Optical phantoms for biomedical polarimetry: a review

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1 Introduction

The use of polarized light in clinical and preclinical applications is expanding, and several recent reviews by Tuchin, Ghosh and Vitkin, Qi and Elson, de Boer et al., and Baumann have illustrated the fast progress of this approach in the medical field.

As polarimetric techniques reach the clinical and commercial stage, there is a need to validate them with replicative systems that could serve as biological proxies and mimic the characteristic trends of typical biological observations. Over the past several decades, a variety of such systems—commonly referred to as phantoms—have been implemented for the use of general optical imaging and sensing; Pogue and Patterson illustrated these tools in an exhaustive review. Here, we focus uniquely on phantoms used for polarimetry in biomedicine; these phantoms were not included in previous reviews and are relevant for all research groups involved in polarimetric studies and instruments development would benefit from sharing a limited set of standardized polarimetric phantoms, as is done earlier in the round robin investigations in ellipsometry.

Keywords: polarization; scattering; anisotropy; tissue phantoms; retardation; depolarization; diattenuation.

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Scattering is generally very high in biological media due to the high density and large variety of sub- and extracellular components (such as organelles, nuclei, collagen fiber bundles, cell membrane, to name a few). Different polarization states of incident radiation—linear, circular, or elliptical—depolarize at different rates. As for the mathematical representation of depolarization, its theoretical premise is generally supported by the Mueller matrix of an intrinsic (or diagonal) depolarizer [Eq. (1a)], satisfying the covariance conditions [Eq. (1b)].

\[
M_\Delta = d_0 \begin{pmatrix} 1 & 0 & 0 & 0 \\ 0 & a & 0 & 0 \\ 0 & 0 & b & 0 \\ 0 & 0 & 0 & c \end{pmatrix}, \quad 0 < d_0 < 1, |a|, |b|, |c| \leq 1.
\]

\[
-a - b - c \leq 1, \quad -a + b + c \leq 1, \quad a + b - c \leq 1.
\]

It follows from Eq. (1a) that \( 1 - |a| \) and \( 1 - |b| \) represent the linear depolarization power (horizontal-vertical and ±45 deg frameworks). Similarly, \( 1 - |c| \) specifies the power of circular depolarization.

From this, the total depolarization power \( \Delta \) can be calculated as...
\[
\Delta = 1 - \frac{\rho + |\rho + |\rho|}{\rho} = 1 - \frac{M_{\rho \rho}}{4}, 0 \leq \Delta \leq 1.
\]

In birefringent media, light experiences changes in propagation speeds for its different polarization components, which leads to phase differences (also called retardation) between those components. Linear retardation is the phase shift between two orthogonal linear polarization states (e.g., 0 deg and 90 deg, or -45 deg and 45 deg). Circular retardation (also referred to as optical rotation) is the difference in phase between the right and the left circular polarized components of light, which happens due to circular birefringence (optical activity). The Mueller matrix of a linear retarder [see Eq. (3)] depends on its phase difference parameter \(\delta\) and on the azimuth \(\theta\) of its fast axis:

\[
R = \begin{pmatrix}
1 & 0 & 0 & 0 \\
0 & \cos^2(\theta) + \sin^2(\theta) \cos \delta & \sin 2\theta \cos 2\theta (1 - \cos \delta) & \sin^2(\theta) + \cos^2(\theta) \cos \delta \\
0 & \sin 2\theta \cos 2\theta (1 - \cos \delta) & \cos^2(\theta) - \sin^2(\theta) \cos \delta & -\sin 2\theta \sin \delta \\
0 & \sin 2\theta \sin \delta & -\cos 2\theta \sin \delta & \cos \delta
\end{pmatrix}
\]

The use of polarimetry in monitoring biological tissue often focuses on quantification of the tissue preferential azimuth (i.e., the orientation of optical axis of uniaxial birefringent medium) related to the arrangement of a collagenous ECM or other cellular assembly. Skeletal muscle and cardiac tissue are both strongly depolarizing and birefringent due to cellular components and layered structure.

Collagen, animal cornea, retina, and optic nerves have all been shown to have large birefringence and preferential alignment through PS-OCT and polarized light microscopy. Several studies using PS-OCT imaging on articular cartilage, which is rich in oriented collagen fibers, have shown changes in collagen retardation in depth. Nerves have also been shown to yield retardation with polarization-sensitive spectroscopy. Since birefringence is the most common source of retardation and signal for this modality, in general most retardance phantoms can be used as PS-OCT phantoms.

Birefringence itself can be divided into “intrinsic birefringence” and “form birefringence.” Typically, biological tissues rich in extracellular matrix (ECM) fibers, for example, skin, cornea, sclera, tendon, uterine cervix, and cardiac tissue, exhibit retardation.

Mueller matrix polarimetry and polarization-sensitive optical coherence tomography (PS-OCT) are techniques capable of quantifying many of the aforementioned parameters of interest. Calculation of the Mueller matrix requires the modulation of both light source and detector into a minimum of four different polarization states for a total of 16 measurements. Once the Mueller matrix is determined, it can be decomposed as a sequence of elementary polarization components: a diattenuator, a retarder, and a depolarizer. PS-OCT is an extension of OCT, a technique based on low-coherence interferometry that can provide high-resolution cross-sectional imaging of biological tissue, and it too can be used to quantify birefringence, diattenuation, and depolarization index, a parameter related to depolarization.

Diattenuation, also called dichroism, is generally considered to have the smallest impact on polarized light propagating in biological media. Diattenuation arises from polarization-selective attenuation of the electrical field. Related to diattenuation is the property of optical activity, also known as circular birefringence, which is characterized by the rotation of the polarization plane of linearly polarized light about the axis of propagation. This property is prevalent for chiral molecules such as glucose, proteins, and nucleic acids.

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2 Optical Phantoms

We have categorized all phantoms by their dominant polarization property—namely, depolarization, retardation, diattenuation, or optical activity. We have also introduced a separate table for biological tissues used as phantoms. Many phantoms exhibit more than one property; hence, they may appear in more than one table, these repeated phantoms are identified by an asterisk (*). The retardation phantoms table includes an induced retardation column. This column is included to differentiate phantoms which are inherently birefringent due to their structure from phantoms that are mechanically stressed, strained, or otherwise manipulated in order to change their birefringence. Many of the phantoms cited in this review have been used by the same investigators in multiple journals, for simplicity, we have not cited all the articles using the same phantoms and limited the review to the ones that were substantially different to each other.

2.1 Biological Phantoms

The construction of polarimetric phantoms is a complex process; hence, biological samples are commonly used in polarization-sensitive optical modalities (Table 1). Collagen-rich tissues, for example, tendons or rat tails, are the most commonly used in polarimetry. As most biological tissues, collagen scatters (and, consequently, depolarizes); more importantly, collagen introduces a phase shift between orthogonal polarization states of incident polarized light due to its strong birefringence. Since many healthy collagen-rich tissues behave as uniaxial birefringent media, the azimuth of optical axis of linear retardation related to collagen alignment can often be measured.21,22,27,59,72

Chicken or cow tendons have been used by many groups to validate polarization-based optical instruments. Azimuth angle is calculated as well as an increase in scalar retardance due to birefringence. Similar to tendon, murine tails also contain collagen fibers which are strongly aligned. Since the azimuth of the collagen fibers preferential orientation can be directly observed, a typical validation test for polarimeters includes positioning a tendon or rat tail at predetermined angles and then measuring samples at different and well-known angular positions.71,58,72

While muscle tissue can be used for the same purposes as collagen-based phantoms, the interpretation of the results is less straightforward due to the increased cellularity of these tissues.61 Studies of myocardium muscle have been conducted by several investigators showing loss of retardation and local order for infarcted tissue. For this reason, samples of myocardium have been used to validate different polarimetric systems. Ghosh et al.57 used Mueller matrix decomposition to calculate depolarization, diattenuation, and retardance of fixed rat myocardial tissue.

Heart valve leaflets are another highly collagenous and anisotropic tissue that have been used as a depolarization and retardation phantom.60 As in previous example, the azimuth of collagen fibers’ preferential orientation can be detected and used for instrument characterization. Changes in depolarization can also be observed by treating the sample with collagenase.58,59

Artificial skin models grown from epidermal keratinocytes forming a multilayered epidermis on top of collagen I hydrogel with dermal fibroblasts have also been used to mimic the interaction of polarized light with the skin.73 Unstained cuts of fixed skin equivalents of varying thickness (range: 5 to 30 μm) were measured in transmission with Mueller microscopy and the values of retardation and depolarization parameters were extracted using logarithmic decomposition of the measured Mueller matrices. The measurements confirmed parabolic dependence of depolarization and linear dependence of retardation on thickness, as follows from differential Mueller matrix formalism.
<table>
<thead>
<tr>
<th>Tissue type</th>
<th>Preparation</th>
<th>Polarization property</th>
<th>Transmission/reflectance</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Axonemes (sea urchin)</td>
<td>Extraction from sea urchin sperm and purification steps</td>
<td>Retardation</td>
<td>R</td>
<td>20</td>
</tr>
<tr>
<td>Bladder (porcine)</td>
<td>Excised, fresh</td>
<td>Depolarization, retardation, diattenuation</td>
<td>R</td>
<td>49</td>
</tr>
<tr>
<td>Brain (porcine)</td>
<td>Phosphate-buffered saline solution (0.02 M)</td>
<td>Depolarization</td>
<td>R</td>
<td>50</td>
</tr>
<tr>
<td>Cartilage (animal)</td>
<td>Excised, fresh</td>
<td>Depolarization, retardation</td>
<td>R</td>
<td>15–18</td>
</tr>
<tr>
<td>Cartilage (porcine)</td>
<td>Excised, fresh</td>
<td>Retardation, depolarization, diattenuation</td>
<td>T</td>
<td>18</td>
</tr>
<tr>
<td>Cervix (porcine)</td>
<td>Fixed in 4% paraformaldehyde and embedded in paraffin</td>
<td>Depolarization, retardation</td>
<td>R</td>
<td>51</td>
</tr>
<tr>
<td>Eye (cornea)</td>
<td>Excised, fresh</td>
<td>Retardation</td>
<td>R</td>
<td>13 and 14</td>
</tr>
<tr>
<td>Eye (optic nerve)</td>
<td>Cryosectioned</td>
<td>Retardation</td>
<td>R</td>
<td>12</td>
</tr>
<tr>
<td>Eye (retina)</td>
<td>Excised, fresh</td>
<td>Retardation</td>
<td>R</td>
<td>14</td>
</tr>
<tr>
<td>Fibroblast (rat)</td>
<td>Suspension</td>
<td>Depolarization</td>
<td>R</td>
<td>52 and 53</td>
</tr>
<tr>
<td>Heart (myocardium)</td>
<td>Excised, fixed</td>
<td>Depolarization, retardation</td>
<td>R</td>
<td>32, 54, 55, and 56</td>
</tr>
<tr>
<td>Heart (porcine myocardium)</td>
<td>Phosphate-buffered saline solution (0.02 M)</td>
<td>Depolarization</td>
<td>R</td>
<td>50</td>
</tr>
<tr>
<td>Heart (rat myocardium)</td>
<td>10% formalin and cut into 1 mm slices</td>
<td>Retardation, diattenuation, depolarization</td>
<td>R</td>
<td>57</td>
</tr>
<tr>
<td>Heart (valve leaflet)</td>
<td>Excised, fresh</td>
<td>Depolarization, retardation</td>
<td>R</td>
<td>58 and 59</td>
</tr>
<tr>
<td>Heart (porcine valve)</td>
<td>Excised, fresh</td>
<td>Retardation</td>
<td>R</td>
<td>60 and 58</td>
</tr>
<tr>
<td>Heart (porcine aorta)</td>
<td>Excised, fresh</td>
<td>Retardation</td>
<td>R</td>
<td>61</td>
</tr>
<tr>
<td>Heart (bovine right ventricle)</td>
<td>Cut into 2 cm × 2 cm × 1 cm sections</td>
<td>Retardation, diattenuation</td>
<td>R</td>
<td>62</td>
</tr>
<tr>
<td>Heart (swine right ventricle)</td>
<td>Excised, fresh</td>
<td>Retardation</td>
<td>R</td>
<td>63</td>
</tr>
<tr>
<td>Heart (rabbit right ventricular wall)</td>
<td>3.7% formaldehyde for 1 day and 20% sucrose solution for an additional 2 days</td>
<td>Retardation</td>
<td>R</td>
<td>64</td>
</tr>
<tr>
<td>Kidney cortex</td>
<td>Phosphate-buffered saline solution (0.02 M)</td>
<td>Depolarization</td>
<td>R</td>
<td>50</td>
</tr>
<tr>
<td>Liver</td>
<td>Phosphate-buffered saline solution (0.02 M)</td>
<td>Depolarization</td>
<td>R</td>
<td>40 and 50</td>
</tr>
<tr>
<td>Melanin granules</td>
<td>Suspension</td>
<td>Depolarization, retardation</td>
<td>R</td>
<td>65</td>
</tr>
<tr>
<td>Microtubules</td>
<td>Extraction from porcine brain and purification steps</td>
<td>Retardation</td>
<td>R</td>
<td>20</td>
</tr>
<tr>
<td>Nerve (lobster leg)</td>
<td>Excised, fresh</td>
<td>Depolarization, retardation</td>
<td>R</td>
<td>19</td>
</tr>
<tr>
<td>Skeletal muscle</td>
<td>Excised, fresh</td>
<td>Depolarization, retardation</td>
<td>R</td>
<td>32, 40, 50, 54–56, 64, and 66–68</td>
</tr>
<tr>
<td>Skin</td>
<td>In vivo</td>
<td>Depolarization, retardation</td>
<td>R</td>
<td>47, 48, and 69</td>
</tr>
<tr>
<td>Skin (calf)</td>
<td>Excised, fresh</td>
<td>Retardation</td>
<td>T</td>
<td>70</td>
</tr>
<tr>
<td>Skin equivalent model</td>
<td>Fixed and cut into few μm slices</td>
<td>Depolarization, retardation</td>
<td>T</td>
<td>71</td>
</tr>
<tr>
<td>Tail (rat)</td>
<td>Frozen and thawed</td>
<td>Depolarization, retardation</td>
<td>R</td>
<td>72</td>
</tr>
<tr>
<td>Tendon</td>
<td>Excised, fresh</td>
<td>Depolarization, retardation</td>
<td>R</td>
<td>45, 50, 60, and 73–76</td>
</tr>
<tr>
<td>Yeast cells</td>
<td>Suspension</td>
<td>Depolarization</td>
<td>R</td>
<td>53</td>
</tr>
</tbody>
</table>
2.2 Depolarizing Phantoms
Several authors have studied the effect of particle size, density, and index of refraction on the polarization of scattered light.\textsuperscript{85,86} As suggested by the results of these studies, the main scatterers in biological tissues are nuclei, organelles, and bulk tissue structures that limit the photon penetration depth and depolarize light traveling through these media.\textsuperscript{53} The cell nuclei and organelles are frequently modeled as spherical scattering particles\textsuperscript{87} of refractive index varying between 1.33 and 1.47. The components of ECM, such as collagen and elastin, have been represented by spherical\textsuperscript{88} or cylindrical\textsuperscript{25} structures.

Work by MacKintosh et al.\textsuperscript{89} showed that circular polarization was maintained for longer depths as compared to linearly polarized light in Mie scattering regime (scatterer size $\geq$ light wavelength in the medium). In one of the relevant studies, Monte Carlo simulations supported this finding by showing that mean penetration depth was $\sim 2$ mean free paths (MFP) for linearly and 10 MFP for circularly polarized light in Mie scattering regime.\textsuperscript{86}

Suspensions of microspheres and other small particles are commonly used to create phantoms with scattering properties (Table 2). The amount of scattering can be adjusted depending on the size and concentration of the microspheres based on the Mie scattering theory. On a smaller scale, nanoparticles have also been widely used to create scattering phantoms in Rayleigh scattering regime. These particles can also be embedded in solid host media, such as gels or polymers, to ensure scattering properties of those materials. In addition, India ink, hemoglobin, and dyes are commonly added to influence the absorbing characteristics.

Several studies, such as Antonelli, Rakovic et al., and Cote and Vitkin,\textsuperscript{75,94,95} have used aqueous polystyrene microsphere

<table>
<thead>
<tr>
<th>Depolarizing agent</th>
<th>Embedding material</th>
<th>Tissue mimicking</th>
<th>Phantom thickness</th>
<th>Transmission/reflectance</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>GNPs (50 nm)</td>
<td>Intralipid</td>
<td>Contrast agent</td>
<td>Semi-infinite</td>
<td>R</td>
<td>90</td>
</tr>
<tr>
<td>Intralipid*</td>
<td>Water, India ink</td>
<td>Bladder wall</td>
<td>Semi-infinite</td>
<td>R</td>
<td>49</td>
</tr>
<tr>
<td>Intralipid</td>
<td>Water</td>
<td>Turbid biological media</td>
<td>Semi-infinite</td>
<td>R</td>
<td>53, 69, and 91</td>
</tr>
<tr>
<td>Intralipid or polystyrene microspheres</td>
<td>Water, Naphthol green</td>
<td>Porcine liver</td>
<td>1 $\mu$m, 1.4 $\mu$m</td>
<td>R</td>
<td>92</td>
</tr>
<tr>
<td>Kapton tape (stacked)*</td>
<td>Layered against a rigid base</td>
<td>Theoretical standard</td>
<td>Semi-infinite</td>
<td>R</td>
<td>93</td>
</tr>
<tr>
<td>Mylar (biaxially oriented polyethylene terephthalate)*</td>
<td>Laid against a plexiglass base</td>
<td>Theoretical standard</td>
<td>Semi-infinite</td>
<td>R</td>
<td>93</td>
</tr>
<tr>
<td>Polystyrene microspheres</td>
<td>Water</td>
<td>Turbid biological media</td>
<td>Semi-infinite</td>
<td>R</td>
<td>24, 40, 53, and 94–97</td>
</tr>
<tr>
<td>Polystyrene microspheres</td>
<td>Intralipid</td>
<td>Turbid biological media</td>
<td>Semi-infinite</td>
<td>R</td>
<td>53 and 2</td>
</tr>
<tr>
<td>Polystyrene microspheres</td>
<td>Polycrylamide, sucrose</td>
<td>Turbid biological media</td>
<td>1 cm$^3$</td>
<td>T</td>
<td>3</td>
</tr>
<tr>
<td>Polystyrene microspheres (0.5 $\mu$m) and fiber glass*</td>
<td>Polycrylamide</td>
<td>Anisotropic sample</td>
<td>$1 \times 2 \times 4$ cm$^3$</td>
<td>T</td>
<td>98</td>
</tr>
<tr>
<td>Polystyrene microspheres and silk fibers*</td>
<td>Water</td>
<td>Anisotropic sample</td>
<td>2.1 cm</td>
<td>R</td>
<td>88 and 99</td>
</tr>
<tr>
<td>Quartz plate (wedged)*</td>
<td>None</td>
<td>N/A</td>
<td>3 mm</td>
<td>T</td>
<td>100</td>
</tr>
<tr>
<td>Melanin granules*</td>
<td>Water</td>
<td>Retina/retinal pigment epithelium</td>
<td>Semi-infinite</td>
<td>R</td>
<td>65</td>
</tr>
<tr>
<td>Silicon phantom (extruded)</td>
<td>Air between layers</td>
<td>Anisotropic sample</td>
<td>2 mm</td>
<td>R</td>
<td>51</td>
</tr>
<tr>
<td>Silicon (amorphous)*</td>
<td>None</td>
<td>Theoretical polarization standard</td>
<td>Semi-infinite</td>
<td>R</td>
<td>93</td>
</tr>
<tr>
<td>Silicon (poly-)*</td>
<td>None</td>
<td>Theoretical standard</td>
<td>Semi-infinite</td>
<td>R</td>
<td>93</td>
</tr>
<tr>
<td>Silicon grating</td>
<td>Silicon wafer</td>
<td>Theoretical standard</td>
<td>Semi-infinite</td>
<td>R</td>
<td>101</td>
</tr>
<tr>
<td>TiO$_2$ nanoparticles (530 nm)</td>
<td>PVC-based transparent material</td>
<td>Biopsy samples</td>
<td>1 mm</td>
<td>T</td>
<td>23</td>
</tr>
<tr>
<td>TiO$_2$</td>
<td>Wax</td>
<td>Skin</td>
<td>2 and 5 mm</td>
<td>R</td>
<td>44</td>
</tr>
<tr>
<td>ZnO nanoparticles (340 nm)</td>
<td>PVCP stock solution</td>
<td>Human skin</td>
<td>0.2 –to 2 mm</td>
<td>T</td>
<td>102 and 103</td>
</tr>
</tbody>
</table>
suspensions as backscattering polarization phantoms. In order to measure the change in scattering (i.e., depolarization) calculated for different suspensions, microsphere diameter was varied.69,90 This class of phantoms has also been shown to depolarize linear polarization less with smaller-diameter microspheres as compared to circular polarization, while, with an increase of the microsphere diameter, circular polarization has been reported to be better preserved as compared to linear polarization.96

While purely aqueous monodispersed suspensions of microspheres are most commonly used in scattering experiments, intralipid has also been used to create depolarizing phantoms.2,53 Intralipid is commonly used as a nutrition supplement and is an emulsion of fatty micelles; therefore, scattering is due to multidispersed spherical structures. Aqueous intralipid suspensions with different dilution factors starting at 1:500 to 1:1 have been used to test depolarization with reflectance polarimetry.93,94,96 An example of such experiment can be seen in Fig. 2, where loss of elliptical polarization is measured as a function of depth in an intralipid suspension as reported by Sridhar and Da Silva.69 While intralipid suspension exhibits monotonic dependence of depolarization on light wavelength, the use of gold nanoparticles (GNPs) suspended in intralipid creates more complicated depolarization behavior.90

Titanium dioxide (TiO2) is another material commonly used to produce scattering in optical phantoms. TiO2 particles have been used in solid host media, such as polydimethylsiloxane or polyurethane, where, before the curing process, these particles are mixed into the polymer. Adjusting the concentration of TiO2 particles makes it possible to change the amount of depolarization.72,104 Zinc oxide (ZnO) particles are also commonly mixed into polymers.102,103 Melanin suspensions of rising concentrations can be used to test depolarization with PS-OCT and model the same phenomenon in the retinal pigmented epithelium. As demonstrated by Baumann et al.,106 the change in depolarization based on melanin concentration has a linear relationship with degree of polarization uniformity (DOPU).

2.3 Retarding Phantoms

Polymer-based materials are a common source of retardation. Due to their molecular structure or preparation process, many polymers possess intrinsic birefringence (i.e., behave as uniaxial crystals).104 Others can be induced to become birefringent by applying mechanical stress to the material.4,105 Many of these polymers are transparent; hence, scattering particles such as microspheres can be added to better simulate biological media. Electrospun polymer fibers, fabricated by charging droplets of polymer at high voltages which creates an interconnected network of small fibers,106 were used by Goth et al.40 to determine the degree of anisotropy of the overall structure. The anisotropic biological elements in the ECM (particularly collagen and elastin) have been simulated with several materials, including silk,88,99 and glass fibers.98,107 An example of fibrous phantom is shown in Fig. 3. Here, the phantom is composed of polystyrene microspheres and well-aligned glass fibers embedded in polycrystalline (glass fibers have a 10-μm diameter and 1.547 refractive index).

Phantoms for PS-OCT require a strong backscattering to generate a high image contrast and have ideally well-defined layers with homogeneous yet different values of birefringence (Table 3). Accordingly, Liu et al. have used a phantom consisting of a long birefringent polymer band laid over four smaller bands of differing birefringence. The optical axes of bottom four bands were oriented at 45 deg with the optical axis of top layer allowing for a depth-dependent change in retardation.106 An example of this retarding phantom is shown in Fig. 4.

Ghosh et al. induced changes in retardation by stretching a polycrystalline phantom. Moreover, changing birefringence, and mixing polystyrene microspheres and sucrose into the polymer, produced phantoms that could be used to characterize retardance, depolarization, and diattenuation.3,98,109 Extruded silicon, silicon wafers with gratings, and other types of silicon (poly and amorphous), as well as different tapes (e.g., Kapton and Mylar) normally used in solar panels, have been used to create phantoms containing different combinations of

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**Fig. 2** Image from Ref. 69. (a) Ruler placed obliquely in a tank containing Intralipid® solution, (b) elliptical channel image at 45 deg after subtraction method 1, (c) elliptical channel image at 45 deg after subtraction method 2. (b) and (c) have a common colorbar represented at the right edge of the figure. Yellow-dotted line represents the Intralipid®-air interface. Each graduation on the ruler (i.e., 1 mm) corresponds to 0.35 mm in actual depth. Wavelength: 633 nm. Text is from Ref. 69.

**Fig. 3** Image from Ref. 107. (a) Cylinder model, (b) sphere-cylinder model, and (c) sphere-cylinder birefringence model.
diattenuation, depolarization, and retardation properties.\(^{51,93,101}\)

Figure 5 shows an example of an experimental setup used to induce birefringence in a polymer through mechanical strain by Wood et al.\(^ {114}\)

In order to account for different geometries and extract geometry-independent metrics of anisotropy, retardance measurements have been taken using an 8-mm-diameter polystyrene sphere of known anisotropy axis azimuth.\(^ {111}\) Fan et al.\(^ {76}\) imaged a plastic cap to determine its retardation with PS-OCT.

### 2.4 Diattenuating Phantoms

The asymmetry of a molecule can result in selective transmission of an incident state of polarized light. Swami et al.\(^ {115}\) measured diattenuation as a parameter to identify the general shape of GNPs (Table 4). Differently shaped GNPs displayed different spectroscopic diattenuation values. Chen et al.\(^ {116}\) and Lung et al.\(^ {117}\) used a quarter-wave plate and a polarizer to test the performance of an analytical model for low

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### Table 3: Retardation phantoms

<table>
<thead>
<tr>
<th>Retardation material</th>
<th>Embedded material</th>
<th>Induced retardation</th>
<th>Tissue mimicking</th>
<th>Phantom thickness</th>
<th>Transmission/reflectance</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Birefringent film</td>
<td>Intralipid, India ink</td>
<td>Structure</td>
<td>ECM</td>
<td>Semi-infinite</td>
<td>R</td>
<td>49</td>
</tr>
<tr>
<td>Electrosyn fibers (0.6 to 1.0 (\mu)m)</td>
<td>None</td>
<td>Structure</td>
<td>Heart valve leaflet</td>
<td>Semi-infinite</td>
<td>R</td>
<td>60</td>
</tr>
<tr>
<td>Human hair</td>
<td>None</td>
<td>Structure</td>
<td>Human hair</td>
<td>N/A</td>
<td>R</td>
<td>15</td>
</tr>
<tr>
<td>Kapton tape (stacked)</td>
<td>Layered against a rigid base</td>
<td>Structure (layers)</td>
<td>Theoretical standard</td>
<td>Semi-infinite</td>
<td>R</td>
<td>93</td>
</tr>
<tr>
<td>Mylar (biaxially oriented polyethylene terephthalate)</td>
<td>Laid against a plexiglass base</td>
<td>Structure</td>
<td>Theoretical standard</td>
<td>Semi-infinite</td>
<td>R</td>
<td>93</td>
</tr>
<tr>
<td>Plastic cap*</td>
<td>None</td>
<td>Structure</td>
<td>Theoretical standard</td>
<td>Semi-infinite</td>
<td>R</td>
<td>76</td>
</tr>
<tr>
<td>Polycarbonate</td>
<td>None</td>
<td>Longitudinal stretch (heating and cooling)</td>
<td>Turbid biological tissue</td>
<td>250 (\mu)m</td>
<td>R</td>
<td>108</td>
</tr>
<tr>
<td>Polyacrylamide polymer (elastic)</td>
<td>None</td>
<td>4 mm stretch</td>
<td>Turbid biological tissue</td>
<td>4 mm</td>
<td>R</td>
<td>109</td>
</tr>
<tr>
<td>Polyacrylamide gels</td>
<td>Polystyrene microspheres, 1 M sucrose</td>
<td>Stretching</td>
<td>Turbid biological tissue</td>
<td>1 (\times) 1 (\times) 4 cm(^3)</td>
<td>T</td>
<td>105</td>
</tr>
<tr>
<td>Polyacrylamide*</td>
<td>Sucrose, polystyrene microspheres</td>
<td>Stretching</td>
<td>Turbid biological tissue</td>
<td>1 (\times) 1 (\times) 1 cm(^3)</td>
<td>T</td>
<td>3</td>
</tr>
<tr>
<td>Polyacrylamide*</td>
<td>Polystyrene microspheres and well-aligned fiber glass</td>
<td>Stretching (1 to 5 mm), birefringence = 0 to 10(^{-5})</td>
<td>Turbid biological tissue</td>
<td>1 (\times) 2 (\times) 4 cm(^3)</td>
<td>T</td>
<td>98 and 107</td>
</tr>
<tr>
<td>Polyethylene (low density)</td>
<td>None</td>
<td>Bending (up to 2.5 MPa)</td>
<td>Turbid biological tissue</td>
<td>1 mm</td>
<td>R</td>
<td>110</td>
</tr>
<tr>
<td>Polystyrene sphere</td>
<td>None</td>
<td>Structure</td>
<td>Infarcted myocardium</td>
<td>8 mm diameter</td>
<td>T</td>
<td>111</td>
</tr>
<tr>
<td>Polystyrene microspheres</td>
<td>Water</td>
<td>Structure</td>
<td>Turbid biological media</td>
<td>Semi-infinite</td>
<td>R</td>
<td>75, 97, 112</td>
</tr>
<tr>
<td>Polyurethane</td>
<td>Particle filled polypropylene</td>
<td>Longitudinal stretch</td>
<td>Theoretical standard</td>
<td>1 mm</td>
<td>R</td>
<td>113</td>
</tr>
<tr>
<td>Silicon (extruded)</td>
<td>Air between layers</td>
<td>Structure</td>
<td>Theoretical standard</td>
<td>2 mm</td>
<td>R</td>
<td>51</td>
</tr>
<tr>
<td>Silicon (amorphous)</td>
<td>None</td>
<td>Structure</td>
<td>Theoretical standard</td>
<td>Semi-infinite</td>
<td>R</td>
<td>93</td>
</tr>
<tr>
<td>Silicon (poly-)</td>
<td>None</td>
<td>Structure</td>
<td>Theoretical standard</td>
<td>Semi-infinite</td>
<td>R</td>
<td>93</td>
</tr>
<tr>
<td>Silk fibers*</td>
<td>Water</td>
<td>Structure</td>
<td>Anisotropic sample</td>
<td>Semi-infinite</td>
<td>R</td>
<td>88 and 99</td>
</tr>
</tbody>
</table>

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Chue-Sang et al.: Optical phantoms for biomedical polarimetry: a review

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diattenuating optical components as they were rotated from 0 deg to 150 deg with a step of 30 deg. Moreover, these authors also used a polymer polarizer baked at 150°C as a sample with both diattenuating and birefringent properties. Chenault and Chipman118 used a rotating sample polarimeter to find linear diattenuation and retardance of the sample calculated from intensity modulation.

2.5 Circular Retardation Phantoms

The effect of circular birefringence is frequently associated with the presence of chiral molecules,119 such as glucose. The aggregation of the presence chiral molecules in media causes the rotation of polarization plane of linearly polarized light as it travels through that volume. Manhas et al.,97 Ortega-Quijano et al.,120 and Ossikovski et al.112 added glucose to a polystyrene microsphere mixture in order to induce chirality and provide optical activity properties to the phantom (Table 5).

Malik et al.124 developed several ocular models to investigate the feasibility of measuring glucose in the eye aqueous humor with polarization-based techniques (Fig. 6). The model shown also accounts for the cornea birefringence utilizing a PMMA-based phantoms overlaying a chamber mimicking the aqueous humor. A similar approach was used by Rawer et al.125 Other intralipid suspension liquid phantoms can be made with absorbers, such as dye, and optically active molecules, such as glucose and l-lysine, to test optical activity in samples.18,121 Antonelli125 used honey to calculate the optical activity of the sample. Pham et al.99 and Chang et al.18 studied the concentration of glucose by measuring the optical rotation angle of circular birefringence (optical activity) in human blood plasma and porcine cartilage samples.

Fig. 5 Image from Ref. 114. Apparatus to create birefringent phantoms.

Fig. 4 Image from Ref. 108. (a)–(c) Intensity, birefringence, and DOP images of the slab and (d and e) cylindrical phantoms. (a) Representative cross-sectional images of the birefringence phantom for galvanometer-scanning system. (b) and (c) En-face images at different depths as indicated by the dashed red lines in (a). Horizontal and vertical scale bars for (a)–(c): 2 mm and 250 μm, respectively. (d) Representative images obtained from one rotational scan with the catheter. Scale bar: 1 mm. (e) Longitudinal sections obtained from a pull-back data set, with its corresponding location indicated by the dashed red line in (d). Radial and horizontal scale bars: 250 μm and 1 mm, respectively. (Text from Ref. 108.)
3 Conclusions
Optical phantoms that can be used for the calibration and benchmarking of polarimetric techniques and for mimicking the optical response of tissues have been used by several investigators. It is to be noted that polarimetric optical phantoms are often unique to each research group and, aside from tests conducted on depolarization with microspheres suspensions, no standardization has been attempted. To our knowledge, only one company offers birefringent phantoms for polarized microscopy (NBS 1963A Birefringent Resolution Target by Thorlabs). As the biomedical applications of polarimetric techniques move toward quantification of directionality and retardation, more standardized phantoms are necessary. The PS-OCT phantoms proposed by Liu et al. are a good example of such approach. The measurements of PS-OCT’s two core parameters, namely,

Table 4  Diattenuation phantoms. *Phantoms that were also tested for other polarization properties in corresponding reference paper.

<table>
<thead>
<tr>
<th>Diattenuation agent</th>
<th>Solvent/preparation</th>
<th>Tissue mimicking</th>
<th>Phantom thickness</th>
<th>Transmission/reflectance</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>GNPs (nonspherical shapes)</td>
<td>CTAB-coated GNPs</td>
<td>Theoretical standard</td>
<td>Semi-infinite</td>
<td>T</td>
<td>115</td>
</tr>
<tr>
<td>Kapton tape (stacked)*</td>
<td>Layered against a rigid base</td>
<td>Theoretical standard</td>
<td>Semi-infinite</td>
<td>R</td>
<td>93</td>
</tr>
<tr>
<td>Mylar (biaxially oriented polyethylene terephthalate)*</td>
<td>Laid against a plexiglass base</td>
<td>Theoretical standard</td>
<td>Semi-infinite</td>
<td>R</td>
<td>93</td>
</tr>
<tr>
<td>Polarizer</td>
<td>None</td>
<td>Theoretical standard</td>
<td>21.59 mm</td>
<td>T</td>
<td>116 and 117</td>
</tr>
<tr>
<td>Polarizer (baked)</td>
<td>150°C for 80 min</td>
<td>Theoretical standard</td>
<td>N/A</td>
<td>T</td>
<td>116 and 117</td>
</tr>
<tr>
<td>Polarizer (rotating)</td>
<td>None</td>
<td>Theoretical standard</td>
<td>N/A</td>
<td>T</td>
<td>118</td>
</tr>
<tr>
<td>Quarter-wave plate</td>
<td>None</td>
<td>Theoretical standard</td>
<td>N/A</td>
<td>T</td>
<td>116 and 117</td>
</tr>
<tr>
<td>Silicon (amorphous)*</td>
<td>None</td>
<td>Theoretical standard</td>
<td>Semi-infinite</td>
<td>R</td>
<td>93</td>
</tr>
<tr>
<td>Silicon (poly-)*</td>
<td>None</td>
<td>Theoretical standard</td>
<td>Semi-infinite</td>
<td>R</td>
<td>93</td>
</tr>
</tbody>
</table>

Table 5  Optical activity phantoms.

<table>
<thead>
<tr>
<th>Optical activity agent</th>
<th>Solvent/preparation</th>
<th>Tissue mimicking</th>
<th>Phantom thickness</th>
<th>Transmission/reflectance</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose (l-lysine)</td>
<td>Distilled water, l-alanine, intralipid suspension, trypan blue dye</td>
<td>Turbid biological media</td>
<td>Semi-infinite</td>
<td>R</td>
<td>121</td>
</tr>
<tr>
<td>Glucose (o-)</td>
<td>Water, 2 μm polystyrene microspheres, lipofundin, blood plasma, SiO₂ nanoparticles</td>
<td>Turbid biological media</td>
<td>40 mm</td>
<td>R/T</td>
<td>70, 97, 112, 120, 122, and 123</td>
</tr>
<tr>
<td>Glucose</td>
<td>Water</td>
<td>Eye aqueous humor</td>
<td>Semi-infinite</td>
<td>R</td>
<td>124–126</td>
</tr>
<tr>
<td>Glucose</td>
<td>Water</td>
<td>Eye aqueous humor</td>
<td>1 x 1 cm²</td>
<td>T</td>
<td>127</td>
</tr>
<tr>
<td>Honey</td>
<td>None</td>
<td>Turbid biological media</td>
<td>Semi-infinite</td>
<td>R</td>
<td>75</td>
</tr>
<tr>
<td>Sucrose</td>
<td>Polyacrylamide, polystyrene microspheres</td>
<td>Turbid biological tissue</td>
<td>1 x 1 x 1 cm³</td>
<td>T</td>
<td>3</td>
</tr>
<tr>
<td>L-(+)-arabinose, M. racemic</td>
<td>Water and polystyrene microspheres</td>
<td>Turbid biological tissue</td>
<td>1 x 1 x 1 cm³</td>
<td>Side T</td>
<td>119</td>
</tr>
</tbody>
</table>

Fig. 6  Optical phantom from Ref. 124. The custom-built ocular model. Glucose concentration in the anterior section is varied through the two infusion tubes.
retardation and azimuth of optical axis can be easily reproduced, and different instruments can be benchmarked using such standardized phantoms. These mixed properties phantoms, particularly ones that include both depolarization and retardation, are needed for many applications. Phantoms that have birefringence of form rather than just intrinsic birefringence are also needed to simulate fibrous tissues, such as the cervix, cardiac tissue, or muscle. Nevertheless, the task of creating general use phantoms is complicated by the heterogeneity of tissues, the complexity of polarized light–tissue interaction, and the strong wavelength dependence of polarization-based techniques.

For these reasons, the use of biological tissue as measurement standards is very common in polarimetric applications, but unless these samples are well-known or measured with an alternative modality (e.g., PS-OCT or second harmonic generation), the scientific rigor of these experiments remains limited.

As new fabrication modalities, such as 3-D printing and lithography, are becoming available to researchers worldwide, we believe that a collaborative effort in the development of a standardized optical phantom for polarimetry could truly benefit the scientific community.

**Disclosures**

The authors have no relevant financial interests in this article and no potential conflicts of interest to disclose.

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Biographies of the authors are not available.