Tensorial tomographic differential phase-contrast microscopy

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Abstract—We report Tensorial Tomographic Differential Phase-Contrast microscopy (T²DPC), a quantitative label-free tomographic imaging method for simultaneous measurement of phase and anisotropy. T²DPC extends differential phase-contrast microscopy, a quantitative phase imaging technique, to highlight the vectorial nature of light. The method solves for permittivity tensor of anisotropic samples from intensity measurements acquired with a standard microscope equipped with an LED matrix, a circular polarizer, and a polarization-sensitive camera. We demonstrate accurate volumetric reconstructions of refractive index, birefringence, and orientation for various validation samples, and show that the reconstructed polarization structures of a biological specimen are predictive of pathology.

Index Terms—Computational microscopy, Quantitative phase imaging, Polarization microscopy, Three-dimensional microscopy

1 INTRODUCTION

Quantitative phase imaging (QPI) is a new emerging class of label-free microscopy method to measure endogenous contrast of cell and tissue by turning specimen-induced phase or polarization changes into images. Due to its low phototoxicity and no photobleaching, QPI has become a valuable tool for studying biology, chemistry, and medicine [1]. The fine anisotropic structures of biological samples are characteristic of their molecular arrangements, such as cell membrane and axon bundles, which can now be detected with high sensitivity at scales smaller than the wavelength of light [2], [3]. As such, polarization-sensitive microscopy is widely used in pathology [4], developmental biology [5], and mineralogy [6], especially in combination with phase microscopy techniques [7].

In the past, numerous 2D label-free polarization microscopy techniques have been invented, from classical qualitative methods such as differential interference contrast (DIC) microscopy [8] and polarized light microscopy [9], [10], to more contemporary polarization microscopy in transmission mode that reports quantitative retardance and 3D orientation of optical path [11], [12], [13]. Diffractive polarization microscopy in particular has attracted many attentions recently. In general there are two categories of methods to achieve quantitative polarization microscopic imaging in combination with diffractive optics: holographic measurements and computational illumination. The first category typically employs interferometry and hence requires highly coherent laser illumination [14], [15], [16], [17]. While those methods can image quantitative Jones matrix at high-speed, holographic systems still remain challenging to align, and coherent speckle artifacts maybe present to decline image quality.

Computational illumination methods, on the other hands, very often use partially coherent light sources, either from a programmable LED array or LCD display, to illuminate the sample and create a data stack, from which the phase and anisotropy images are then computationally recovered [7], [18], [19], [20], [21]. This has the flexibility for creating not only angular diversity in the illumination to enable high spatial-bandwidth product images [19], [20], but also polarization diversity to further incorporate the depolarization effects [7], for instance.

To image thicker samples, three-dimensional imaging method are desired to obtain a depth-resolved representation. One well-established technique is polarization sensitive optical coherence tomography [22], which creates cross-sectional images for tissues by point-scanning. Recently reported polarization-sensitive diffraction tomography approaches [23], [24] create volumetric reconstructions of polarization properties that highlights biologically specific structures in model organisms [23]. While often impressive, these methods are usually based on Michelson interferometer setup that requires precise mechanics to either rotate the sample or steer the beam illuminated from different angles, which are challenging to implement. Alternate approaches use high numerical aperture (NA) objective to scan through the semi-transparent samples [25], creating “confocal-like” depth sections of the entire mouse brain. However, such methods require expensive oil-immersion lenses and additional high-quality illumination units [25].

To overcome the challenges of existing techniques, we propose T²DPC, a new 3D differential phase contrast-based polarization sensitive tomographic microscope technique. Differential phase contrast microscopy (DPC) is a low-cost label-free method that can create quantitative tomographic reconstruction of refractive index by capturing a focal stack of the sample of interest illuminated with partially coherent light [26]. Based on DPC principles, we demonstrate how we
can outfit a standard microscope with a programmable LED matrix, a circular polarizer, and a polarization sensitive camera to solve for the permittivity tensor maps of anisotropic samples. In the rest of this article, we first derive a forward model describing how vectorial light propagation through tensorial samples for our experimental setup. We then evaluate our method by analyzing tomographic reconstructions of refractive index, birefringence, and orientation for various calibration samples, and demonstrate that the reconstructed polarization properties of a biological specimen is predictive of pathology.

2 PROPOSED METHOD

Here we describe principle and experimental implementation of T²DPC.

2.1 Experimental setup

The experimental setup is illustrated in Fig.1(a), whose backbone is an inverted microscope. The sample was illuminated from the bottom by an LED matrix with $25 \times 25$ addressable pixels (WS2812B-2020) and a pitch of 3.125 mm. The DPC illumination patterns are depicted in Fig.1(b). We note that there are more advanced illumination patterns, such as ring and gradient patterns, for instance, for improved DPC contrast and robustness [27], [28]. Here, we use semicircular illumination patterns as a first demonstration, as they are standard [26], [28] and give better signal-to-noise ratio (SNR) due to higher illumination radiance. The maximum illumination angle is chosen to match the NA of the objective. To generate a polarized illumination source, we placed a left circular polarizer (Edmund CP42HE) between the LED array and sample. The anisotropic specimen is placed on a mechanical stage (actuator from Thorlabs Z825b) to translate the sample along the optical axis to create a focal stack. After getting diffracted by the sample, light propagates through a 4f system consisting of an objective (Olympus PLN; 20x, 0.4NA for validation samples, and 10x, 0.25NA for biological samples) and a 180-mm tube lens.

![Fig. 1. Workflow of T²DPC. (a) An illustration of the experimental setup. Light from LED array is polarized with a left circular polarizer (LCP) before illuminating the sample. After interacting with the permittivity tensor of the sample, light propagates through a 4f imaging system. A camera with polarization sensitive sensor is place at back focal plane of the tube lens to record $0^\circ$, $45^\circ$, $90^\circ$, $135^\circ$ oriented components of the vectorial light. (b) displays the illumination patterns we use. (c) plots a few representative transfer functions for different polarization measurement configuration under different illumination patterns. (d) shows reconstructed tomographic permittivity tensor of a human heart tissue sample.](image-url)
(Thorlabs AC508). An image is formed in the back focal plane of the tube lens, and captured with a polarization sensitive sensor array (Sony IMX250MZRI), which detects intensity of four linearly polarized components of the light field. The polarized CMOS sensor achieves this by placing micro-lens and wire-grid linear polarizer oriented at 0°, 45°, 90°, and 135° in front of each individual camera pixels [29], as illustrated in Fig.1(a). Each pixel has a pixel size (pitch) of 3.45 µm; hence, a two-by-two “super-pixel” contains all four polarizations measurements, with an effective pitch of 6.9 µm. A micro-controller (ARM Cortex-M3) along with a voltage level shifter (Todiy SN74AHCT) are used to control the LED illumination and hardware trigger the motorized actuator.

2.2 Principle of T2DPC

2.2.1 Notation

Throughout this article, we use vector symbol \( \vec{z} \) and matrix symbol \( \mathbf{z} \) to represent interaction between polarized light and specimen tensors. Further, we use Mathematical Script font with curly brackets to describe spatial operations, such as \( \mathcal{F}\{\cdot\} \), which denotes Fourier transform. Bold letters in lower case are used to describe vectors in frequency (\( \mathbf{u} \)) or space (\( \mathbf{r} \)). Further, we use \( \vec{z} \) to indicate frequency domain counterparts of variables first defined in space domain.

2.2.2 Light propagation

A sample’s vectorial optical property can be described by its \( 3 \times 3 \) permittivity matrix [30]

\[
\mathbf{\varepsilon} = \begin{bmatrix} \varepsilon_{xx}(\mathbf{r}) & \varepsilon_{xy}(\mathbf{r}) & \varepsilon_{xz}(\mathbf{r}) \\
\varepsilon_{yx}(\mathbf{r}) & \varepsilon_{yy}(\mathbf{r}) & \varepsilon_{yz}(\mathbf{r}) \\
\varepsilon_{zx}(\mathbf{r}) & \varepsilon_{zy}(\mathbf{r}) & \varepsilon_{zz}(\mathbf{r}) \end{bmatrix}, \tag{1}
\]

where \( \mathbf{r} = (x, y, z) \) is the voxel position in three dimension. Under the first Born approximation [24], [25], the relation between scattered light field \( \mathbf{E}^s \) and illumination field \( \mathbf{E}^0 \) can be written as

\[
\mathbf{E}^s(\mathbf{r}) = \iiint \mathbf{G}(\mathbf{r} - \mathbf{r}') \mathbf{V}(\mathbf{r}') \mathbf{E}^0(\mathbf{r}') d\mathbf{r}', \tag{2}
\]

where \( \mathbf{V}(\mathbf{r}') = \mathbf{\varepsilon} - \mathbf{\varepsilon}_0 \) is the sample scattering potential matrix, \( \mathbf{\varepsilon}_0 \) is the permittivity tensor of the background medium, and \( \mathbf{G}(\mathbf{r}) \) is the dyadic Green’s tensor [31]. Assuming each quasi-monochromeric LED source with wavelength \( \lambda \) generates a field at the sample plane, with a lateral frequency \( \mathbf{u}' = (u'_x, u'_y) \) and axial frequency \( \eta = u'_z; |\mathbf{u}'|^2 + \eta^2 = \lambda^{-2} \). After the vectorial electric field is diffracted by the sample, the optical field propagates through the imaging system, which can be modeled as a low-pass filter with a pupil Jones matrix \( \mathbf{P}(\mathbf{u}) \) in the frequency domain [20]. Hence, if we denote the illumination as \( \mathbf{E}^0(\mathbf{r}, \mathbf{u}') \), the detected intensity on the image plane with \( l^{th} \) analyzer (linear polarizers at \{0°, 45°, 90°, 135°\}) with single LED illumination from angle \( \mathbf{u}' \) can be written as [20], [32]

\[
l^l(\mathbf{r}, \mathbf{u}') = |\mathbf{d}_l^T \mathcal{F}^{-1}_2 \mathbf{P}(\mathbf{u}) \mathcal{F}_2(\mathbf{E}^s(\mathbf{r}, \mathbf{u}') + \mathbf{E}^0(\mathbf{r}, \mathbf{u}'))|^2. \tag{3}
\]

\( \mathbf{d}_l \) is the Jones vector for \( l^{th} \) analyzer. For a linear polarizer oriented at \( \alpha, \mathbf{d} = [\cos \alpha, \sin \alpha]^T \) [20], [33]. The intuition behind this is that after linear analyzer, the vectorial field reduces to a scalar pointing along one polarization direction [20].

For DPC measurements, we assume different LEDs are mutually incoherent. Thus, the measured intensity from each pattern illumination is incoherent sum of intensities from each individual point sources. To approximate this summation with an integral, the measured intensity from \( n^{th} \) illumination pattern \( S^n(\mathbf{u}_0) \) is

\[
l^{l,n}(\mathbf{r}) = \int \int S^n(\mathbf{u}') l^l(\mathbf{r}, \mathbf{u}') d\mathbf{u}'. \tag{4}
\]

2.2.3 Forward model and inverse problem

In our first demonstration of T2DPC, we make a few approximations to the model described in Section 2.2.2 to obtain a less ill-posed inverse problem for image reconstruction with the proposed experimental measurement strategy.

Following Saba et.al. [24], we first make a paraxial approximation. Assuming weak polarization along optical axis of the illumination, and negligible interaction between traverse and axial polarization caused by the sample, we can approximate the \( 3 \times 3 \) permittivity matrix with a \( 2 \times 2 \) one,

\[
\mathbf{\varepsilon} = \begin{bmatrix} \varepsilon_{xx}(\mathbf{r}) & \varepsilon_{xy}(\mathbf{r}) \\
\varepsilon_{yx}(\mathbf{r}) & \varepsilon_{yy}(\mathbf{r}) \end{bmatrix}. \tag{5}
\]

Although this approximation can be inaccurate for certain crystals illuminated at high angle [34], a finite element analysis-based study showed that it is accurate up to a 25° illumination angle [24]. In addition, we assume the background media is isotropic, which has a diagonal permittivity tensor \( \mathbf{\varepsilon}_0 = \mathbf{\varepsilon}_0 \). This also simplifies the Green’s tensor to a diagonal matrix with same component for each polarization [24]

\[
\mathbf{\mathcal{G}}(\mathbf{r}, \mathbf{r}') = \begin{bmatrix} G(\mathbf{r}, \mathbf{r}') & 0 \\
0 & G(\mathbf{r}, \mathbf{r}') \end{bmatrix} \tag{6}
\]

in which

\[
G(\mathbf{r}, \mathbf{r}') = \frac{e^{j k_0 |\mathbf{r} - \mathbf{r}'|}}{4 \pi |\mathbf{r} - \mathbf{r}'|} \tag{7}
\]

is the same as the scalar Green’s function.

We further assume the sample we image is homogeneous. That is, there exist a principal axis, where the permittivity matrix becomes diagonal [30], [34], [35]. This is widely assumed for various crystals and biological samples [12], [36]. Under this assumption, the permittivity matrix is symmetric, and can be decomposed into [36],

\[
\mathbf{\varepsilon} = \begin{bmatrix} \cos \theta & \sin \theta & 0 \\
-\sin \theta & \cos \theta & 0 \\
0 & 0 & \epsilon_e \end{bmatrix} = \begin{bmatrix} \cos \theta & -\sin \theta \\
\sin \theta & \cos \theta \end{bmatrix} \begin{bmatrix} \epsilon_o \end{bmatrix}. \tag{8}
\]

Here \( \epsilon_e \) and \( \epsilon_o \) are permittivity along ordinary and extraordinary axis, and \( \theta \) is the orientation to the extraordinary axis, or the slow axis (extraordinary axis has a higher refractive index). Following the convention [35], we rename elements in Eq.9 as

\[
\begin{align} \epsilon_1 &= \epsilon_{xx} \\
\epsilon_2 &= \epsilon_{yy} \tag{9a} \\
\epsilon_3 &= \epsilon_{xy} = \epsilon_{yx} \end{align} \tag{9b}
\]

With left circularly polarized illumination, the scattering
potential along $x - y$ polarization is

$$\begin{bmatrix} V_1 \\ V_3 \\ V_2 \end{bmatrix} \frac{1}{j} = \begin{bmatrix} V_1 + jV_3 \\ V_3 + jV_2 \end{bmatrix}, \tag{10}$$

where

$$\begin{align*}
V_1 &= 4\pi k_0^2 (c_1 - c_0) \\
V_2 &= 4\pi k_0^2 (c_2 - c_0) \\
V_3 &= 4\pi k_0^2 c_3,
\end{align*}$$

and $k_0$ is the wavenumber of the isotropic background medium.

Finally, following Chen et al. [26], we make two more approximations to the illumination and scattering process. First we assume the illumination at sample plane from each LED is a plane wave, which is widely assumed in LED-based computational microscopy [37]. Second, since high-quality objectives (Olympus PLN) are used in this experiment, we disregard pupil aberration in this first demonstration, and model the pupil as a low-pass filter $P(u)$ with cut-off frequency equivalent to the NA of the objective lens [26]. Joint reconstruction of pupil jones matrix for anisotropically aberration correction is left to future work [20]. The T^2DPC forward model we use can then be expressed as

$$\tilde{I}^{l,m}(u, \eta) = \tilde{I}_\theta^{l,m}(u, \eta) + H_{Re}^m(u, \eta) \cdot \tilde{V}_{Re}(u, \eta) + H_{Im}^m(u, \eta) \cdot \tilde{V}_{Im}(u, \eta), \tag{12}$$

where $\tilde{I}_\theta^{l,m}(u, \eta)$ is 3D Fourier transform of the measured focal stack, and 3D Fourier transform of the DC term represents the background intensity illuminated with $l^{th}$ pattern and analyzed with $l^{th}$ analyzer; $l \in \{0^\circ, 45^\circ, 90^\circ, 135^\circ\}$. $\tilde{I}_\theta^{l,m}(u, \eta)$ is $\tilde{V}_{Re}(u, \eta)$ and $\tilde{V}_{Im}(u, \eta)$ are 3D Fourier transforms of $V_{Re}^l(r)$ and $V_{Im}^l(r)$, respectively, and are related to Eq. 11 via

$$\begin{align*}
V_{Re}^{0}(r) + jV_{Im}^{0}(r) &= V_1(r) + jV_3(r) \\
V_{Re}^{45}(r) + jV_{Im}^{45}(r) &= \frac{\sqrt{2}}{2} (V_1(r) + V_3(r)) \\
&\quad + j \frac{\sqrt{2}}{2} (V_2(r) + V_3(r)) \\
V_{Re}^{90}(r) + jV_{Im}^{90}(r) &= V_3(r) + jV_2(r) \\
V_{Re}^{135}(r) + jV_{Im}^{135}(r) &= \frac{\sqrt{2}}{2} (-V_1(r) + V_3(r)) \\
&\quad + j \frac{\sqrt{2}}{2} (V_2(r) - V_3(r)),
\end{align*}$$

where $H_{Re}^m = j \cdot H_{Im}^m$ are the transfer functions for the real and imaginary part of the scattering potential when illuminated with $m^{th}$ pattern $S^m(u')$, respectively [26], [40]. $G(u, \eta)$ is the Fourier transform of Green's function $G(r)$ in Eq.(7). For concise expression, we now denote $v \in \mathbb{C}^{N \times M \times 3}$ as a vector representation of $V_1, V_2, V_3$, with $N, M, Q$ are width, height, and depth of the 3D sample, respectively. The sizes of reconstructed digital images in Fig. 2 are $512 \times 512 \times 100 \times 3, 400 \times 400 \times 100 \times 3,$ and $1024 \times 1024 \times 80 \times 3$, with physical voxel sizes of $0.345 \times 0.345 \times 0.8 \mu m^3$, $0.345 \times 0.345 \times 0.8 \mu m^3$, and $0.69 \times 0.69 \times 1 \mu m^3$, respectively. The measurements have the same digital image size as the reconstruction, with 20x magnifications used in Fig. 2-3 and a 10x magnification for Fig. 4. We further introduce the operator $A_U(\cdot)$ as the forward model operator for all the polarization configurations, where $U$ denotes the illumination angular support: $U = [u_{min}, u_{max}] \cap [\eta_{min}, \eta_{max}]$. To reconstruct the permittivity matrix, the inverse problem is formulated as

$$v = \arg \min \mathcal{L}(v), \tag{16}$$

with the loss function

$$\mathcal{L}(v) = \sum_{(u, \eta) \in U} ||A_U(v) - \tilde{I}(u, \eta)||^2 + \gamma \sum_{i} tv(v).$$

$tv(\cdot)$ is the isotropic total variation operator applied on $V_1, V_2, V_3$. $\rho$ is a regularization penalization parameter empirically set to be $1 \times 10^{-7}$ for all the experiments. The forward model is implemented in Pytorch [41], an auto-differentiation library, and the loss is minimized using the Adam optimizer [42]. Due to the memory limitation, we use stochastic gradients to approximate the full gradient,

$$\frac{\partial}{\partial v} \mathcal{L}(v) \approx \frac{\partial}{\partial v} \mathcal{L}_{\mathbb{U}_{\delta}}(v),$$

where $\mathbb{U}_{\delta} \subset \mathbb{U}$ is a subset of the support of $A_U(v)$ chosen randomly at each iteration.

2.2.4 Deriving polarization properties from T^2DPC reconstruction

To obtain polarization properties such as orientation and birefringence from T^2DPC reconstructions, we start by explicitly writing out the following relation described in Eq.(8),

$$\begin{align*}
\epsilon_1 &= \epsilon_e \cos \theta^2 + \epsilon_o \sin \theta^2 \tag{18a} \\
\epsilon_2 &= \epsilon_e \sin \theta^2 + \epsilon_o \cos \theta^2 \tag{18b} \\
\epsilon_3 &= (\epsilon_e - \epsilon_o) \sin \theta \cos \theta, \tag{18c}
\end{align*}$$
Fig. 2. Reconstruction of a two-layer monosodium urate (MSU) crystal sample. (a) shows a few representative images of raw measurements, when focused in the middle of the sample. First row shows images captured with different illumination patterns. Second row shows images captured with analyzers oriented at different directions. (b) plots reconstruction slices of MSU sample at two unique z-positions. The reconstructed orientation for each pixel is coded with color. (c) A 3D view of reconstructed birefringence of the sample, with its field-of-view labeled in (b). (d) Zoom-in for the orientation reconstructions regions labeled in (b)

from which, we can compute

\[
\begin{align*}
\epsilon_1 + \epsilon_2 &= \epsilon_o + \epsilon_e \\
\epsilon_1 - \epsilon_2 &= (\epsilon_e - \epsilon_o) \cos(2\theta) \\
2\epsilon_3 &= (\epsilon_e - \epsilon_o) \sin(2\theta).
\end{align*}
\]

Thus, we have

\[
\begin{align*}
\epsilon_\theta &= \bar{\epsilon} - \frac{1}{2}\Delta \epsilon \\
\epsilon_e &= \bar{\epsilon} + \frac{1}{2}\Delta \epsilon,
\end{align*}
\]
where

\[
\begin{cases}
\Delta \varepsilon = \varepsilon_e - \varepsilon_o = \sqrt{(\varepsilon_1 - \varepsilon_2)^2 + 4\varepsilon_3^2} \\
\bar{\varepsilon} = \frac{\varepsilon_1 + \varepsilon_2}{2}.
\end{cases}
\]

Then the refractive index along the ordinary and extraordinary axes can be computed as \( n_{o,e} = \sqrt{\varepsilon_{o,e}} \), along with the averaged refractive index \( \bar{n} = n_o + n_e \) and birefringence \( \Delta n = n_e - n_o \) reported in Fig. 3 and Fig. 4. Finally, the orientation of the slow or extraordinary axis \( \theta \in [0, \pi] \) reported in Fig. 2 can be derived using the relation

\[
\tan 2\theta = \frac{2\varepsilon_3}{\varepsilon_1 - \varepsilon_2}.
\]

Hence,

\[
\theta = \begin{cases}
\frac{1}{2} \arctan \tfrac{2\varepsilon_3}{\varepsilon_1 - \varepsilon_2}, & \text{if } \varepsilon_1 - \varepsilon_2 > 0 \\
\frac{1}{2} \arctan \tfrac{2\varepsilon_3}{\varepsilon_1 - \varepsilon_2} + \frac{\pi}{2}, & \text{otherwise}.
\end{cases}
\]

3 Experimental Results

To evaluate the performance of proposed method, we show reconstructions of a variety of calibration samples, validating different aspects of the proposed method using different calibration samples. Finally, we show reconstruction of a heart tissue sample containing amyloid, an indicator of a lethal heart disease called cardiac amyloidosis.
3.1 Quantitative orientation measurement

To validate the reconstructed orientation accuracy, we imaged two-layer monosodium urate (MSU) samples separated by 12 μm. The focal stack is taken with 0.8μm step size using an 0.4 NA objective. Figure 2(a) shows...
the images taken when focused in middle of two layers. The first row shows representative images captured with the zero-degree analyzer pixels, and different illumination patterns. In contrast, the second row shows images when sample is illuminated with the same pattern, but analyzed with different linear polarizers. The pattern and analyzer orientation are labeled at top-left corners of each individual images. Figure 2(b) shows reconstruction slices from two different z-positions. The spatially resolved orientation is computed using Eq.23. To best visualize orientation results, we follow the convention [17], [19], [20] to display the multidimensional polarization data using an HSV colormap, where value displays birefringence, orientation is coded in hue, and saturation is set to one. Zoom-ins of select regions are also presented in (d). These results suggest that the reconstructed orientations of line-shape MSU samples follow the structural directions of the sample, in agreement with prior works [17], [19], [20]. Figure 2(c) shows a 3D view of the reconstructed birefringence for the field-of-view (FOV) labelled in (b). In addition, supplement Fig.1 presents 2D reconstructions of the MSU sample using a classic LC-PolScope method [11]. The same depth and zoom-in regions shown in Fig.2 are displayed here to highlight the superior depth sectioning ability of the proposed $T^2$DPC method. Although LC-PolScope can give accurate orientation and birefringence reconstructions from focused depth, artifacts are present in the images due to out-of-focus regions of the 3D sample.

3.2 Validation of refractive index and birefringence

In this section we show reconstruction results of a sample consisting of two layers of 3-µm polystyrene microspheres (1.60 at 520 nm [43]) immersed in 1.58-index oil. Similar to the configuration used to image MSU sample, we use 0.4NA objective lens, and 0.8µm z-scan step size. Figure 3(a) shows brightfield images captured when focused at different axial positions of the sample. Note that since polysterene spheres are transparent, and have a refractive index close to that of the immersion fluid, the beads almost disappear when they are in focus. These images confirm that the two layers of beads are separated by 8.8µm. Figure 3(c-d) presents reconstructed refractive index and birefringence for both layers. Edge birefringence is a well-established phenomenon [44], and we see expected ring-shaped birefringence reconstructions that match structures reported in prior literature [20], [25]. Figure 3(e) displays cross-sections of the reconstructed volume at two regions labelled in (a). We see the reconstructed two-layers are accurately separated by 8.8µm with a better resolution comparing with the brightfield focal stack in (b). In addition, we see two line-shaped birefringence reconstructions. We suspect this is due to the edge birefringence at the boundary of oil and glass (both slide and cover glass). (f) plots a line profile of refractive index and birefringence reconstructions along x-axis, averaged from ten beads. The reconstructed refractive index is slightly lower than the ground truth. The reconstructed birefringence value matches results reported with other techniques [20] (0.4 rad for a single layer of 10µm microspheres; with projection approximation [35], retardance $\delta_o$ for thickness $d$ and birefringence $\delta_n$ are related as $\delta_o = 2\pi nd/\lambda$).

3.3 Detecting cardiac amyloidosis from heart tissue sample

Finally, we applied our method to image a thinly-sliced heart sample commonly viewed under polarized light microscope in pathology lab. In current practices, the cardiac tissue is thinly sectioned and stained with congo red dye. Under white light illumination, if we sandwich the sample in between a pair of cross-polarized generator and analyzer, the birefringence of amyloid, an indicator of a deadly disease called cardiac amyloidosis, can create a vibrant apple-green color [45], as shown in Fig.4(b). To enlarge the field-of-view, we switch to a 10x objective for this sample, and use 1.0µm step size. Figure 4(a) shows a brightfield image when the sample in focus. Figure 4(c)-(d) shows the reconstructions of refractive index and birefringence. As the sample is thinly sliced, we only present a center slice of the sample. We can clearly see that the birefringence structure in (d) is very similar to (b), which can be used to as a tool to help diagnosis amyloidosis. (e) and (f) display reconstructed slices at different depth positions for the region labeled in (c) and (d).

4 Conclusion and Discussion

In summary, we have introduced $T^2$DPC, a new method to record polarized measurements with computational illumination strategies, and provide quantitative tomographic permittivity matrix reconstructions for a variety of calibration samples and biological specimens without an interferometry setup. Using relatively low NA objectives, we demonstrate by volumetric modeling the sample, $T^2$DPC has superior depth section ability compared with 2D methods. To extend the functions of $T^2$DPC, several incremental improvements can be made. First, to relieve the ill-posedness of the problem, we have approximated the permittivity tensor with its lateral components. Although widely used [18], [19], [20], [24], this simplification disregards out-of-plane anisotropy, which can contain valuable biological and diagnostic information [25], [46]. Using more angular and polarization-diverse patterns can potentially allow reconstruction of uni-axial, or even more general permittivity tensors, from which out-of-plane orientations can be derived. This can be practically implemented by placing different generators in front of each individual LEDs and turn on them sequentially. In addition, deploying measurement strategies from diffraction tomography-type setups [38], [47] can also hopefully record enough informative measurements for tomographic reconstructions without scanning the sample [23], [24]. Further, adopting a vectorial multi-scattering forward model [24] can hopefully extend the method to image thicker samples.

Finally, as a first demonstration, currently we use a stochastic gradient-based method to solve the inverse problem. While it is easy to implement, it can also result in slow convergence. As the propagation through imaging system part of the forward model can be efficiently inverted [26], we anticipate deploying variable splitting methods [48] can significantly improve the convergence speed, as well as allows flexible use of regularization methods may not have an explicit form [49], for example. We hope to explore in these directions to ensure successful translation of $T^2$DPC to future clinical and scientific studies.
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