**In vivo** volumetric imaging of the human upper eyelid with ultrahigh-resolution optical coherence tomography

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**Abstract.** The upper eyelid is a biological tissue with complex structure, essential for the maintenance of an optically clear ocular surface due to its physical (blinking) effect. The Meibomian glands (MGs) are structures that lie beneath the surface of the inner eyelid and are partially responsible for the production of the superficial oily layer of the tear film. The MGs are only superficially visible under magnification when the eyelid is everted. We present for the first time in vivo 3-D images of healthy and inflamed human MGs. Tomograms were acquired from the tarsal plate of everted human eyelids with a 1060-nm ultrahigh-resolution optical coherence tomography (UHROCT) system, with \( \sim 3 \, \mu m \times 10 \, \mu m \) (axial \times lateral) resolution in biological tissue at the rate of 91,911 A-scans/s. Comparison with histology shows that the UHROCT images reveal a spatial distribution of structures that appear to correspond with the MGs’ acini and ducts (in healthy subjects), and accumulation of heterogeneous, highly scattering biological material and clear fluids in the visibly blocked glands. Noninvasive, volumetric high-resolution morphological imaging of the human tarsal area could have a significant impact in the clinical diagnostics of inflammatory and noninflammatory lid edema, hematomas, cysts, tumors, injuries, infections, congenital anomalies, eyelid adhesions, and disturbances in the position of the lid and in better understanding of the physiological response of the upper eyelid to these various conditions and causes.

Optical coherence tomography (OCT) is an imaging modality that allows for volumetric, noninvasive imaging of the morphology of biological tissue with micrometer-scale resolution at imaging depths of 1 to 2 mm below the tissue surface. Over the past 10 years, commercial and research grade ultrahigh-resolution OCT (UHROCT) systems have been used in ophthalmology to acquire noninvasively and in vivo high-resolution images of the human anterior chamber, specifically, the cornea. Here, we present, to our knowledge for the first time, in vivo 3-D UHROCT tomograms of the human tarsal plate in healthy subjects and an inflamed Meibomian gland of the human upper eyelid.

A high-speed, 1060 nm UHROCT system was developed for in vivo imaging of the human anterior and posterior eye. When applied to imaging anterior structures of the human eye, this system has a major advantage over other UHROCT systems operating at 800 nm or 1300 nm: the acquired images do not require post-processing for dispersion compensation, since water has a dispersion null in the \( c = 1020 \, \text{nm} \), \( \Delta \lambda = 110 \, \text{nm} \). The sample arm of the system is connected to an optical imaging probe, consisting of three achromatic lenses and a pair of galvanometric scanners, mounted on a modified slit lamp biomicroscope. The interference signal is detected with a custom, high-performance spectrometer (P&K Optica, Inc.), interfaced to a 1024 pixel linear array InGaAs camera (SUI, Goodrich Corp.) with 92 kHz readout rate. The UHROCT system provides 3 \( \mu m \) axial and \( \sim 10 \, \mu m \) lateral resolution in biological tissue and 99 dB SNR for 1.3 mW power of the imaging beam. Images are processed with MATLAB (Mathworks) and Amira (Visage Imaging, Inc.).

The 2-D and 3-D tomograms were acquired in vivo from...
the everted eyelids of three human subjects. The imaging procedure was approved by the University of Waterloo Human Ethics Committee. Two of the subjects had normal, healthy eyelids, while the third had an inflamed MG (chalazion). The subjects were prescreened and their eyelids everted by a licensed optometrist. Volumetric images (1000 × 512 × 256), corresponding to 4-mm × 1 mm × 2 mm physical dimensions (horizontal × depth × vertical), were acquired from different locations in the eyelid, in direction from the fold toward the marginal area, perpendicular to the tissue surface and across the orientation of the MG’s main ducts.

Figure 1 shows representative UHROCT 2-D images [(b) and (c)] of a healthy human eyelid. For comparative purposes, a representative upper eyelid histology (periodic acid Schiff (PAS) staining) image is also shown (a). The UHROCT 2-D images were obtained perpendicular to the known anatomic course of the MG’s main ducts, as illustrated by the black line on the histology image [Fig. 1(a)]. The UHROCT image dimensions were 1000 × 400 (A-scans × pixels), corresponding to 4-mm lateral and ~0.8-mm depth scan. The tomograms show relatively homogeneous superficial tissue, presumably, the inner surface connective tissue of the tarsal plate [Fig. 1(b) black arrow] and a network of low-reflective lobes with connections ducts presumably cross sections of the MG acini [Fig. 1(c) white arrow] and ductules [Fig. 1(b) white arrow] adjacent to an additional area of relatively homogeneous tissue (presumably, outer surface connective tissue of the tarsal plate).

Two videos, presented in Fig. 2, show a cross-sectional view (a) and an en face view (b) of a representative volumetric image set of the human upper eyelid. The image sequence in Fig. 2(a) was acquired in the direction from the fold of the everted eyelid toward the eyelashes and exhibits cross sections of ductlike structures (low-reflective, dark brown, large circular features) and associated smaller irregularly shaped lobes (pale brown). The video in Fig. 2(b) shows the same structures in en face view. The lobelike structures appear to form clusters near the ends of the ductlike structures, leading us to conclude that they likely correspond to the MG’s acini and ductules, respectively, as seen in histological observations of the eyelid structure. The ductlike structures appear similar to large blood vessels, although there are major differences: the cross section of an MG’s main duct increases in direction from the fold toward the eyelashes, and the main ducts of all MGs in the human eyelid are oriented parallel to each other, which corresponds to the morphological features observed in the en face tomograms [Fig. 2(b)].

Figure 3 shows a selection of representative en face images from the video in Fig. 2(b). The tomogram shown in Fig. 3(a) was acquired ~200 μm below the tissue surface, and there appears to be a large, ductlike structure [presumably the MG main duct; Fig. 3(b) black arrow]. To the temporolateral (right) side, there appears to be additional duct structure that is positioned deeper from the inner surface of the eyelid. On the nasolateral (left) side of the large ductlike structure, a cluster of low-reflective (pale brown), irregularly shaped lobular features (presumably acini) appears, visible on the highly reflective background. A magnified (2 × 1) view of the section in Fig. 3(a) marked with the white rectangle is shown in Fig. 3(b) where the lobular features are better observed. The sequence of en face images [Figs. 3(c) 3(b)] obtained from different depths within the everted eyelid shows the spatial distribution of the ductlike and lobular features within the tissue.

Volumetric images were also acquired from a clinically diagnosed chalazion (nodular swelling in the tarsal plate), and representative tomograms are shown in Fig. 4. The images were acquired in the direction from the fold of the everted eyelid, across the visible, superficial mass of the chalazion, toward the eyelashes. Figure 4(a) shows the structural appearance of the relatively unaffected tissue adjacent to the mass of the chalazion. In Fig. 4(b) a small region of highly reflective tissue, enveloped in a thin membrane and surrounded by optically clear material (likely fluid; black arrow) is visible. Figure 4(c) shows a cystlike feature of almost round shape, with a thick membrane [Figs. 4(c) and 4(d) black arrow], filled with optically clear material, most likely fluid. The subsequent UHROCT tomograms acquired across the bulk of the swelling [Figs. 4(b) 4(d)] show that as the size of the mass increases, its shape becomes more irregular and it is filled with clusters of highly reflective material [Fig. 4(d) gray arrow]. On the basis of current knowledge of the histopathological mechanisms associated with this type of swelling, the
observed highly reflective spots within the cyst may be agglomerations of inflammatory cells such as lymphocytes, epitheloid cells, or multinucleated giant cells, while the clear fluid may be liberated sebaceous material.

In summary, a state-of-the-art, high-speed, UHROCT system was used to acquire for the first time in vivo volumetric images of the everted human upper eyelid. The 3-D tomograms revealed the apparent intricate structure of the healthy Meibomian glands’ ducts and acini, which were previously seen only with ex vivo histological analysis. This work also demonstrates the clinical relevance and potential of the UHOCTopt technology for noninvasive diagnostics of abnormalities in the upper eyelid, by revealing the internal structure of a chalazion, something previously requiring a biopsy.

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References