Published in final edited form as:

Opt Lett. 2020 September 01; 45(17): 4919–4922. doi:10.1364/OL.400855.

***In vivo* imaging of the depth-resolved optic axis of birefringence in human skin**

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Abstract

Recent progress has enabled the reconstruction of the local (i.e., depth-resolved) optic axis (OAx) of biological tissue from measurements made with polarization-sensitive optical coherence tomography (PS-OCT). Here, we demonstrate local OAx imaging in healthy human skin *in vivo*. The images reveal dense, weaving patterns that are imperceptible in OCT intensity tomograms or conventional PS-OCT metrics and that suggest a mesh-like tissue organization, consistent with the morphology of dermal collagen. Using co-registered polarization-sensitive optical coherence microscopy, we furthermore investigated the impact of spatial resolution on the recovered OAx patterns and confirmed their consistency. OAx orientation as a contrast mechanism merits further exploration for applications in dermatology.

Polarization-sensitive optical coherence tomography (PS-OCT) measures the depth-dependent polarization states of light backscattered in tissue [1, 2]. Skin is rich in birefringent fibrillar collagen and causes sufficient retardation to cause a continuous variation of the observed polarization states versus depth. The reduction of retardation in thermally denatured collagen has served to determine injury depth in burn patients with PS-OCT [3]. PS-OCT has also been used to characterize scar tissue in patients [4] and scar formation in animal models [5]. Despite early promise, PS-OCT has found relatively little application in dermatology, possibly because it has lacked the detailed insight into collagen morphology afforded by non-linear [6] and polarized light microscopy [7, 8].

The conventional cumulative retardance and cumulative optic axis (OAx) orientation of PS-OCT describe the linear retardance characterizing the round-trip propagation from the sample surface to a given depth. Although straightforward to compute, cumulative signals can be difficult to interpret, especially in samples with an OAx orientation that varies with

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Disclosures. The authors declare no conflicts of interest.

depth, as encountered in the mesh-like arrangement of fibrous bundles occurring in healthy skin [9].

Computing the local retardance and local OAx orientation more accurately reveals the sample's birefringence at each depth, free from the cumulative effect of the preceding layers. Demonstrated for imaging skin and oral mucosa *in vivo* in humans [10, 11] and for scar formation in animals [5], the scalar amount of local retardance provides an assessment of the presence of birefringent collagen bundles at each depth but discloses little detail on their spatial arrangement.

Reconstructing the local OAx orientation, corresponding to the physical azimuthal orientation of linearly birefringent sample elements with respect to the beam's axis at each depth, has recently received renewed attention [12–17]. It requires an additional recursive depth-correction to compensate for the cumulative effect of the preceding tissue layers. We have developed algorithms for the robust reconstruction of the local OAx orientation with standard-resolution PS-OCT [15], with optical coherence microscopy (OCM) [17], and through intravascular catheters [16].

In this Letter, we report on local OAx imaging in healthy human skin *in vivo*, for the first time to the best of our knowledge. Our local OAx orientation images reveal compelling patterns that are suggestive of the known mesh-like arrangement of collagen bundles in skin and that vary depending on skin location. To rule out that the observed OAx orientations are an artificial result of unresolved tissue features, we performed co-registered imaging with higher-resolution PS-OCM.

The PS-OCT and PS-OCM systems employed in this study have previously been described in detail [15, 17]. In short, our PS-OCT is based on a phase-stabilized, 1310-nm swept-source OCT system that instantaneously measures full Jones matrices by depth-multiplexing two input polarization states (11 μm axial resolution in air, 50 kHz A-line rate). Alternately, our 800-nm spectrometer-based PS-OCM relies on inter-A-line polarization modulation to sequentially measure Jones matrices (1.5 μm axial resolution in air, 20 kHz A-line rate). Imaging with either system was performed using a slightly tilted glass window in front of the objective lens for the skin to lightly push against and reduce motion artifacts. The thickness of the window was 2.0 mm and 1.0 mm in the PS-OCT and PS-OCM, respectively. The gap between the skin surface and the window was filled with index-matching gel to reduce image artifacts [18]. PS-OCT was performed with either of two objective lenses (LSM02, LSM03, Thorlabs Inc., USA) providing lateral resolutions of 11 μm and 22 μm , respectively.

Reconstruction of local retardance and OAx was originally developed for conventional-resolution PS-OCT [15] and then adapted for high-resolution PS-OCM imaging [17]. In brief, standard processing provided cumulative complex-valued Jones matrix tomograms. Transformed back to the wavenumber domain, Jones matrices were made transpose-symmetric, which enforces all optic axes to lie within the horizontal plane of the Poincaré sphere [16] and corrected for chromatic system polarization effects. The effect of transmission through system components was removed by left and right multiplying with the

inverse of the square root of the sample surface matrix, leaving only a global offset in the orientation angle [17]. Using concurrent matrix decomposition [15], the retardation vectors were isolated to construct SO(3) rotation matrices that represent the cumulative retardance in Mueller formalism. After incoherent three-dimensional (3D) spatial filtering of the cumulative SO(3) matrices, recursive depth-correction recovered the local, i.e., depth-resolved, rotation matrix. The angle of its rotation vector within the horizontal plane of the Poincaré sphere corresponds to twice the local OAx orientation, resulting in the common π -ambiguity, and its length indicates the amount of local retardance. The full width at half maximum of the truncated Gaussian filtering kernels for PS-OCT and PS-OCM were $2.5 \times 2.5 \times 3.5$ and $9 \times 9 \times 4$ voxels ($x \times y \times z$), respectively.

Throughout this manuscript, images have been flattened to compensate the intentionally slanted surface. The intensity images were spatially filtered with a 2D median filter in the lateral directions (3×3 pixels, $x \times y$). The OAx orientation is mapped to color, overlaid with brightness encoding local retardance, and masking regions with low depolarization index (< 0.45), as previously described [17]. To ease the interpretation of cross-sections and *en-face* images, their respective positions are indicated by solid red lines in *en-face* images, and by dashed lines or arrows in cross-sectional images, respectively.

Fig. 1 shows a representative result from the back of a healthy adult volunteer's hand, measured with PS-OCT *in vivo*. The lower-NA objective lens, LSM03, was used for a large FOV of 10×10 mm. The volumetric scanning, consisting of 800 A-lines in both scanning directions, took 14.4 s. Considering the positive birefringence of collagen, the OAx orientation in Fig. 1 (b) represents the apparent slow axis orientation of the linear retarder describing cumulative round-trip propagation from the sample surface to a given depth. The cumulative OAx frequently wraps, resulting in abrupt 90° changes in the cumulative OAx orientation, but otherwise exhibits a smooth variation that is difficult to associate with the assumed underlying tissue morphology [13]. In contrast, the local OAx orientation characterizes the direction of linear retardance of a depth-resolved thin tissue section, independent of the preceding layers (Fig. 1 (c)). It relates more closely to the tissue's local birefringence and reveals weaving, bundle-like structures with alternating OAx orientation throughout the dermis, reminiscent of the fibrous, principally collagen, bundles composing this tissue [9] (see Visualization 1). Consecutive zones of distinct OAx orientation are indiscernible in the intensity signal, highlighting the complementary nature of these two contrast mechanisms. *En-face* views of intensity and local OAx orientation at a depth of $335 \mu\text{m}$ (assuming a refractive index of skin of 1.43 [18]) are displayed in Figs. 1 (d) and (e), respectively, revealing alternating patches of continuous OAx orientation, without obvious preferential orientation (see Visualization 2).

Fig. 2 shows images of the volar and dorsal forearm imaged in the same volunteer with PS-OCT using the same objective lens (see also Visualizations 3–4). Each volumetric scan, with an FOV of 5×5 mm and 500 A-lines in both scanning directions, took 6.0 s. The *en-face* OAx orientation maps reveal a mesh-like network, which is only faintly perceptible in corresponding intensity *en-face* images (which contain surface topography-generated artifacts [18]). On the volar forearm, a majority of the tissue features an OAx orientation orthogonal to the axis of the forearm, i.e., along its circumferential direction, whilst the

remainder is largely aligned in parallel. On the dorsal forearm, the fibers do not seem to exhibit an apparent preferential orientation. In both locations, we observed hair follicles encircled by a full revolution in the OAx orientation, marked by representative white dashed circles in Fig. 2, and consistent with collagen fibers wrapping around the follicle. Similar patterns of dermal collagen surrounding hair follicles have been observed with SHG in mouse skin [19]. The pattern convincingly prevails at different depths, affirming the robustness of our reconstruction method. We employed this feature to adjust the unknown global offset in OAx orientation by matching the tangents around the circle with the color wheel.

To consider the impact of spatial resolution on the local OAx imaging, we carefully co-registered PS-OCT and PS-OCM *in vivo* images of a small skin area on the back of the volunteer's hand (dorsal). PS-OCM took ~ 4 min to scan 1500 polarization-modulated A-line pairs in each lateral direction, over an area of 1×1 mm. Using the higher-NA objective lens, LSM02, to reduce the otherwise substantial difference in lateral resolution between the two systems, we acquired an FOV of 3×3 mm with 500 A-lines in each direction with PS-OCT. The time between imaging with the two systems was < 15 min. A thin fiducial marker, made of cut-out opaque tape, was attached to the skin to help identify the FOV of PS-OCM within the $9 \times$ larger FOV of PS-OCT.

Matching PS-OCT and PS-OCM intensity and local OAx orientation maps are displayed in Fig. 3. The higher spatial resolution of PS-OCM reveals rich details in the intensity signal that are lost with the lower resolution of PS-OCT but is accompanied by shallower imaging depth due to the limited depth of field. The orientation of collagen bundles, invisible in PS-OCT, can often be inferred from PS-OCM intensity *en-face* images, but is dramatically enhanced in the local OAx orientation maps. The (cellular) epidermis is weakly birefringent and does not induce noticeable local retardance. Only in the papillary dermis, i.e., the uppermost layer of the dermis [9], and below, does the local retardance become pronounced and OAx well defined. The OAx orientation map measured with PS-OCM reveals weaving patterns similar to those observed in PS-OCT. The local OAx orientation maps of PS-OCM undoubtedly reveal finer strands of continuous OAx orientation than for PS-OCT. However, comparing the co-registered OAx orientation maps shows an overall good correspondence. The OAx orientation of strands that exceed the size of the lower resolution point-spread function match very well. Low-resolution measurements of the OAx orientation in areas composed of smaller strands with varying orientation seem to reveal the average orientation, akin to low-pass filtering, especially in *en-face* views (dashed white circles in Fig. 3 (h, i)). This is further corroborated by using a large spatial filtering kernel, matching the dimensions of the filter used in PS-OCT processing, for the reconstruction of PS-OCM data, as shown in Fig. 3 (e, j, o). Although incoherent averaging of higher resolution data preserves finer details, it mimics closely the PS-OCT OAx maps. Within cross-sections, it can be appreciated that small fibers are frequently averaged out in the PS-OCT maps, as indicated by the orange and cyan arrows in Figs. 3 (c–d). Yet, the combined effect of differently oriented small fibers may also lead to a minimum in local retardance at low resolution, when in fact the tissue remains composed of highly birefringent but distinctly oriented fibrous material (cyan arrows). Reassuringly, we did not detect instances of artificially high local retardance or OAx orientations entirely dissociated from those measured at higher

resolution. Comparing the measured local retardance, converted to tissue birefringence, across the matched volumes between the two systems at their native resolution, showed very close agreement. Although the resolution of PS-OCM should suffice to resolve collagen bundles in skin (4–10 μm , [20]), the arrangement of collagen on a still smaller scale likely leads to similar effects in the high-resolution PS-OCM signal. Nevertheless, we conclude from this co-registration experiment that while the lower resolution leads to loss of detail and occasionally exhibits artificially low local retardance, the PS-OCT OAx maps are remarkably consistent with the higher resolution measurements.

Owing to the birefringence of fibrous bundles (principally collagen), local OAx orientation measured with standard resolution PS-OCT provides insight into the tissue organization that is difficult to obtain from intensity or local retardance images and is otherwise only available to intensity imaging at much higher spatial resolution. PS-OCT provides faster scanning and OAx reconstruction (18 min versus 5.5 h, using MATLAB R2020a on a PC equipped with Intel Core i9–7900X 3.3GHz processor and 32GB memory), and larger FOV, depth of field, and imaging depth than PS-OCM, highlighting its potential for skin imaging in a clinical setting. It provides a metric that can serve for quantitative analysis by computing, e.g., the anisotropy or heterogeneity of the OAx distribution [21, 22]. Whilst we only imaged healthy skin in this set of proof-of-concept experiments, better methods to quantify collagen organization *in vivo* offer future advances in the management of skin pathologies, in the optimization of wound healing therapies that reduce scarring, and in the investigation of the effects of aging and of cosmetic products [23, 24].

In summary, we performed proof-of-concept local OAx imaging of healthy human skin *in vivo*. Local OAx orientation provides a compelling contrast mechanism to investigate the organization of collagen in skin and overcomes limitations of previous cumulative and scalar local retardance imaging with PS-OCT.

Acknowledgment.

We acknowledge fruitful discussions with Dr. Danuta Sampson and Dr. Peijun Gong.

Funding. National Institutes of Health (P41EB-015903, R03EB-024803).

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Fig. 1. Back of a healthy adult volunteer's hand, measured with PS-OCT. Intensity (a), cumulative (b) and local (c) OAx orientation in the B-scan cross-sectional view (Visualization 1), and intensity (d) and local OAx orientation (e) in *en-face* view (Visualization 2). EP, epidermis. DE, dermis. The OAx orientation maps, (b) and (c), are cropped at 1 mm in the axial direction.

Fig. 2.

Intensity and local OAx orientation of the volar (left panel) and dorsal (right panel) forearm of a healthy adult. (a) and (b) are cross-sectional B-scan views (Visualization 3), and (c-f) are *en-face* views (Visualization 4). (g) and (h) are cross-sections along the oblique axis of a hair follicle, marked in (d) and (f). Representative hair follicles are highlighted by dashed circles. The axis of the forearm (volar and dorsal) is indicated by the arrow in (c).

Fig. 3.

The co-registered result of dorsal hand measured with PS-OCT and PS-OCM. (e, j, o) are PS-OCM OAx maps reconstructed with a large spatial filter matching that of PS-OCT. (a-e) are B-scan views (Visualization 5), (f-j) and (k-o) are *en-face* views (Visualization 6) at two indicated depths. The dashed mesh in (c) and (d), 0.2×0.2 mm in size, helps to visually co-register the local OAx images. EP, epidermis. DE, dermis. FM, fiducial marker.