High-resolution polarization-sensitive optical coherence tomography for zebrafish muscle imaging

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Abstract: Zebrafish are an important animal model, whose structure and function information can be used to study development, pathologic changes and genetic mutations. However, limited by the penetration depth, the available optical methods are difficult to image the whole-body zebrafish in juvenile and adult stages. Based on a home-made high-resolution polarization-sensitive optical coherence tomography (PS-OCT) system, we finished in vivo volumetric imaging for zebrafish, and various muscles can be clearly discerned by scanning from dorsal, ventral, and lateral directions. Besides structure information, polarization properties extracted from PS-OCT images provide abundant function information to distinguish different muscles. Furthermore, we found local retardation and local optic axis of zebrafish muscle are related to their composition and fiber orientation. We think high-resolution PS-OCT will be a promising tool in studying myopathy models of zebrafish.

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1. Introduction

Myopathies are involved in a variety of diseases and conditions caused by mutations in many genes [1,2] or triggered by environmental stimuli [3]. Due to the diversity and severity of myopathies, it is necessary to understand the mechanism of myopathies. Benefited from the high similarity in the structure and genetics compared with humans, the zebrafish ranging from the embryonic period to adulthood is an important animal myopathy model [4–6].

In recent years, various optical imaging modalities, such as confocal microscopy [7], light sheet microscopy [8], and two-photon microscopy [9], have been investigated for imaging the zebrafish muscle in the embryo or larval stage. However, most of these optical modalities imaging zebrafish muscles require specific fluorescent labeling. Moreover, zebrafish will gradually lose their transparency in the first two weeks of development, which seriously decreases their penetration depth in juvenile and adult stages. Other imaging methods with good penetration depth, such as photoacoustic imaging [10], optical coherence tomography (OCT) [11], and space-gated microscopy [12], have been used to investigate juvenile or adult zebrafish. OCT functional extensions were also used to gain additional contrast in imaging zebrafish, such as dual modality functional optical coherence tomography and photoacoustic microscopy (OCT-PAM) [13] and pump-probe OCT (PPOCT) [14]. However, due to lacking marker information of musculature, it is hard for these imaging methods to detect the details of zebrafish muscle.

Polarization characteristics are found to provide abundant function information for describing the change of muscle. Several polarization imaging methods have been used to image zebrafish muscle, such as polarization contrast optical diffraction tomography [15], Mueller-matrix scanning microscopy [16], and polarized light microscope [17]. However, as mentioned above, their penetration depth is not enough to detect juvenile and adult zebrafish.

Polarization-sensitive optical coherence tomography (PS-OCT), as a functional extension of OCT, not only benefits from the advantages of OCT, such as, in vivo, non-labeling, high resolution, high sensitivity, higher penetration depth than microscopies, but also provides the
polarimetric properties for biological samples. It has been demonstrated its ability in analyzing the birefringence and fiber orientation of musculatures, including smooth muscle [18], skeletal muscle [19], and myocardium [20]. One preliminary study based on PS-OCT with an optical frequency domain reflectometer by P. Rossignoli et al. [21] was to image a 30-day old zebrafish, but they only proved the difference of polarization properties between birefringent and non-birefringent tissues limited by the parameters of their system.

In this study, a high-resolution home-made PS-OCT system was developed for imaging juvenile and adult zebrafish in vivo and obtaining multiple polarization characteristics of its muscles. In order to analyze different muscle tissues, the zebrafish was imaged from lateral, dorsal, and ventral directions. The birefringence and the optic axis of the zebrafish muscles were calculated, and it was proven that their values are related to the composition and fiber orientation of muscles. Further, we scanned the muscle of injured zebrafish and proved the high sensitivity to muscle changes of our PS-OCT system.

2. Methods and materials

2.1. PS-OCT system

The schematic of our spectral domain PS-OCT system is shown in Fig. 1(a). Output light, emitting from a superluminescent diode (SLD) (BLM2-D, Superlum) with the center wavelength of 840 nm and the bandwidth of 100 nm, passes through a three-paddle polarization controller (PC) and is coupled into a linear polarizer (POL) to obtain vertically polarized light. The polarization direction of the light is made by the PC to align with the direction of the polarizer. The vertically polarized incident light is separated into the sample and reference arms by a 50:50 non-polarizing beam splitter (BS). The reference light passes a quarter-wave plate (QWP1) angled at 22.5°, a dispersion compensator (DC) consisting of a pair of prisms, a neutral density filter (ND), and is reflected by a reference mirror (RM). DC is used to balance the dispersion between reference and sample arms. After light passes through the QWP1 again, the reference light is linearly polarized at 45°.

Another quarter-wave plate (QWP2) is added in the sample arm and oriented at 45°, providing circularly polarized light to the sample scanned by a 2D galvanometer scanning mirror (GV).
and the scanning lens (SL). Light power on the sample is ~4 mW. After backscattering from the sample and passing through the QWP2 again, the sample light with polarization change returns to the BS and interferes with the reference beam. Then, the interference light is split by a polarizing beam splitter (PBS) into two orthogonal polarization states and acquired by two commercial spectrometers (SP) (Cobra-S 800, Wasatch Photonics) through single-mode fibers.

Our system is performed with an axial resolution of ~3.4 μm in air and a transverse resolution of ~13 μm in the focal plane. Each spectrometer operates at 25 kHz line rate.

2.2. Data acquisition and processing

PS-OCT images are calculated from the spectral data after background removal, resampling to k-space, zero padding, and Fourier transformation. In order to ensure horizontal and vertical polarized spectral interferograms identical, two commercial spectrometers are accurately aligned. For suppressing the strong backscattering from scales of zebrafish, an additional spectral shaping filter is used to reduce the backscattering from scales and improve the signal-to-noise ratio.

2.3. System validation

We verified the accuracy of the PS-OCT system by imaging an octadic-wave plate (OWP). The octadic-wave plate (OWP) is a polarization element that can be used to manipulate the polarization state of light. The OWP is placed in the light path of the OCT system, and the polarization properties of the sample are measured. The polarization properties of the sample include the intensity of reflectivity (I), accumulative retardation (δ), accumulative optic axis (θ), and degree of polarization uniformity (DOPU).

The complex interferometric signals are obtained in horizontal (H) and vertical (V) polarization channels [23] by,

\[
\tilde{A}_{H,V}(z) = A_{H,V}(z) \exp[i\Phi_{H,V}(z)].
\]  

Where z is the depth coordinate, A and Φ are the magnitude and phase of the complex signal. The corresponding Stokes vector \( \vec{S} \), which describes the polarization state of light, is given by [24],

\[
\begin{bmatrix}
    I \\
    Q \\
    U \\
    V
\end{bmatrix} = \begin{bmatrix}
    \tilde{A}_H \tilde{A}_V^* + \tilde{A}_V \tilde{A}_H^* \\
    \tilde{A}_H \tilde{A}_V^* - \tilde{A}_V \tilde{A}_H^* \\
    \tilde{A}_H^* \tilde{A}_V + \tilde{A}_V^* \tilde{A}_H \\
    i(\tilde{A}_H \tilde{A}_V^* - \tilde{A}_V \tilde{A}_H^*)
\end{bmatrix} = \begin{bmatrix}
    A_H^2 + A_V^2 \\
    A_H^2 - A_V^2 \\
    2A_H A_V \cos \Delta \Phi \\
    2A_H A_V \sin \Delta \Phi
\end{bmatrix}.
\]  

Where, \( \Delta \Phi = \Phi_H - \Phi_V \). \( \tilde{A}^* \) is the complex conjugation of \( \tilde{A} \). Here, it must be noted that we used the full Stokes averaging method [25] to suppress speckle noise by using a 3D kernel including pixels within 40 μm. The intensity of reflectivity (I), accumulative retardation (δ), accumulative optic axis (θ), and degree of polarization uniformity (DOPU) can be calculated [23] by:

\[
\begin{align*}
I(z) &= |A_H(z)|^2 + |A_V(z)|^2 \\
\delta(z) &= \arctan \left( \frac{A_V(z)}{A_H(z)} \right) = \frac{1}{2} \cos^{-1} \left( \frac{Q}{I} \right) \\
\theta(z) &= \frac{180^\circ - \Delta \Phi}{2} = 90^\circ - \frac{1}{2} \tan^{-1} \left( \frac{V}{U} \right) \\
DOPU(z) &= \sqrt{\left( \frac{Q}{I} \right)^2 + \left( \frac{U}{I} \right)^2 + \left( \frac{V}{I} \right)^2}
\end{align*}
\]

(3)

δ and θ are used to describe the birefringence of the sample. DOPU is used to describe the depolarization [26]. δ and θ are accumulated parameters from the sample surface to the measurement depth and they are restricted to limited ranges, which make δ and θ have banded pattern in strongly birefringent samples. Therefore, it is not intuitive for interpreting the retardation and optic axis at a specific location. Compared with the accumulative retardation and accumulative optic axis, local retardation and local optic axis [27] can extract depth-resolved polarization properties of the sample.

2.3. System validation

We verified the accuracy of the PS-OCT system by imaging an octadic-wave plate (OWP). The accumulative retardation and optic axis orientation of 20k A-scans were calculated by Eq. (3) and...
averaged. Firstly, we validated the accuracy of measuring retardation and optic axis orientation by varying the axis orientation of OWP at a fixed path length difference of 1.69 mm. The full range of axis orientation is covered by rotating the optic axis of OWP from 0° to 180° with a step of 10°, while the retardation stayed at a constant value of 45°. The comparison of measured and theoretical values of retardation and axis orientation are shown in Fig. 2(a). As shown in Fig. 2(a), the measured retardation (blue squares) slightly fluctuates around theoretical value (blue dashed line) with the increase of the axis orientation of OWP. The mean value and standard deviation of the retardation are 45.51° and 4.31°, respectively. Moreover, measured axis orientation (pink triangles) increases almost linearly with the increase of OWP orientation (pink dashed line).

Further, we validated the system stability of measurement in different imaging depths. We changed the path length difference between the sample and the reference mirror and maintained the axis orientation of the OWP at a constant value. We measured each retardation and axis orientation in the depth range of 0.1-1.6 mm with a step depth of 0.14 mm and the results are shown in Fig. 2(b). As shown in Fig. 2(b), the measured retardation and axis orientation are both approximately constant against the depth. The mean retardation value and standard deviation of the OWP are 46.38° and 1.25°, and the corresponding parameters of optic axis orientation are 41.22° and 2.13°. A slight drift of axis orientation may be induced by signal attenuation with depth. These results are similar to those of other PS-OCT systems [23,28], which prove the accuracy and stability of our PS-OCT system.

2.4. Sample preparation

Wild-type zebrafish of strain AB (n = 3, male, older than 60 days) were used in our study. Zebrafish were firstly anesthetized in a 0.024% tricaine solution by immersion until they became unresponsive to touch. After that, the zebrafish was placed in agar gel filled with 0.012% tricaine solution. The agar gel had two kinds of depths to hold zebrafish for dorsal, ventral, and lateral scanning.

After OCT imaging, the zebrafish was sacrificed by using 0.4% tricaine and ice water and fixed in 4% paraformaldehyde solution. The zebrafish was embedded inside paraffin sections and cut at a thickness of 20 µm. All the slices were stained with hematoxylin and eosin as imaging references. All experimental protocols using animals were approved by the Institutional Animal Care Committee of Nankai University.
3. Experimental results

3.1. Intensity images from three directions

To obtain OCT images of all kinds of muscles, we scanned zebrafish from dorsal, ventral, and lateral directions by our PS-OCT system. The placements of fish under three scanning directions are shown in Fig. 3.

Each OCT data set contains \(2500 \times 1000 \times 2048\) pixels \((12.5 \text{ mm} \times 5 \text{ mm} \times 2.3 \text{ mm})\), whose scanning time is \(~2 \text{ min}\) for per polarization channel. Typical en-face OCT images of zebrafish are shown in Fig. 4. Figs. 4(a)–4(b) are the results of lateral scanning at different depths. Figs. 4(c) and 4(d) are the results of dorsal and ventral scannings, respectively. Benefited from millimeter-scale imaging depth and micron-scale resolution, the details of organs and tissues of zebrafish, such as eye (E), brain (B), semicircular canal (SeC), gill filaments (GF), pectoral fin muscle (PecFM), facial lobe (FL), dorsal fin (DF), anal fin (AF), epaxial muscles (EM), hypaxial muscles (HM), spinal cord (SpC) and body cavity (BC), can be clearly observed in the en-face images [Figs. 4(a)–4(b)]. Other details of dorsal tissues [e.g. brain and supracarinate muscles (SM)] and ventral tissues [e.g. sternohyoid (St), pectoral fin muscle (PecFM), pelvic fin muscle (PelFM), pelvic fin (PelF) and abdominal wall (AW)] can be shown by dorsal and ventral scannings [Figs. 4(c)–4(d)]. The feasibility of the penetration depth and imaging quality of our PS-OCT system is confirmed visually based on these results.
3.2. PS-OCT images of zebrafish

The typical intensity image of zebrafish and its corresponding images of polarization parameters, including accumulative retardation, accumulative optic axis, DOPU, local retardation and local optic axis by lateral scanning, are shown in Fig. 5. In the intensity image [Fig. 5(a)], iris (I), cornea (Co), adductor mandibulae (AM), opercle (O), gill (G), pectoral fin (PecF), scales (Sc), skin (Sk), trunk musculature (TM), swim bladder (SB) and vertebral column (VC), can be clearly identified.

In the DOPU image [Fig. 5(b)], most DOPU values of skin and pectoral fin are high and homogeneous. As shown in Fig. 5(c), the accumulative retardation values of the above tissues tend to zero, which indicates the low birefringence of those tissues. In contrast, the accumulative retardation values of high birefringence tissues, such as trunk musculature and adductor mandibulae, fluctuate between 0 and $\pi/2$ along the depth direction, caused by phase wrapping effects for the accumulation results of retardation [26]. The local retardation [Fig. 5(d)] presents the quantitative analysis of birefringence. The local retardation values of muscle tissues with high birefringence are higher than the values of skin, pectoral fin, and gill due to different collagen contents. As shown in the figure of the accumulative optic axis [Fig. 5(e)], low birefringence tissues, such as skin and gill, can reflect the directivity within the tissues visually. However, the accumulative optic axis is incorrect for samples of optic axis varying along with the depth [27] or high birefringence [29]. Unfortunately, the polarization characteristics of the zebrafish muscle belong to these two conditions. As shown in Fig. 5(e), we can observe the phase jump of accumulative optic axis marked with white arrows in the trunk musculature. This phase jump may be mistakenly interpreted in studying zebrafish and can be corrected by calculating the local optic axis [27] shown in Fig. 5(f). Therefore, the depth-resolved local optical axis is extracted to analyze the muscles in the subsequent research. Skin of zebrafish has higher DOPU and lower local retardation than its muscle, so please note that the color maps of DOPU [Fig. 5(b)] and local retardation [Fig. 5(d)] are specially designed to highlight the different contrast characteristics of zebrafish, and the color map of DOPU is no longer used in the subsequent figures.
3.3. Segmentation of the muscle based on the DOPU threshold

To prevent interference of scales and skin, it is necessary to extract the muscles from OCT images before analyzing them. Segmented intensity images based on the thresholds of intensity and DOPU are shown in Figs. 6(a) and 6(b), respectively, where the blue regions below the threshold are regarded as muscles. As shown in Fig. 6(a), some muscles with high intensity are segmented mistakenly. As a comparison, when the DOPU threshold is set to be 0.86, clear boundaries between the skin and the muscle can be achieved in Fig. 6(b). That is because compositions of skin and muscle have different polarization characteristics. 3D intensity rendering OCT images before and after segmentation based on the DOPU threshold are shown in Fig. 6(c) and 6(d), respectively. As shown in these two figures, before segmentation, the scales (Sc) cover the zebrafish completely. After segmentation, the structure of myoseptum (M) and anal fin musculature (AFM) can be clearly observed and only with a few scales left on the body.

![Fig. 6. Segmentation of the muscle. (a) and (b) are intensity images used to show the segmentation results of muscles based on the thresholds of intensity and DOPU, respectively. (c) and (d) are 3D intensity rendering OCT images of zebrafish before and after segmentation, respectively, where scales (Sc), myoseptum (M), and anal fin musculature (AFM) are pointed out by different green marks. Scale bars are 500 µm.](image)

3.4. Birefringence analysis of the zebrafish muscle by lateral scanning

OCT images obtained by lateral scanning can cover the intact upper-half zebrafish, which provides sufficient information to study various kinds of muscles in detail. Based on the PS-OCT images, we can further analyze the birefringence of the zebrafish muscle quantitatively.

It has been known that the red muscle has more lipid than the white muscle [30] and the birefringence of lipid is lower than that of muscle [31], which implies red and white muscles should have different birefringence characteristics.

Figure 7(a) displays an en-face image of accumulative retardation, and the red muscle (RM) area is pointed out by a low-value boundary marked by two black dashed curves. We randomly selected two positions marked by red and green lines in Fig. 7(a) to further investigate the birefringence of the zebrafish muscle. Figs. 7(b) and 7(c), Figs. 7(d) and 7(e), and Figs. 7(f) and 7(g) are their intensity images, accumulative retardation images, and local retardation images, respectively. For highlighting the polarization characteristics of the muscle, the contents in the body cavity were removed. In addition, in order not to make images too messy, color bars of Fig. 7 and the following figures in this paper are the same as those of Fig. 5.

The white muscle, including epaxial muscles (EM) and hypaxial muscles (HM), can be observed in Figs. 7(b) and 7(c). It is easy to identify the infracarinate muscles (IM) based on the boundary with HM and the position in zebrafish. To our knowledge, the lipid has stronger
Fig. 7. Birefringence analysis of different muscles of zebrafish by lateral scanning. (a) An en-face image of accumulative retardation, in which the red muscle (RM) area is marked by two black dashed curves, and two randomly selected positions marked by red and green lines are used to further investigate the birefringence of zebrafish muscle. (b) and (c) are their intensity images, (d) and (e) are accumulative retardation images, and (f) and (g) are local retardation images. Epaxial muscles (EM), hypaxial muscles (HM), infracarinates muscles (IM), and red muscle (RM) are pointed out in (b)-(g). RM area is marked by pink dashed circles in (b) and (c). Different muscles are separated by black dashed curves in (d)-(g). (h) is the counting histogram of EM, HM, and RM in the regions marked by white dashed rectangular boxes in (f). (i) is a 3D rendering image of local retardation, where RM, anal fin musculature (AFM), and myoseptum (M) are marked by black dashed curves, the black arrow, and black dot-dashed curves, respectively. Scale bars are 500 µm.
scattering than the muscle, which attenuates the intensity of the tissues under the lipid [31]. Based on these characteristics corresponding to the red muscle (RM), we roughly marked the RM area by a pink dashed circle. However, it is hard to discern the clear boundary of RM from these intensity images.

In their accumulative retardation images [Figs. 7(d) and 7(e)], more details of muscles can be used to identify different muscles. RM and IM areas can be clearly discerned by black dashed curves, and RM shows lower values than other muscles due to its low birefringence.

Local retardation images [Figs. 7(f) and 7(g)] can be used to analyze the birefringence of different muscles quantitatively. We selected a region including 30 (x) × 80 (z) pixels (~0.16 mm × 0.1 mm) from each tissue marked by white dashed boxes in Fig. 7(f). The counting histograms of EM, HM, and RM are plotted in Fig. 7(h), in which we can observe the numerical distribution of RM is lower than those of EM and HM. We compared the local retardation of white muscles (including EM and HM) of zebrafish with previous studies in mouse skeletal muscles fiber [19] and found that they are on the same order of magnitude (~10^{-2} rad). Further, we referred to the article [32] to convert local retardance to birefringence. The birefringence of white muscles of zebrafish is about 1.1×10^{-3}, which is smaller than that of the porcine tendon (~4×10^{-3}). This may be caused by the different shape and composition of muscle fibers in different animals. By extracting the muscle based on the DOPU threshold, a 3D rendering image of local retardation [Fig. 7(i)] is given, in which RM, AFM, and myoseptum(M) are shown with low birefringence because of their high lipid content. They are marked by black dashed curves, the black arrow, and black dot-dashed curves in Fig. 7(i), respectively. Compared with the 3D intensity rendering image shown in Fig. 6(d), RM can be clearly observed in Fig. 7(i), which demonstrated the role of the polarization contrast.

3.5. Local optic axis analysis of zebrafish by lateral scanning

After distinguishing the red and white muscles by the above birefringence analysis, we further analyzed the local optic axis of the muscle. Figures 8(a) and 8(b) are given to show the local optic axis images with the same position of Figs. 7(d) and 7(e), respectively. The separated areas with red dashed curves are the same as the areas in Figs. 7(d) and 7(e).

As shown in Figs. 8(a) and 8(b), the distribution of local optic axis values is not uniform in EM and HM areas, and different muscle tissues have different local optic axis patterns. These results may be caused by various orientations of muscle fibers. A 3D rendering image of local optic axis is shown in Fig. 8(c) with a transparency of 0.5, where the internal muscle can be easily observed. We can identify the EM and the HM based on different local optic axis patterns. As
the fibers of EM and HM extend to the dorsal and ventral sides of zebrafish, respectively, the local optic axes of them gradually change. Besides those, the local optic axes of the pectoral fin muscle (PecFM) and anal fin musculature (AFM) have clear difference compared with those of the adjacent muscles.

For validating the similarity between the local optic axis and the orientation of muscle fiber, we randomly selected an area in a red dashed rectangular box in Fig. 8(c) and compared it with its tissue section [Fig. 8(d)]. As shown in Fig. 8(d), the orientation distributions of various muscle fibers are differentiated with yellow dot-dashed curves, which are similar to the distributions of the optic axis split in Fig. 8(c). The pink dashed curves were plotted to roughly indicate the local optic axis and the orientation of muscle fiber in Fig. 8(c) and 8(d), respectively. In general, we can observe the consistency of the local optic axis and the orientation of muscle fiber. Similar results have been proved in the previous studies of mouse skeletal muscle fiber [19].

We selected two en-face images at different depths, as shown in Figs. 9(a) and 9(b), to further analyze the relationship between the local optic axis and the orientation of muscle fibers. Their corresponding streamline diagrams of the local optic axis, overlaying on the intensity images, are shown in Figs. 9(c) and 9(d). The red dashed curves are used to separate the different optic axis modes of EM and HM, which is the rough boundary between EM and HM. The PecFM and the AFM are marked with red dashed circles and square, respectively. As shown in Fig. 9(c), the local optic axis pattern of PecFM is different from those of adjacent muscles. More definite evidence, implying a corresponding relationship between the optic axis and the orientation of muscle fibers, can be clearly seen in the contrast enhanced en-face intensity image [Fig. 9(e)].

![Fig. 9.](image)

Fig. 9. (a) and (b) are two en-face local optic axis images at different depths. (c) and (d) are the corresponding streamline diagrams of the local optic axis. (e) is the contrast enhanced en-face intensity image of Fig. 4(a). (f) is the enlarged view of green dashed-line rectangular area in (e). Pink arrows in (e) and (f) indicate muscle fibers, whose orientations are similar to the local optic axes indicated in (c) with a pink arrow. (g) is the histological section corresponding to the red dashed-line rectangular area in (d). Pink solid, dotted, and dot-dashed lines describe the orientations of different muscle fibers. (a), (c) and (e) locate at the same depth of the same fish as Fig. 4(a). Scale bars are 500 µm.
Figures 9(a), 9(c) and 9(e) locate at the same depth of the same fish as Fig. 4(a). The enlarged view of the green dashed-line rectangular area in Fig. 9(e) is shown in Fig. 9(f). We can observe several muscle fibers indicated by pink arrows, whose orientations are similar to their local optic axis pointed out by a pink arrow in Fig. 9(c). For further verification, we compared with the local optic axes of the red dashed-line rectangular area in Fig. 9(d) with the corresponding histological section [Fig. 9(g)]. In Fig. 9(g), the AFM fibers (pointed out by a pink solid curve) are almost vertically aligned. The HM fibers (pointed out by a pink dotted curve) far away from the AFM are inclined. The muscle fibers (pointed out by a pink dot-dashed curve) between AFM and HM are mostly horizontally aligned. These fiber orientations of muscles exhibit good consistency with the local optic axis shown in Fig. 9(d). The polarization coordinates used for local optic axis measurement are added in Figs. 8(c), 9(a) and 9(b).

3.6. Results from ventral and dorsal scanning directions

Compared with lateral scanning, scanning from dorsal and ventral directions can give a more specific description for SM and IM. Two sets of typical en-face images by ventral and dorsal scanning are shown in Fig. 10 and Fig. 11, respectively, in which include the intensity, accumulative retardation, local retardation, accumulative optic axis, the local optic axis with corresponding streamline diagrams, and 3D rendering images of local optic axis. Most of the ventral tissues and organs, such as opercle (O), sternohyoid (St), pectoral fin muscle (PecFM), abdominal wall (AW), and pelvic fin muscle (PelFM), can be seen in Fig. 10. The epaxial muscles (EM) and supracarinate muscles (SM) can be seen in Fig. 11. The tissues and organs are separated by red dashed curves.

![Fig. 10. En-face PS-OCT images by ventral scanning. (a)-(e) are intensity, accumulative retardation, local retardation, accumulative optic axis, local optic axis overlaid with corresponding streamline images, respectively. The opercle (O), sternohyoid (St), pectoral fin muscle (PecFM), abdominal wall (AW), and pelvic fin muscle (PelFM) are separated by red dashed curves. (f) is the 3D rendering image of local optic axis. Scale bars are 500 µm.](image)

The most striking feature of these images is their symmetry along the sagittal central axis. As shown in Figs. 10(a) and 11(a), the intensity images show the symmetry of the bilateral organ structure. The accumulative retardation images [Figs. 10(b) and 11(b)] and the local retardation images [Figs. 10(c) and 11(c)] show the symmetrical birefringence of the bilateral muscles. The local retardation values of muscles are larger than those of the skin and fins. The accumulative optic axis images [Figs. 10(d) and 11(d)] and the local optic axis [Figs. 10(e) and 11(e)] show the symmetrical orientation of bilateral muscle fibers. The 3D rendering images of local optic axis of ventral and dorsal scannings are shown in Figs. 10(f) and 11(f), respectively.

It should be noted that the accumulative retardation fluctuates with depth. The symmetry of accumulative retardation not only indicates the symmetrical birefringence of zebrafish muscles but also implies the starting points of the accumulative retardation fluctuation in the symmetric
positions are at the same depth. More importantly, it is demonstrated that the scanning direction of our system is strictly perpendicular to the sagittal central axis of the zebrafish. It is well known that different accumulative retardations of the same muscle will be obtained by scanning the long axis of the muscle fibers with different angles, which result in different local retardations. Different from the lateral muscles, the distribution of dorsal and ventral muscles of zebrafish is more diverse and the orientation of the muscle fibers varies more with depth. Therefore, scanning as perpendicular to the long axis of muscle fiber as possible is very important to provide accurate and repeatable polarization characterizations of the zebrafish muscle.

### 3.7. Characterization of the injured zebrafish based on PS-OCT images

In order to demonstrate that the variation of muscle can be characterized by PS-OCT images, an injured zebrafish was scanned. A wild type adult zebrafish (male, older than 90 days) was anesthetized and was stabbed by using a sterile needle in its trunk muscle. After that, the zebrafish was put back into the fresh water. After one day, the zebrafish was anesthetized again and scanned by our PS-OCT system from the dorsal direction.

OCT images with the injured area marked by a black dashed rectangular box are shown in Fig. 12, in which include intensity (a), accumulative retardation (b), and local retardation images (c). Based on these results, we can observe different intensity and birefringence characterizations between the injured and the healthy muscles. As shown in Fig. 12(a), the injured area has less muscle texture than adjacent muscle tissue, which indicates the muscle fibers have been broken. As noted in the literature [17], the reduction of birefringence in zebrafish is related to the muscle damage. The change of the accumulative retardation tends to be flat in the injured area in Fig. 12(b) and the local retardation of the injured area stays at a low-value level in Fig. 12(c), which both indicate the disappearance of its birefringence because of the injury of the muscle.
4. Discussions

Combining the results scanned by three directions of zebrafish, nearly all kinds of muscles are imaged \textit{in vivo} by our PS-OCT system. The calculated results of birefringence and optic axis, reflecting polarization characteristics of muscles, can be used to distinguish different muscle tissues. Based on these characteristics, we obtained 3D image reconstruction and rendering images. It is proven by the results of injured fish that our PS-OCT system has enough sensitivity to detect the variation of muscle.

Benefit from high resolution and better penetration, more tissues and organs can be detected in OCT images than other images. To the best of our knowledge, this is the first time to use a high-resolution PS-OCT system to image the muscle for \textit{in vivo} juvenile and adult zebrafish. Due to melanin pigmentation and physical size increasing with its development, other optical imaging technologies can image the larval zebrafish, but is hard to image the adult zebrafish. Ultrasound, magnetic resonance imaging, and X-ray computed tomography are slow and have not enough resolution to image zebrafish muscle clearly. We proved that muscles can be characterized and distinguished based on high-resolution PS-OCT images, and the polarization characteristics can be served as valuable specific labels of zebrafish muscle.

As functional parameters, polarization characteristics can reflect the condition of the muscle. The birefringence and the local optic axis are nearly uniform in the same kind of muscle, and disorders of the birefringence and local optic axis are related to the degree of muscle diseases [19]. Calculated results of the birefringence and local optic axis for zebrafish muscles in this paper manifest the possibility of quantitative analysis for muscle diseases, which prove PS-OCT has great potential in assessing the condition of the muscle and helping establish the pathological zebrafish model.

We also imaged other organs of zebrafish by using our PS-OCT system, such as brain and eyes. We will further study their polarization characteristics. Besides, based on the comprehensive functional analysis in PS-OCT images, zebrafish with different genetic models or muscle diseases, such as dag1 and dmd mutants [33], will be studied in the future. Further investigations will be done to establish the polarization characterization model of the zebrafish and assess zebrafish diseases and toxicological models.

5. Conclusion

We have demonstrated the ability of our PS-OCT system for imaging various muscles of juvenile and adult zebrafish from three directions’ scanning. Our methods provide the quantitative birefringence assessment of zebrafish muscle and the mapping relationship between the local optic axis and the orientation of zebrafish muscle fibers. The PS-OCT images of the injured zebrafish illustrate the high sensitivity of our PS-OCT system for detecting the variation of zebrafish muscles. Based on the findings and conclusion in this study, we believe high-resolution PS-OCT has great potential in studying zebrafish muscle models.

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Disclosures

The authors declare that there are no conflicts of interest related to this article.

References

1. S. Lange, F. Xiang, A. Yakovenko, A. Vihola, P. Hackman, E. Rostkova, J. Kristensen, B. Brandmeier, G. Franzen, B. Hedberg, L. G. Gunnarsson, S. M. Hughes, S. Marchand, T. Sejersen, I. Richard, L. Edström, E. Ehler, B. Udd, and M. Gautel, “The kinase domain of titin controls muscle gene expression and protein turnover,” Science 308(5728), 1599–1603 (2005).

2. H. Jungbluth, S. Treves, F. Zorzato, A. Sarkozy, J. Ochala, C. Sewry, R. Phadke, M. Gautel, and F. Muntoni, “Congenital myopathies: disorders of excitation-contraction coupling and muscle contraction,” Nat. Rev. Neurol. 14(5), 151–167 (2018).

3. W. R. Saliba, L. H. Goldstein, G. S. Habib, and M. S. Elias, “Toxic myopathy induced by the ingestion of loquat leaf extract,” Ann. Rheum. Dis. 63(10), 1355–1356 (2004).

4. J. R. Guyon, L. S. Steffen, M. H. Howell, T. J. Pusack, C. Lawrence, and L. M. Kunkel, “Modeling human muscle disease in zebrafish,” Biochim. Biophys. Acta, Mol. Basis Dis. 1772(2), 205–215 (2007).

5. D. P. O'Sborn, H. L. Pond, N. Mazaheri, J. Dejaardin, C. J. Munn, K. Mushref, E. S. Cauley, I. Moroni, M. B. Pasquinelli, E. A. Sellars, A. Carapezza, N. B. Hill, J. N. Partlow, R. K. Willaert, J. Bharj, R. A. Malamiri, H. Galehdari, G. Shariati, R. Maroofian, M. Mora, L. E. Swan, T. Voit, F. J. Conti, Y. Jamshidi, and M. C. Manzini, “Mutations in INPP5K cause a form of congenital muscular dystrophy overlapping Marinesco-Sjögren syndrome and dystroglycanopathy,” Am. J. Hum. Genet. 100(3), 537–545 (2017).

6. K. M. Lebold, C. V. Löhr, C. L. Barton, G. W. Miller, E. M. Labut, R. L. Tanguay, and M. G. Traber, “Chronic vitamin E deficiency promotes vitamin C deficiency in zebrafish leading to degenerative myopathy and impaired swimming behavior,” Comp. Biochem. Physiol., Part C: Toxicol. Pharmacol. 157(4), 382–389 (2015).

7. A. M. Fallata, R. A. Wyatt, J. M. Levesque, A. Dufour, C. M. Overall, and B. D. Crawford, “Intracellular localization in zebrafish muscle and conserved sequence features suggest roles for gelatinase A moonlighting in sarcomere maintenance,” Biomedicines 7(4), 93 (2019).

8. A. Kaufmann, M. Mickoleit, M. Weber, and J. Huisken, “Multilayer mounting enables long-term imaging of zebrafish development in a light sheet microscope,” Development 139(17), 3242–3247 (2012).

9. S. Gao, Y. Tang, and W. Lin, “Development of a two-photon turn-on fluorescent probe for cysteine and its bio-imaging applications in living cells, tissues, and zebrafish,” New J. Chem. 42(17), 14075–14078 (2018).

10. T. Y. Li, X. Xiang, T. Chen, C. Gao, H. Fu, L. Wang, L. Deng, L. Zeng, and P. Zhang, “In vivo monitoring and high-resolution characterization of the prednisolone-induced osteoprotic process on adult zebrafish by optical coherence tomography,” Biomed. Opt. Express 10(3), 1184–1195 (2019).

11. M. Jang, H. Ko, J. H. Hong, W. K. Lee, J.-S. Lee, and W. Choi, “Deep tissue space-gated microscopy via acousto-optic interaction,” Nat. Commun. 11(1), 710 (2020).

12. R. Haindl, A. J. Deloria, C. Sturtzel, H. Sattmann, W. Rohringer, B. Fischer, M. Andreana, A. Unterhuber, T. Schwerte, M. Distel, W. Drexler, R. Leitgeb, and M. Liu, “Functional optical coherence tomography and photoacoustic microscopy imaging for zebrafish larvae,” Biomed. Opt. Express 11(4), 2137–2151 (2020).

13. W. Kim and B. E. Applegate, “In vivo molecular contrast OCT imaging of methylene blue,” Opt. Lett. 40(7), 1426–1429 (2015).

14. L. van Rooij and J. Kalkman, “Polarization contrast optical diffraction tomography,” Biomed. Opt. Express 11(4), 2109–2120 (2020).

15. A. Le Gratiet, M. d’Amora, M. Duocastella, R. Marongiu, A. Bendandi, S. Giordani, P. Bianchini, and A. Diaspro, “Zebrafish structural development in Mueller-matrix scanning microscopy,” Sci. Rep. 9(1), 19974 (2019).

16. J. Berger, T. Sztal, and P. D. Currie, “Quantification of birefringence readily measures the level of muscle damage in zebrafish,” Biochim. Biophys. Res. Commun. 423(4), 785–788 (2012).

17. Q. Li, K. Kurnowska, P. B. Noble, A. Cairncross, A. James, M. Villiger, and D. D. Sampson, “Robust reconstruction of local optic axis orientation with fiber-based polarization-sensitive optical coherence tomography,” Biomed. Opt. Express 9(11), 5437–5455 (2018).

18. Y. Wang, K. Zhang, N. B. Wasala, D. Duan, and G. Yao, “Optical polarization tractography revealed significant fiber disarray in skeletal muscles of a mouse model for Duchenne muscular dystrophy,” Biomed. Opt. Express 6(2), 347–352 (2015).

19. Y. Wang, K. Zhang, D. Duan, and G. Yao, “Heart structural remodeling in a mouse model of Duchenne cardiomyopathy revealed using optical polarization tractography [Invited],” Biomed. Opt. Express 8(3), 1271–1276 (2017).

20. P. Rossignoli, N. Tiso, M. Santagiustina, E. Autizi, E. Grisan, and L. Palmieri, “Polarization sensitive optical coherence tomography for zebrafish imaging,” in 2015 Fotonica AEIT Italian Conference on Photonics Technologies (IET) (2015), pp. 1–4.

21. B. Baumann, M. Augustin, A. Lichtenegger, D. J. Harper, M. Muck, P. Eugui, A. Wartak, M. Pircher, and C. K. Hitzenberger, “Polarization-sensitive optical coherence tomography imaging of the anterior mouse eye,” J. Biomed. Opt. 23(8), 1 (2018).
23. E. Götzinger, M. Pircher, and C. K. Hitzenberger, “High speed spectral domain polarization sensitive optical coherence tomography of the human retina,” Opt. Express 13(25), 10217–10229 (2005).
24. N. Ortega-Quijano, T. Marvdashti, and A. K. E. Bowden, “Enhanced depolarization contrast in polarization-sensitive optical coherence tomography,” Opt. Lett. 41(10), 2350–2353 (2016).
25. Q. Xiong, N. Wang, X. Liu, S. Chen, H. Liang, S. Chen, and L. Liu, “Single input state polarization-sensitive optical coherence tomography with high resolution and polarization distortion correction,” Opt. Express 27(5), 6910–6924 (2019).
26. B. Baumann, “Polarization sensitive optical coherence tomography: A review of technology and applications,” Appl. Sci. 7(5), 474 (2017).
27. C. Fan and G. Yao, “Mapping local optical axis in birefringent samples using polarization-sensitive optical coherence tomography,” J. Biomed. Opt. 17(11), 110501 (2012).
28. D. J. Harper, M. Augustin, A. Lichtenegger, P. Eugui, C. Reyes, M. Glösmann, C. K. Hitzenberger, and B. Baumann, “White light polarization sensitive optical coherence tomography for sub-micron axial resolution and spectroscopic contrast in the murine retina,” Biomed. Opt. Express 9(5), 2115–2129 (2018).
29. C. Fan and G. Yao, “Correcting optical-axis calculation in polarization-sensitive optical coherence tomography,” IEEE Trans. Biomed. Eng. 57(10), 2556–2559 (2010).
30. G. Kaneko, H. Shirakami, Y. Hirano, M. Oba, H. Yoshinaga, A. Khieokhajonkhet, R. Nagasaka, H. Kondo, I. Hirono, and H. Ushio, “Diversity of lipid distribution in fish skeletal muscle,” Zool. Sci. 33(2), 170–178 (2016).
31. W. Y. Oh, S. H. Yun, B. J. Vakoc, M. Shishkov, A. E. Desjardins, B. H. Park, J. F. de Boer, G. J. Tearney, and B. E. Bouma, “High-speed polarization sensitive optical frequency domain imaging with frequency multiplexing,” Opt. Express 16(2), 1096–1103 (2008).
32. L. Chin, X. Yang, R. A. McLaughlin, P. B. Noble, and D. D. Sampson, “En face parametric imaging of tissue birefringence using polarization-sensitive optical coherence tomography,” J. Biomed. Opt. 18(6), 066005 (2013).
33. A. Daya, R. Donaka, and D. Karasik, “Zebrafish models of sarcopenia,” Dis. Models Mech. 13(3), dmm042689 (2020).