Anisotropy and multiple scattering in thick mammalian tissues

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A dual-channel Mach–Zehnder interferometer using heterodyne detection allowed us to measure simultaneously parallel and perpendicular polarization components through various mammalian tissues at a wavelength of $\lambda = 633$ nm. By contrast with liver tissue, skeletal muscles of a few millimeters thickness exhibit strong anisotropic properties that change the direction of the linear polarization of the light. This rotation of the initial plane of polarization is to be distinguished from the depolarization that is due to the multiple light scattering that goes along with large temporal fluctuations. Complementary photos under linearly polarized light illustrate the behavior difference between liver (isotropic medium) and muscle (anisotropic medium).

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1. INTRODUCTION

For medical applications such as optical tomography or in situ spectroscopic measurements, it is necessary to know how light propagates in tissues. In optical coherence tomography based on the detection of ballistic (straightforward) photons, the best images of the tissue structure up to a 1-mm depth have been obtained by combining spatial collimation and low-coherence interferometry.\(^\text{1-3}\) In this domain, the use of the polarization state is awaited as a potential means to improve the contrast of fibrous structures in biological tissue,\(^\text{4}\) to characterize the birefringence of the myocardium muscle,\(^\text{5}\) or to image the birefringence of bovine tendon.\(^\text{6}\)

For investigations in tissue thicker than 1 mm, in which pure ballistic photons can no longer be detected, one must extract information from scattered light. Fortunately, coherent methods using a long-length coherence source remain efficient to detect diffuse light for imaging purposes.\(^\text{7}\) Considered as a possible means to improve the selection of forward-transmitted light through dense scattering media, polarimetry has been combined with lock-in detection\(^\text{8-10}\) or time-resolved detection schemes.\(^\text{11,12}\) Besides, in mammalian tissue, even more than in latex suspensions, the preservation of the incident polarization has been observed in the multiple-scattering regime over distances much larger than those in the ballistic regime,\(^\text{12,13}\) but little is known about this phenomenon. Finally, temporal fluctuations of the light intensity due to the Brownian motion are an additional difficulty in imaging biological tissues that contain a large amount of water.\(^\text{14}\)

In a recent study on the multiple-scattering regime, we observed that the polarization contrast in thick liver samples decreases progressively as the thickness of the sample increases from 1 to 6 mm, while large temporal fluctuations of the polarization are observed.\(^\text{13}\) In the present paper we report on a more complete description of light passing through liver as an example of isotropic tissue, in comparison with the case of muscle tissue; the two tissues are studied in identical experimental conditions. The original part of the work reported here deals with the influence of the strong anisotropy of thick muscles in polarized incident light, which dominates the multiple-scattering effect at the millimeter scale. This anisotropy in polarized light in vertebrate striated muscles has long been observed on a thin slice by light microscopy.\(^\text{15}\) In the contractile unit, known as sarcomere, light I bands (actin-containing) are isotropic to polarized light (\(I = \text{isotropy}\)). Anisotropy in polarized light is located in the dark A band (\(A = \text{anisotropy}\)), which contains myosin molecules. The striation pattern of a sarcomere is repeated regularly with a periodicity of $\approx 2.6 \mu m$ over the entire muscular cell length.\(^\text{15}\) One could imagine that such a regular structure might be taken into account for diffuse tomography or in-depth spectroscopic applications.

For the sake of simplicity, we will measure only the diagonal components $p_{yy}$ and $p_{xx}$ of the coherence matrix\(^\text{15}\)

$$\rho = \begin{pmatrix} p_{yy} & p_{xy} \\ p_{yx} & p_{xx} \end{pmatrix}$$

to describe the polarization $P$ of the transmitted light for a linear incident polarization. In practice, it is often considered that the polarization contrast, computed directly from $p_{yy}$ and $p_{xy}$ only, can completely describe the state of polarization of the scattered light.\(^\text{11,12}\) For tissue samples in which scattering particles are not spherical, however, a more precise description using the four Stokes
parameters of the polarization matrix should be considered when the nondiagonal components \( p_{xy} \) and \( p_{yx} \) are nonzero. Because of the time fluctuations observed in our tissue samples, it was not possible to measure accurately the four Stokes parameters by mechanically switching a quarter-wave plate, a half-wave plate, and a polarizing filter in sequence. Therefore we used a dual-channel setup that can measure simultaneously parallel and perpendicular components with good accuracy and high sensitivity. In any case, these components will be sufficient to identify qualitatively an optical anisotropy by distinguishing a rotation of the polarization plane (when \( p_{yx} < p_{xx} \)) from a progressive depolarization.

2. MATERIALS AND METHOD

To analyze the polarization of the transmitted light, we measured only the contrast of the linear polarization, using the dual-channel interferometer diagrammed in Fig. 1. The long-coherence laser source was a CW, linearly polarized, 7-mW He-Ne laser. In the measuring arm of the interferometer, two acousto-optical modulators, AOM’s, introduced a frequency shift of 1.3 kHz. On the detector, such a frequency shift yielded an oscillating signal that was easy to amplify. The polarized beamsplitting cube, set after the sample, separated linear parallel and perpendicular components of the light emerging from the sample. Similarly, the reference beam, circularly polarized by a quarter-wave plate, was split into its linear components in order to produce with the signal waves a heterodyne beat on both detectors. We achieved the calibration by inserting a half-wave plate at 45° and replacing the sample with a series of calibrated neutral densities. The detected voltage enabled us to obtain the extinction in terms of optical thickness (as \( OT = \ln(10) \cdot OD \)) corresponding to \(-\ln(I/I_0)\). We measured the contrast of polarization \( P \) given by the formula

\[
P = \frac{I_{yy} - I_{xx}}{I_{yy} + I_{xx}}.
\]

Carefully cut after being frozen, liver and muscle slices of beef and calf were mounted between two parallel flat glass sheets at checked thicknesses ranging from 1 to 5 mm. Muscle samples were cut perpendicular to the main fiber direction. The incident laser beam width of \( \sim 1 \) mm integrated the properties of a great number of muscle cells over the whole sample thickness. To study the spatial distribution of the polarization, we measured both parallel \( OT_{yy} \) and perpendicular \( OT_{xx} \) components at 18 different places on each sample. For each place, we computed the contrast of polarization \( P \) during 50 s. Thus, at a sampling rate of 2 Hz, we measured 100 \( P \) values at each place, giving 1800 \( P \) values for the total data acquisition on each sample.

In the photo created for macroscopic comparison (Fig. 5 below), muscle and liver samples were set into the same holder. Linearly polarized illumination was provided by combining a white-light source with a large polarizing sheet (HN22; Polaroid). To analyze both parallel and perpendicular polarized components of the light passing through the sample, we juxtaposed two polarizing sheets (HN22) against the holder side in front of the camera.

3. RESULTS

Figure 2 shows the average of all polarization contrast \( P \) values obtained at 18 places through calf muscle and beef liver samples, with \( L \) ranging from 1 to 5 mm. On each sample, standard deviations were measured over all 1800 \( P \) values. Data concerning muscle and liver samples are given on the same figure to facilitate comparison. From this precise point of view, light should be rapidly depolarized in muscle even for the 1-mm-thick sample, whereas depolarization is progressive through liver, leading to a polarization transport length close to or slightly above 5 mm.

A thick muscle sample is not perfectly homogeneous; the direction of muscle fibers can change over a few millimeters. Therefore the anisotropy due to myosin molecules should be partially degraded through thick samples. Actually, at a given place through 1 mm of muscle, the intensities of both parallel and perpendicular transmitted components were perfectly steady for a few minutes. This steady state leads to very steady \( P \) values. However, from one place to another, \( P \) values appeared very different, as shown in the right column in Fig. 3. In this figure the vertical dashed lines positioned every 50 s indicate the moment when measurement places have been changed. For more clarity, we selected here only ten random places on each slice. When the place of the measuring beam on the sample was changed, \( P \) values ranged between \(-1\) and \(+1\) because the direction of the emerging linear polarization plane varied between the incident direction (angle \( = 0 \)) for \( I_{yy} > 0 \) and \( I_{xx} = 0 \)) and the crossed direction (angle \( = \pi/2 \) for \( I_{yy} = 0 \) and \( I_{xx} > 0 \)). Even if we could not determine a complete coherence matrix, negative values for \( P \) (i.e., \( I_{xx} > I_{yy} \)) corresponded necessarily to a rotation of the polarization plane between \( \pi/4 \) and \( \pi/2 \). Figure 4 shows the recording of both parallel (\( OT_{yy} \)) and crossed (\( OT_{xx} \)) components of the intensity extinction on 18 different places on the 1-mm-thick muscle sample. In this example, the corre-
sponding intensity in the crossed direction can be close (see the first and fourteenth places in this example) or identical (the fourth place in this example) to the maximum intensity measured in the direction parallel to the incident polarization. Consequently, our experiments allowed us to retrieve a clear and strong birefringence in polarized light in the striated muscle on the millimeter scale. The rotation of the polarization plane, different from one place to another, is probably linked to the direction of its striated structure. In any case, we understand better now that the large standard deviation given in Fig. 2 for the 1-mm-thick muscle sample corresponds to a large data dispersion depending on the places and not on time fluctuations. Furthermore, when the muscle thickness increases, the multiple scattering dominates the birefringence, and light becomes depolarized with increased fluctuations. Depolarization seems almost complete when \( L = 5 \) mm. The same phenomenon was observed in chicken breast and beef muscle.

To compare the maintenance of the polarization after propagation through muscle with propagation through homogeneous and isotropic tissue, we show in each plot on the left side of Fig. 3 ten different polarization measurements observed through beef liver samples during 50 s for each place. When \( L = 1 \) mm, most of the \( P \) values were close to 1 and none below 0. Moreover, time fluctuations recorded between the dashed lines in Fig. 3 appeared larger in liver (on the left) than in muscle (on the right). For all studied thicknesses of liver, we checked that fluctuations observed during 15 min at 18 different places were similar to fluctuations observed during 15 min at the same place. When \( L = 5 \) mm, \( P \) values are distributed symmetrically around 0 (but are not perfectly random) while the signal fluctuations are as large as in the case of thin samples (see also Fig. 2). Consequently, on the millimeter scale, liver appeared as an optically isotropic tissue, in which the light behavior (dominated by scattering) was almost identical from one place to another. Results were similar for pork and beef liver samples.

For a direct macroscopic comparison between muscle and liver samples juxtaposed into the same holder (\( L = 1 \) mm), an additional experiment was carried out by combining a white-light source with parallel and crossed polarizing sheets. In the photo (Fig. 5), the calf muscle appears highly granular with an average extinction that is almost identical for the two polarization channels. On the contrary, extinction in liver is rather homogeneous.
However, additional more accurate experiments on muscle cut perpendicular to and along the fiber direction might be useful to determine which fiber structure and which fiber orientation influence the rotation of the polarization plane. Finally, we are planning experiments to observe in vivo whether the muscle birefringence changes during the contraction.

5. CONCLUSION

Muscle and liver tissues offer different optical characteristics within a range of thicknesses lower than the polarization transport length (i.e., from 1 to 5 mm) as observed with a long-coherence interferometer based on a polarization-sensitive heterodyne detection method. On the other hand, these new data could be useful for tissue diagnostics in reflectometry, as mentioned previously. On the one hand, consequences of the muscle birefringence in polarized light should be taken into account for optical diffuse tomography or in-depth spectroscopic applications since muscles are numerous in different parts of the human body. Elsewhere, tissues other than muscle that have a very regular structure, such as the white matter in nervous tissue, could be investigated to verify how they influence light behavior. Thus future realistic models applied to light propagation should take into consideration the diversity of tissues, which will result in an improvement in the use of diffuse tomography.

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