation factors px and py, 0 < px, py < 1, in a single optical train was simulated. The polar decomposition input and output R, ∆, and diattenuation index, D, were recorded for a number of simple cases: index/parameter values were combinations of 0, 0.5, and 1, with no pixel variation, and are listed in Table 5. Finally, in the third study, simulated optical
maps were created by Lu-Chipman decomposition of the same matrix train as in the second study: depolarizer, retarder, and diattenuator. In this simulation $R=0.9$, $D=0.88$ and $\Delta=0.3$. The input retardance was kept constant while depolarization and diattenuation were varied from 0-20% coefficient of variation (CV, pixel standard deviation/pixel mean across the image), by only adding random numbers to non-zero diattenuation and depolarization matrix elements. For example, a diattenuation map input with mean $D=0.88$ and normally-distributed random pixel variation producing a standard deviation of 0.088 would have a CV of 10%.

3. Theory

3.1. Stokes parameters and Mueller matrix

Determination of the experimental Mueller matrix from 36 acquired images listed in Table 1 and previously described [22], was done according to Table 2, to create 16 Mueller matrix element maps. Alternative image acquisition strategies calculate the experimental Mueller matrix with fewer images and less intrinsic error [24], but for initial feasibility determination, this strategy was followed. Improved acquisition strategies will be implemented in the future. The experimental Mueller matrix associated with each pixel was filtered according to the method of Cloude to ensure the matrices were physically realizable [25]. Image addition and subtraction solves the equation $\mathbf{S}' = \mathbf{M}\mathbf{S}$ at each pixel location, where $\mathbf{S}$ and $\mathbf{S}'$ are the Stokes vectors from the PSG and reflected signal, respectively. Mueller matrix elements were calculated pixel by pixel and normalized by $M_{11}$.

### Table 2. Calculation of 16 Mueller Matrix element maps

| $M_{11}$ | $M_{21}$ | $M_{31}$ | $M_{41}$ |
|----------|----------|----------|----------|
| $HH + HV + VH + VV$ | $HH + HV - VH + VV$ | $PH + PV - MH - MV$ | $RH + RV - LH + LV$ |
| $M_{22}$ | $M_{32}$ | $M_{42}$ |
| $HH - HV + VH - VV$ | $PH - PV - MH + MV$ | $RH - RV - LH + LV$ |
| $M_{33}$ | $M_{43}$ |
| $HP - HM + VP - VM$ | $PR - PL - MR + ML$ |
| $M_{44}$ |
| $HR - HL + VR - VL$ |

3.2. Mueller matrix decomposition (MMD)

Following the MMD technique of Lu and Chipman [26] with extra parameters from Manhas et al. [7], the 4×4 Mueller matrix is decomposed into the product of three matrices corresponding to a depolarizer ($M_{\Delta}$), a retarder ($M_{R}$), and a diattenuator ($M_{D}$):

$$M = M_{\Delta}M_{R}M_{D}. \quad (1)$$

The corresponding three individual Mueller matrices have the formats

$$M_{\Delta} = \begin{bmatrix} 1 & \bar{0}^T \\ P_{\Delta} & m_{\Delta} \end{bmatrix}, \quad (2a)$$

$$M_{R} = \begin{bmatrix} 1 & \bar{0}^T \\ \bar{0} & m_{R} \end{bmatrix}, \quad (2b)$$

and

$$M_{D} = \begin{bmatrix} 1 & \bar{D}^T \\ \bar{D} & m_{D} \end{bmatrix}, \quad (2c)$$

with element symbols explained below.
The diattenuation magnitude can be obtained directly from algebraic calculation of Mueller matrix elements,

\[ D = \sqrt{\frac{M_{12}^2 + M_{13}^2 + M_{14}^2}{M_{11}}} \].

(5)

The diattenuation vector is presented as:

\[ \vec{D} = \frac{1}{M_{11}} \begin{bmatrix} M_{12} \\ M_{13} \\ M_{14} \end{bmatrix} \].

(6)

The 3×3 submatrix \( m_D \) of \( M_D \) can be written as:

\[ m_D = \sqrt{1 - D^2} I + \frac{1 - \sqrt{1 - D^2}}{D^2} \vec{D} \vec{D}^T \].

(7)

Where \( I \) is the identity matrix.

After determining \( M_D \), the product of \( M_\Delta \) and \( M_R \) is calculated as:

\[ M_\Delta M_R = MM_{D^{-1}} = M' \].

(8)

Consider \( m \) as a 3×3 submatrix of \( M \) and \( m' \) a submatrix of \( M' \). Then, from Eq. (8):

\[ m' = m_\Delta m_R, \]

and

\[ m_\Delta^2 = m'(m')^T. \]

(9)

(10)

The submatrix \( m_\Delta \) will be calculated based on the Cayley-Hamilton theorem. If \( \lambda_1, \lambda_2, \lambda_3 \) are eigenvalues of \( m'(m')^T \) then the eigenvalues of \( m_\Delta \) are \( \sqrt{\lambda_1}, \sqrt{\lambda_2} \) and \( \sqrt{\lambda_3} \). An expression for \( m_\Delta \) is then:

\[ m_\Delta = \pm \left\{ m'(m')^T + \left( \sqrt{\lambda_1}, \sqrt{\lambda_2}, \sqrt{\lambda_3} \right) I \right\}^{-1} \times \left\{ \left( \sqrt{\lambda_1} + \sqrt{\lambda_2} + \sqrt{\lambda_3} \right) m'(m')^T + \sqrt{\lambda_1, \lambda_2, \lambda_3} I \right\}. \]

(11)

Subsequently, the minus sign is used if the determinant of \( m' \) is negative, otherwise the plus sign is used.

Calculation of \( M_\Delta \) requires \( P_\Delta \), given as:

\[ \vec{P}_\Delta = \frac{\vec{P} - mD}{1 - D^2}, \]

in which \( \vec{P} \) is a polarizance vector of \( M \) and has the formula

\[ \vec{P} = \frac{1}{M_{11}} \begin{bmatrix} M_{21} \\ M_{31} \\ M_{41} \end{bmatrix}. \]

(13)

Now that \( M_\Delta \) is computed, the net depolarization \( (\Delta) \) is then given by Eq. (14):

\[ \Delta = 1 - \left( \frac{\text{tr}M_\Delta - 1}{3} \right). \]

(14)
The retardance matrix is expressed as:

\[ M_R = M_{\Delta}^{-1}(M'). \]  \hspace{1cm} (15)

From there, the retardance can be obtained by:

\[ R = \cos^{-1}\left(\frac{\text{tr}M_R}{2} - 1\right). \] \hspace{1cm} (16)

Singular Mueller matrices are treated by singular value decomposition, according to equations presented in Appendix B of [26].

The total retardance matrix from the decomposition process can be further expressed as a combination of a linear retarder (\(\delta\)) and a circular retarder (\(\Psi\)) [7]:

\[ \delta = \cos^{-1}\left(\sqrt{\left[M_R(2,2) + M_R(3,3)\right]^2 + \left[M_R(3,2) - M_R(2,3)\right]^2} \right)^{1/2} - 1; \] \hspace{1cm} (17)

and

\[ \Psi = \tan^{-1}\left(\frac{[M_R(3,2) - M_R(2,3)]}{[M_R(2,2) - M_R(3,3)]}\right). \] \hspace{1cm} (18)

4. Results

4.1. Experimental results

4.1.1. Power loss through polarizing elements

Power measurements of the illumination beam show non-ideal behavior of the polarizer and quarter waveplate in the polarization state generator, and small departures from rotational invariance (Fig. 2). The total beam intensity without polarizing elements was 264.5 ± 3.4 μW, cut to 128.2 ± 3.74 μW (48.5% loss) and 126.4 ± 3.5 μW (47.8% loss) with the PSG set for linear and circular polarization, respectively (Fig. 2(A)). As the linear polarizer was rotated from 0 to 180° in 15° increments, the signal intensity fluctuated slightly within the range of 122-134 μW. These measurements could be used in the future to apply multiplicative correction factors to the polarized reflectance images with different polarization configurations (Table 1) used to construct the experimental Mueller matrix coefficient maps.

![Fig. 2. (A) Illumination intensity before and after generation of polarization states. (B) Linear polarization intensity at 15° polarizer rotation intervals. Error bars represent standard deviation of 10 consecutive measurements.](image)

4.1.2. Speckle reduction

To reduce laser speckle from the reflected signal, a speckle reducer was inserted in the illumination path (Fig. 1). Reflectance images of the Spectralon diffuse reflector were smoother after (Fig. 3(B))
than before (Fig. 3(A)) addition of the diffuser. The PSG and PSA were in the parallel (HH) configuration to accept more reflectance signal. Slight vignetting at the image edges was caused by the diameter limit of the incident Gaussian beam. Speckle produced an extended, highly skewed pixel histogram (Fig. 3(C)), compared to a normal pixel intensity distribution after the speckle reducer (Fig. 3(D)). The pattern of speckle from the reflectance standard, while interesting, was of less interest to this study than the illumination pattern with speckle reduction. Indeed, to capture the full Gaussian profile of the speckle reduced signal of Fig. 3(B),(D), the speckle peaks of Fig. 3(A),(C) acquired with the same camera exposure were necessarily saturated. The image CV was 60.0 ± 0.2% before (saturated) and 25.1 ± 0.4% after speckle reduction (n=5 images per group, p<0.001, Student’s t-test).

Fig. 3. Representative images (A,B) and histograms (C,D) of a Spectralon surface in the absence (A,C) and presence (B,D) of the speckle reducer. The scale bar for both images is indicated.

4.1.3. Mueller matrix imaging calibration

To evaluate the accuracy of the polarimeter, the experimental Mueller matrix, polar decomposition (PD) submatrices and derived optical parameters from an imaged quarter waveplate were compared to the theoretical Mueller matrix and expected parameters (Table 3). Experimental matrix elements agreed with theoretical matrix elements from 96.1-100%. Linear retardance from PD was 1.6% lower than the expected value of 1.57 radians, and orientation from PD was lower than expected by 0.17°, within the resolution of the hand-adjusted rotation holder.

4.1.4. Mueller matrix imaging of a reflectance standard, polarizer and waveplate

The Spectralon reflectance standard with and without quarter waveplate were imaged and PD parameters were compared to the waveplate only, revealing high depolarization from Spectralon (Fig. 4). The depolarization coefficient of the SDRS alone and with waveplate were similar at $\Delta=0.958 \pm 0.013$ and $\Delta=0.964 \pm 0.012$, respectively, whereas the waveplate alone had $\Delta=0.013 \pm 0.008$. All depolarization maps were highly uniform (Fig. 4(A),(D),(G)). The retardance map of the waveplate was uniform and close to 1.57 ($R=1.544 \pm 0.005$), but derived
Table 3. The experimentally measured Mueller matrix, decomposed submatrices and parameters of the quarter-wave plate with fast axis oriented vertically.

| raw MM       | filtered MM | theoretical M |
|--------------|-------------|---------------|
| 1.000 0.001 0.030 -0.001 | 1.013 -0.001 0.024 0.001 | 1 0 0 0 |
| 0.001 1.000 0.023 0.034 | 0.003 0.99 0.022 0.034 | 0 1 0 0 |
| 0.002 0.039 0.039 -0.960 | 0.004 0.040 0.033 -0.970 | 0 0 0 -1 |
| 0.006 0.003 1.000 0.015 | 0.012 0.002 0.99 0.021 | 0 0 1 0 |

 retardance values from the SDRS and SDRS + QWP were farther from the expected value with large pixel-level variation at $R=1.16 \pm 0.75$ and $R=1.51 \pm 0.70$, respectively. Diattenuation maps were uniform and close to 0 for the three conditions.

4.1.5. Mueller matrix imaging of bovine articular cartilage

Bovine articular cartilage was imaged in reflectance, and the experimental Mueller matrix element maps (Fig. 5) and Mueller matrix averaged over the entire tissue in the field of view (Table 4) were calculated. Many of the element maps showed lattice-like texture on the imaged articular surface, including on- and off-diagonal maps of $M_{22}$, $M_{32}$, $M_{42}$, $M_{43}$ and $M_{44}$, indicating phase anisotropy. In contrast, the first row ($M_{12}$, $M_{13}$, $M_{14}$) and column ($M_{21}$, $M_{31}$, $M_{41}$), relating to diattenuation, had poorer contrast and less image texture. Notable observations include large and disparate values for $M_{22}=0.224$, $M_{33}=0.372$, and $M_{44}=0.122$ and large values of the non-diagonal elements $M_{42}=0.22$ and $M_{43}=-0.17$.

Table 4. Filtered experimental Mueller matrix of adult bovine articular cartilage (mean±SD).

| Parameters                              | Symbol | Measured values     | Expected values |
|-----------------------------------------|--------|---------------------|-----------------|
| Depolarization (dimensionless)          | \Delta | 0.033±0.009         | 0               |
| Total retardance (rad)                  | $\Psi$ | 1.544±0.005         | 1.57            |
| Linear retardance (rad)                 | $\delta$ | 1.544±0.005         | 1.57            |
| Circular retardance (rad)               | $\Theta$ | 89.83±0.831         | 90°             |
| Diattenuation (dimensionless)           | $D$    | 0.026±0.006         | 0               |

4.1.6. Polar decomposition Mueller matrix maps of articular cartilage

Polar decomposition parameters of articular cartilage were calculated as more fundamental parameters (i.e., free from orientation-dependence) of the experimental matrix maps [17,28]. The depolarization map was uniform and high with depolarization coefficient $D=0.663 \pm 0.035$ (Fig. 6(A)). The diattenuation parameter was also uniform and low compared to a linear polarizer, with $D=0.214 \pm 0.033$ (Fig. 6(C)).
Fig. 4. Mueller matrix decomposition parameters of (A,B,C) spectralon diffused reflectance standard, (D,E,F) quarter-wave plate and (G,H,I) spectralon coupled with quarter-wave plate. (A,D,G) Depolarization, (B,E,H) retardance and (C,F,I) diattenuation maps were compared between the objects. Scale and colormap bars are indicated.

Fig. 5. Normalized Mueller matrix images of a cartilage explant. The scale bar is indicated. The dashed area in $M_{11}$ indicates the extent of cartilage tissue, cut with a razor blade to make a defined corner.
Table 5. Tested inputs of polar decomposition.

| Inputs to MMD | Outputs of MMD | Singular? |
|--------------|----------------|-----------|
| D 0.5 0.5 0.5 | D 1 1 0 | Yes ($\lambda_1=\lambda_2=\lambda_3=0$) |
| 0.5 1 0.5 0.5 | 0.5 1 0 | Yes ($\lambda_1=\lambda_2=\lambda_3=0$) |
| 0.5 0.5 1 0.5 | 0.5 1 | No |
| 1 1 0.5 0.5 | 1 1 0 | Yes ($\lambda_1=\lambda_2=\lambda_3=0$) |
| 0 0.5 0.5 0 | 0.5 0.5 | No |
| 0.5 0.5 0.5 | 0.5 0.5 | No |
| 0.5 0.5 0 | 0.5 0 | No |

Fig. 6. MMD parameters of bovine articular cartilage as (A) depolarization, (B) retardance, (C) diattenuation and (D) azimuth maps. Scale bar is indicated.
Total retardance and orientation maps revealed dissimilar lattice textures (Fig. 6(B),(D)). The retardance map texture patterns were similar to (but inverted from) $M_{22}$ and $M_{44}$ maps. Table 5 compares articular cartilage PD parameters to the SDRS, quarter waveplate, and polarizer. Values indicate the presence of depolarization, retardance and diattenuation in the articular cartilage specimen. Added Gaussian noise and median filtering did not greatly affect the average $R$, $D$, and $\Delta$ from the articular cartilage (Fig. 7).

![Fig. 7. Retardance, diattenuation, and depolarization values are insensitive to small added noise and filtering. The raw images (A) unaltered, (B) with standard deviation $= 0.05$ noise added, and (C) median filtered at a radius of 6 pixels were processed to produce optical maps. The mean ± standard deviation of pixel values are presented for each map. Scale bar and grayscale colorbars (one per column) are indicated.]

### 4.2. Simulation results

For a simulated retarder and depolarizer, polar decomposition produced accurate uniform retardance maps over the entire range of uniform depolarization. When the depolarization index was varied across pixels, uniform retardance maps were most accurate for $\Delta < 0.8 \pm 0.05$, and $\Delta < 0.6 \pm 0.2$ (Fig. 8). For a more general simulated specimen consisting of non-ideal diattenuator, depolarizer, and a linear retarder, several inputs and polar decomposition results were evaluated, listed in Table 5. The singular cases produced an erroneous retardance of $R = 0$, but otherwise PD parameter values were accurate.

A constant retardance along the same optical path as uniform non-ideal depolarization and diattenuation centered at $\Delta = 0.3$ and $D = 0.88$ produced accurate maps for all three parameters, following PD. With higher pixel-level Gaussian variation of $\Delta$ and $D$, more pixels in the retardance map become inaccurate, with the majority of those resulting from evaluation of singular matrices during PD (Fig. 9(A) and 10). Diattenuation index maps appeared similar to the input, while PD-derived depolarization index maps were increasingly inaccurate for pixel CV $> 5\%$. 
Fig. 8. Results of MMD on a simulated specimen with uniform retardance $R=1.57$ and variable depolarization $\Delta$ centered at values between 0 to 1 with Gaussian-distributed random pixel variation creating pixel value standard deviations of 0.05 or 0.2, on a mean of $\Delta=0.3$. Error bars represent the reconstructed $R$ map standard deviation across all $40 \times 40$ pixels.
Fig. 9. Simulation results for polar decomposition. Input and polar decomposition (PD) output (A) retardance maps, $R$, (B) diattenuation maps, $D$, and (C) depolarization maps, $\Delta$, with a range of pixel standard deviation values (expressed as coefficient of variation, %) for input $\Delta$ and $D$. The mean ± standard deviation of each map is displayed in or below each image.
Fig. 10. Simulation results for PD of a field of view of 50 × 50 pixels, with constant retardance $R$ but varying depolarization, $\Delta$, and diattenuation, $D$. More pixels had singular matrix calculations with higher variation in $\Delta$, leading to inaccurate $R$. Meanwhile the depolarization values were highly skewed from the input mean of 0.3. Colorbars with pixel values are presented in the histograms. The depolarization index histogram contains an inset to visualize pixel values from 0 to 1.
5. Discussion

This study describes a Mueller polarimetric imaging system for backscattered light from thick tissues, calibration with standard reflectance and polarization optics, simulation, and measurement of polarization properties of articular cartilage in reflection. Speckle reduction with a diffuser actuated by an electroactive polymer allowed good contrast to reveal lattice textures from articular cartilage reflectance maps. We note that the camera exposure should be larger than the speckle reducer actuation period (3.3 ms) to allow time-averaging of the moving speckle pattern. Error in Mueller matrix measurements and polar decomposition parameters was <4% indicating typical accuracy for measurements involving manual adjustment of optical elements on rotating mounts. Importantly, measurements of depolarization and retardance from a diffuse reflector, articular cartilage, and simulated depolarizers confirmed accurate calculation of retardance only for moderate and low depolarization and more spatially uniform depolarization. Bovine articular cartilage measurements appeared to meet this criterion for accurate reconstruction of retardance by polar decomposition. Finally, the Mueller matrix measurements of articular cartilage showed rich parameter map information about the tissue birefringence, depolarization and diattenuation.

High depolarization made calculation of retardance less accurate as demonstrated by the Spectralon diffuser with waveplate images and simulated depolarizer results. This finding agrees with Bueno et al. who reported that larger depolarization produces more error in computing retardance of human retinas [27–29]. In older subjects, this effect was attributed to loss of transparency/more scattering in the ocular medium or retinal disease such as glaucoma, that led to inaccurate retinal retardance measurements. Similarly, Spectralon obscured retardance from the quarter waveplate in polar decomposition maps. Also, large variation in depolarization contributing to a simulated experimental Mueller matrix led to high uncertainty in retardance calculated from polar decomposition. However, while depolarization in the articular cartilage was moderately high at $\Delta = 0.66 \pm 0.04$, the standard deviation was low, as the CV was $\sim 6\%$. This was apparently small enough to avoid spurious retardance maps, which were robust to added noise and smoothing of the raw images.

The texture of articular cartilage from Mueller matrix images and polar decomposition maps is consistent with previous studies using simple polarized contrast but contains greater spatial information. Several generalizations are possible from experimental Mueller matrix element maps. For the articular cartilage map, the Mueller matrix was non-diagonal, unlike that of several cancer specimens in reflection [30], and other tissues [31], indicating polarization effects besides partial depolarization. $M_{22}$ and $M_{33}$ were unequal, indicating anisotropy of linear depolarization. The linear retardance of aligned type II collagen fibers present in the superficial zone of articular cartilage [1, 2, 20] may partially explain anisotropic depolarization. The feasibility of collecting Mueller matrix images and polar decomposition maps from articular cartilage indicates a potential use of Mueller matrix polarimetry for explant experiments culturing articular cartilage for growth, maturation, and biomechanical studies. The image-based simulation of polar decomposition may be employed in the future to understand observed textures in polarimetric tissue maps, derived from microstructural elements such as collagen fibers of known orientation, birefringence and organization.

Depolarization and retardance of bovine articular cartilage fall within the range of values from other fibrous connective tissues. The depolarization of the cartilage sample measured in this study was 0.66, in the same range as other fibrous tissues including muscle ($\Delta = 0.92$), heart ($\Delta = 0.84$), healthy skin ($\Delta = 0.72$), and chicken articular cartilage ($\Delta = 0.5 - 0.7$) [10, 32]. Multiple scattering from nanoscale biomolecules including collagen, and relatively low absorption in articular cartilage (that does not contain melanocytes or erythrocytes) partially explain the relatively high depolarization. Compared to PS-OCT studies measuring retardance of bovine cartilage, the retardance of primarily superficial zone articular cartilage from this study was 0.93 rad, compared to 0.94 rad for bovine articular cartilage measured by polarization-sensitive
optical coherence tomography [33], 0.52-1.57 rad in chicken cartilage [34], and 0.66-1.48 rad from porcine articular cartilage [35]. This retardance is primarily associated with aligned type II collagen microstructure, with a lesser amount (around 6%) due to co-aligned glycosaminoglycans [36].

The presented instrument, acquisition method, simulation and analysis have strengths and weaknesses, but extend previous work in articular cartilage using simple polarimetric contrast imaging. In our previous work [1, 21], we described the appearance of a lattice-like pattern from bovine articular cartilage imaged with Michelson’s contrast parameter previously used for polarimetric imaging of skin by Jacques et al [37]. This polarimetric contrast texture was reduced following surface scrape, enhanced by removing deeper tissue, aligned with split lines in the superficial zone, and depended strongly on superficial zone thickness. The present work demonstrates the feasibility of collecting Mueller matrix images that capture a similar lattice-like texture from bovine articular cartilage, without further exploring clinical utility or parameter sensitivity to native tissue variability or pathophysiology. The optimal configurations of input polarization states minimizing errors in Mueller matrix have been determined through error propagation and simulation to be the vertices of Platonic solids within the Poincare sphere: for example, a tetrahedron for $4 \times 4$ (input x output) measurements and an octahedron for $6 \times 6$ measurements [38]. Our choice of linear ($H, V, P, M$) and circular ($R, L$) polarization basis states is indeed an octahedron, with vertices along the great circles describing complete linear and circular polarization. In the future, if the imaging setup is modified to use photoelastic modulators, illumination/sensing states will be shifted to elliptical polarization, avoiding linear and circular polarization where input vector phase errors become probable and large. Manipulating polarizers and waveplates on manual mounts over 36 configurations is laborious but produced reasonably small measurement errors (Table 3, Fig. 4), and has been used before [22] with the advantage that six images occur at a minimum intensity, providing a visual accuracy check for polarization state generator/analyzer settings. The choice of laser illumination was not conventional but will allow future addition of laser speckle contrast and digital holographic microscopy modalities to the instrument, which we anticipate will be useful for dynamic biomechanical studies and surface roughness characterization of articular cartilage in direct comparison to polarimetric features. The image-based simulation presented in Figs. 9 and 10 allows prediction of Mueller matrix parameter maps from theoretical distributions of polarimetric parameters, with uniform distributions used in this work a convenient starting point.

6. Conclusion

In summary, we demonstrated that imaging bovine articular cartilage with laser-based Mueller matrix imaging polarimetry and decomposition is feasible, captures image texture related to tissue birefringence, and does not lead to large errors that obscure decomposition parameters. For Mueller matrix imaging using a nonscanned laser, speckle removal is important to obtain high quality images, though we propose that the speckle pattern can be easily re-introduced for speckle contrast imaging. High spatial variation in depolarization produces inaccurate retardance calculations during Mueller matrix decomposition. These basic findings establishing feasibility to image articular cartilage with Mueller matrix imaging suggest future steps to determine the utility of the approach to characterize articular cartilage explants, grafts and tissue removed during orthopedic surgery. Future work includes optimizing the image acquisition for speed and accuracy, adding modalities to the instrument, such as laser speckle contrast imaging, and determining sensitivity of Mueller matrix parameters to cartilage explant growth, maturation, or damage during in vitro culture. Mueller matrix approaches may be adopted for use during arthroscopic surgery if they can operate within the constraints and needs of that clinical environment.

Acknowledgments. R. Huynh thanks the Catholic University of America for funding through the New Millennium Fellowship.
Disclosures. The authors declare no conflicts of interest.

Data availability. Data underlying the results presented in this paper are not publicly available at this time but may be obtained from the authors upon reasonable request.

References

1. R. N. Huynh, G. Nehmetallah, and C. B. Raub, “Noninvasive assessment of articular cartilage surface damage using reflected polarized light microscopy,” J. Biomed. Opt. 22(6), 065001 (2017).
2. R. N. Huynh and C. B. Raub, “Noninvasive surface damage assessment of bovine articular cartilage explants by reflected polarized light microscopy,” Annual International Conference IEEE Eng Med Biol Soc 2016 (2016), pp. 2897–2900.
3. T. T. Tower and R. T. Tranquillo, “Alignment maps of tissues: I. Microscopic elliptical polarimetry,” Biophysical Journal 81(5), 2954–2963 (2001).
4. R. Oldenbourg and T. Ruiz, “Birefringence of macromolecules: Wiener’s theory revisited, with applications to DNA and tobacco mosaic virus,” Biophysical Journal 56(1), 195–205 (1989).
5. A. Pierangelo, S. Manhas, A. Benali, C. Fallet, J. L. Totobenazara, M. R. Antonelli, T. Novikova, B. Gayet, A. De Martino, and P. Validire, “Multispectral Mueller polarimetric imaging detecting residual cancer and cancer regression after neoadjuvant treatment for colorectal carcinomas,” J. Biomed. Opt. 18(4), 046014 (2013).
6. A. Pierangelo, A. Benali, M. R. Antonelli, T. Novikova, P. Validire, B. Gayet, and A. De Martino, “Ex-vivo characterization of human colon cancer by Mueller polarimetric imaging,” Opt. Express 19(2), 1582–1593 (2011).
7. S. Manhas, M. K. Swami, P. Buddhawant, N. Ghosh, P. K. Gupta, and J. Singh, “Mueller matrix approach for determination of optical rotation in chiral turbid media in backscattering geometry,” Opt. Express 14(1), 190–202 (2006).
8. L. Trifonyuk, A. Sdobnov, W. Baranowski, V. Ushenko, O. Olar, A. Dubolazov, L. Pidkamin, M. Sidor, O. Vanchuliak, A. Motrich, M. Gorsky, and I. Meglinski, “Differential Mueller matrix imaging of partially depolarizing optically anisotropic biological tissues,” Lasers Med Sci 35(4), 877–891 (2020).
9. S. Alali and A. Vitkin, “Polarized light imaging in biomedicine: emerging Mueller matrix methodologies for bulk tissue assessment,” J. Biomed. Opt. 20(6), 061104 (2015).
10. M. Sun, H. He, N. Zeng, E. Du, Y. Guo, S. Liu, J. Wu, Y. He, and H. Ma, “Characterizing the microstructures of biological tissues using Mueller matrix and transformed polarization parameters,” Biomed. Opt. Express 5(12), 4223–4234 (2014).
11. M. W. Kudonen, M. J. Escuti, N. Hagen, E. L. Dereniak, and K. Oka, “Snapshot imaging Mueller matrix polarimeter using polarization gratings,” Opt. Lett. 37(8), 1367–1369 (2012).
12. N. Hagen, K. Oka, and E. L. Dereniak, “Snapshot Mueller matrix spectropolarimeter,” Opt. Lett. 32(15), 2100–2102 (2007).
13. M. Borovkova, M. Peyvaste, O. Dubolazov, Y. Ushenko, V. Ushenko, A. Bykov, S. Deby, J. Rebinder, T. Novikova, and I. Meglinski, “Complementary analysis of Mueller-matrix images of optically anisotropic highly scattering biological tissues,” J. Eur. Opt. Soc.-Rapid Publ. 14(1), 20 (2018).
14. G. Yao and L. V. Wang, “Two-dimensional depth-resolved Mueller matrix characterization of biological tissue by optical coherence tomography,” Opt. Lett. 24(8), 537–539 (1999).
15. J. Chue-Sang, Y. Bai, S. Stoff, M. Gonzalez, N. Holness, J. Gomes, R. Jung, A. Gandjbakhche, V. Chernomordik, and J. Ramella-Roman, “Use of Mueller matrix polarimetry and optical coherence tomography in the characterization of cervical collagen anisotropy,” J. Biomed. Opt. 22, 086010 (2017).
16. J. P. Angelo, T. A. Germer, and M. Litorja, “Structured illumination Mueller matrix imaging,” Biomed Opt Express 10(6), 2861–2868 (2019).
17. I. Saytashv, S. Saha, J. Chue-Sang, P. Lopez, M. Laughrey, and J. C. Ramella-Roman, “Self validating Mueller matrix Micro-Mesoscope (SAMMM) for the characterization of biological media,” Opt. Lett. 45(8), 2168–2171 (2020).
18. C. Okoro and K. Toussaint, “Second-harmonic patterned polarization-analyzed reflection confocal microscope,” J. Biomed. Opt. 22, 086007 (2017).
19. H. E. Panula, M. M. Hyttinen, J. P. Arokoski, T. K. Langsjo, A. Pelttari, I. Kiviranta, and H. J. Helminen, “Articular cartilage superficial zone collagen birefringence reduced and cartilage thickness increased before surface fibrillation in experimental osteoarthritis,” Annals of the Rheumatic Diseases 57(4), 237–245 (1998).
20. C. B. Raub, S. C. Hsu, E. F. Chan, R. Shirazi, A. C. Chen, E. Chnari, E. J. Semler, and R. L. Sah, “Microstructural remodeling of articular cartilage following defect repair by osteochondral autograft transfer,” Osteoarthritis and Cartilage 21(4), 860–868 (2013).
21. R. N. Huynh, B. Pesante, G. Nehmetallah, and C. B. Raub, “Polarized reflectance from articular cartilage depends upon superficial zone collagen network microstructure,” Biomed Opt Express 10(11), 5518–5534 (2019).
22. J. S. Baba, J. R. Chung, A. H. DeLaughter, B. D. Cameron, and G. L. Cote, “Development and calibration of an automated Mueller matrix polarization imaging system,” J. Biomed. Opt. 7(3), 341–349 (2002).
23. J. M. Sanz, C. Extremiana, and J. M. Saiz, “Comprehensive polarimetric analysis of Spectralon white reflectance standard in a wide visible range,” Appl. Opt. 52(24), 6051–6062 (2013).
24. S. Alali, A. Gribble, and I. A. Vitkin, “Rapid wide-field Mueller matrix polarimetry imaging based on four photoelastic modulators with no moving parts,” Opt. Lett. 41(5), 1038–1041 (2016).
25. S. Cloude, *Polarisation: Applications in Remote Sensing* (Oxford University Press, 2010).
26. S.-Y. Lu and R. A. Chipman, “Interpretation of Mueller matrices based on polar decomposition,” *J. Opt. Soc. Am. A* 13(5), 1106–1113 (1996).
27. J. Bueno, “Polarimetry in the human eye using an imaging linear polariscope,” *J. Opt. A: Pure Appl. Opt.* 4(5), 553–561 (2002).
28. J. Bueno, “The influence of depolarization and corneal birefringence on ocular polarization,” *J. Opt. A: Pure Appl. Opt.* 6, 1464 (2004).
29. N. Lippok, B. Braaf, M. Villiger, W. Y. Oh, B. J. Vakoc, and B. E. Bouna, “Quantitative depolarization measurements for fiber-based polarization-sensitive optical frequency domain imaging of the retinal pigment epithelium,” *J. Biophotonics* 12, e201800156 (2019).
30. M. R. Antonelli, A. Pierangelo, T. Novikova, P. Validire, A. Benali, B. Gayet, and A. De Martino, “Mueller matrix imaging of human colon tissue for cancer diagnostics: how Monte Carlo modeling can help in the interpretation of experimental data,” *Opt. Express* 18(10), 10200–10208 (2010).
31. V. Sankaran, J. T. Walsh Jr., and D. J. Maitland, “Comparative study of polarized light propagation in biologic tissues,” *J. Biomed. Opt.* 7(3), 300–306 (2002).
32. I. Ahmad, A. Khaliq, M. Iqbal, and S. Khan, “Mueller matrix polarimetry for characterization of skin tissue samples: A review,” *Photodiagnosis and Photodynamic Therapy* 30, 101708 (2020).
33. D. K. Kasaragod, Z. Lu, J. Jacobs, and S. J. Matcher, “Experimental validation of an extended Jones matrix calculus model to study the 3D structural orientation of the collagen fibers in articular cartilage using polarization-sensitive optical coherence tomography,” *Biomed Opt Express* 3(3), 378–387 (2012).
34. P. G. Ellingsen, M. B. Lilledahl, L. M. Aas, L. Davies Cde, and M. Kildemo, “Quantitative characterization of articular cartilage using Mueller matrix imaging and multiphoton microscopy,” *J. Biomed. Opt.* 16(11), 116002 (2011).
35. C. M. Chang, Y. L. Lo, N. K. Tran, and Y. J. Chang, “Optical characterization of porcine articular cartilage using a polarimetry technique with differential Mueller matrix formulism,” *Appl. Opt.* 57(9), 2121–2127 (2018).
36. K. Kiraly, M. M. Hyttingen, T. Lapvetelainen, M. Elo, I. Kiviranta, J. Dobai, L. Modis, H. J. Helminen, and J. P. A. Arokoski, “Specimen preparation and quantification of collagen birefringence in unstained sections of articular cartilage using image analysis and polarizing light microscopy,” *The Histochemical Journal* 29(4), 317–327 (1997).
37. S. L. Jacques, J. C. Ramella-Roman, and K. Lee, “Imaging skin pathology with polarized light,” *J. Biomed. Opt.* 7(3), 329–340 (2002).
38. D. Layden, M. F. Wood, and I. A. Vitkin, “Optimum selection of input polarization states in determining the sample Mueller matrix: a dual photoelastic polarimeter approach,” *Opt. Express* 20(18), 20466–20481 (2012).