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Maturation of the Human Fovea: Correlation of Spectral-Domain Optical Coherence Tomography Findings With Histology

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Abstract

Purpose—To correlate human foveal development visualized by spectral-domain optical coherence tomography (SDOCT) with histologic specimens.

Design—Retrospective, observational case series.

Methods—Morphology and layer thickness of retinal SDOCT images from 1 eye each of 22 premature infants, 30 term infants, 16 children, and 1 adult without macular disease were compared to light microscopic histology from comparable ages.

Results—SDOCT images correlate with major histologic findings at all time points. With both methods, preterm infants demonstrate a shallow foveal pit indenting inner retinal layers (IRL) and short, undeveloped foveal photoreceptors. At term, further IRL displacement forms the pit and peripheral photoreceptors lengthen; the elongation of inner and outer segments (IS and OS, histology) separates the IS band from retinal pigment epithelium. Foveal IS and OS are shorter than peripheral for weeks after birth (both methods). By 13 months, foveal cone cell bodies stack >6 deep, Henle fiber layer (HFL) thickens, and IS/OS length equals peripheral; on SDOCT, foveal outer nuclear layer (which includes HFL) and IS/OS thickens. At 13 to 16 years, the fovea is fully developed with a full complement of SDOCT bands; cone cell bodies >10 deep have thin, elongated, and tightly packed IS/OS.

Conclusions—We define anatomic correlates to SDOCT images from normal prenatal and postnatal human fovea. OCT bands typical of photoreceptors of the adult fovea are absent near birth because of the immaturity of foveal cones, develop by 24 months, and mature into childhood. This validates the source of SDOCT signal and provides a framework to assess foveal development and disease.

High-Resolution Spectral-Domain Oct (SDOCT) with reported resolution of <5 μm has enabled visualization of retinal anatomy and clinical evaluation of retinal pathology in the adult retina.^{1,2} SDOCT displays alternating bands of hyper- and hyporeflectivity (Figure 1, Top) that correspond with histologically defined retinal layers (Figure 1, Bottom).^{3–11} The SDOCT bands have been labeled from inner to outer: nerve fiber layer (NFL); ganglion cell layer (GCL); inner plexiform layer (IPL); inner nuclear layer (INL); outer plexiform layer/photoreceptor synapse layer (OPL/PSL);¹² outer nuclear layer, which includes Henle fiber layer (ONL+HFL);^{12,13} external limiting membrane (ELM); inner segment and outer segment junction, which a recent report shows is inner segment band (IS);¹⁴ outer segments (OS); and retinal pigment epithelium (RPE). Note that the term OPL/PSL above refers to the hyperreflective band on SDOCT that has been conventionally assigned the term OPL. In classic histology OPL includes both the photoreceptor synapses and axons as they extend out from the foveal center.¹⁵ On SDOCT the axons, however, are hyporeflective and have been indistinguishable from the photoreceptor nuclei. Thus, the hyporeflective band, labeled ONL+HFL here, includes the axons and nuclei and has been conventionally termed ONL. These revised terms are based on the publications of Curcio and associates,⁵ Lujan and associates,¹³ and Spaide and Curcio.¹⁴

Maldonado and associates recently reported absence and variations in many of these layers as evidence of the dynamic morphologic changes associated with development of human fovea from SDOCT imaging.¹⁶ These in vivo changes of inner and outer retinal layers at the foveal center generally appeared consistent with cellular redistributions reported in histologic studies;^{17–23} however, a cross-sectional analysis with direct comparison to histologic specimens was not performed. There are numerous reports of macular abnormalities in infants on SDOCT^{24–33} and it would be appropriate to compare to infant histology rather than to the adult eye.

We present a direct correlation using human histologic specimens³⁴ and SDOCT images, and create a timeline for onset of retinal layers. This correlation will be required for the clinical assessment of normal and pathologic development of infant retina by SDOCT. This also aids in defining and validating SDOCT bands for the adult eye.

Methods

SDOCT Subjects

Twenty-two premature infants, 30 term infants, 16 children, and 1 adult were enrolled between January 20, 2009 and January 27, 2012 under research protocols approved by the Duke University Health System Institutional Review Board. Parents or guardians of subjects consented to participation in these observational studies using SDOCT imaging. Imaging was performed at the time of standard dilated fundus examination in neonatal intensive care unit (NICU), in outpatient clinic, or during an examination under anesthesia for pediatric eye care. In the newborn nursery, both the dilated fundus examination and the SDOCT imaging were part of the research protocol.^{16,24,27} Subjects' medical records were reviewed for health history and gestational age. Images from a normal adult subject consented in an SDOCT imaging study were used for comparison. Table 1 describes the age range for each study phase and demographics of each subject.

SDOCT Imaging Procedure

After dilated examination by the ophthalmologist, a research portable, handheld SDOCT unit (Bioptigen Inc, Research Triangle Park, North Carolina, USA) was used to image both eyes of all subjects per protocol as previously published.^{27,29} In awake infants, no sedation or lid speculum was used and several macular volume scans centered on the fovea were captured at 0 or 90 degrees.²⁷

SDOCT Image Processing and Retinal Layer Thickness Measurements

SDOCT images were converted into Digital Imaging and Communications in Medicine (DICOM) format and evaluated qualitatively by experienced SDOCT graders using OSIRIX medical imaging software (OSIRIX Foundation, Geneva, Switzerland) for the presence of fovea, all retinal layers, and any pathologic abnormality. Poor quality or scans with retinal pathology, including cystoid macular edema or subretinal fluid, as defined in our prior publications, were excluded.^{24,28} Reproducibility of the grading was established by inter-reader agreement. Graders were masked to other clinical data. The highest-quality scans from each subject containing the center of the fovea were selected from each session, based on subjective assessment of resolution. Because of varying eye size with age, B-scans ranged from 6.3 to 10.3 mm in length with 630 to 1000 A-scans per B-scan. Pixel size data were verified by age or each eye.²⁷ To avoid intra-eye variation, a random number generator was used to select 1 imaging session of 1 eye per subject per age group for analysis.

To quantify retinal layers, the semiautomated segmentation of a single central scan was performed using a custom software, DOCTRAP (Duke OCT Retinal Analysis Program) version 16.1,^{16,35,36} based in MATLAB (Mathworks, Natick, Massachusetts, USA). The segmentation lines were placed on the inner aspect of inner limiting membrane (ILM), GCL, INL, OPL/PSL, ONL+HFL, IS band, RPE, and Bruch membrane. Therefore, the outlined retinal layers included ILM+NFL, GCL+IPL, INL, OPL/PSL, ONL+HFL, IS+OS, and RPE. External limiting membrane (ELM) was not visible in the young infants and thus not routinely segmented. Retinal foveal thickness was defined as the thickness of the entire neurosensory retina from the ILM to the inner aspect of the RPE. To improve image resolution SDOCT images were summed and illustrated in figures. A custom MATLAB script was implemented on the segmentation output to compute thickness of entire retina and

each segmented layer at the foveal center, and then at 250- μm intervals to 2000 μm at each phase. To assess pit development, we also computed foveal/parafoveal ratio (parafoveal = 1000 μm from fovea) for retinal thicknesses²⁸ as illustrated in Table 2.

Histologic Specimens and Imaging

To anatomically correlate segmented SDOCT retinal layers, we used histologic specimens from 22 weeks gestation to 13 years and an adult eye for comparison. Histology specimens were obtained with the aid of the University of Washington Lion's Eye Bank and were described in the previous paper.³⁴ Donors had no history of pathology that would adversely affect development. Eyes were enucleated 2 to 8 hours after death, the cornea and lens removed, and in most cases the globe was immersed in a mixture of 4% paraformaldehyde and 0.5% glutaraldehyde in pH 7.4 phosphate buffer. In a few eyes the entire globe was embedded in paraffin and sectioned serially at 12 μm . In other eyes the horizontal meridian was frozen-sectioned serially at 12 μm , allowing immunolabeling at a known locus with a wide variety of antibodies. In each method, every tenth slide was stained with 1% azure II–methylene blue to locate the fovea. For comparison of SDOCT and histology, light micrographs were sized so that they were in representative matching dimensions to the SDOCT. The majority of images, as annotated in the figures, are thus shown at approximately 2:1 axial/lateral ratio to aid in viewing layers and to mimic typical SDOCT viewing.

Results

Retinal Layer Thickness and Changes During Development

There is a progressive increase in neurosensory retinal thickness across 7 phases of development from 30 weeks postmenstrual age (PMA) to 16 years of age (Figure 2; demographics in Table 1). The segmentation of layers provides a precise delineation of tissue changes in the living human retina and a range of normal thicknesses (standard deviation bars in Figure 2) during development.

Total neurosensory retinal thickness (Figure 2) consistently is least in the fovea and greatest in the periphery. This difference is smallest in phase 1 (foveal/parafoveal ratio 0.66), and up to phase 4 the pit deepens and the pit shoulders increase in height to create the greatest thickness difference (foveal/parafoveal ratio 0.36). Note that for all layers, the foveal/parafoveal ratio would be small when a layer is thinner at the fovea, would equal 1 for a flat layer, and would be >1 when the layer bulges at the fovea. Between phases 4 and 7, overall retinal thickness increases and the foveal pit becomes more shallow, reducing the foveal/parafoveal ratio (0.64).

The NFL (Figure 2) is consistently thin in the fovea, and changes very little with age. The GCL+IPL layers (Figure 2) are thinner centrally and thicker peripherally in all phases. By phase 5, these layers in the fovea become the thinnest in development (foveal/parafoveal ratio 0.48 in phase 1, 0.05 to 0.07 in phases 5–7). During phases 3–5, the prominent elevation of GCL+IPL on the foveal edge or shoulder decreases and moves peripheral to the fovea. The hyporeflective INL (Figure 2) starts out almost flat (foveal/parafoveal ratio 0.99) and then develops pronounced thinning at the foveal center by phase 3 but also has later thinning of the shoulder through phase 7 (foveal/ parafoveal ratio 0.27 to 0.24 in phases 3–7).

The hyperreflective OPL/PSL (Figure 2) has a nearly flat contour through phase 5. During phases 6–7, there is a small additional thinning in the fovea accompanied by a pronounced thickening on the shoulders (foveal/parafoveal ratio phase 7, 0.99). The hyporeflective ONL plus Henle fiber layer (Figure 2) has a subtle concavity in phase 1 with mild increase in

thickness across the retina through phase 4. The ONL+HFL shows a profound increase at the fovea from phases 4 to 7, creating a bulge (foveal/parafoveal ratio 1.66 in phase 7). The IS+OS layer (Figure 2) is not present in phase 1 and gradually thickens in the midperiphery but not centrally up to phase 4 (foveal/ parafoveal ratio 1.15 in phase 4), and thickens at the fovea between phases 4 and 7 (foveal/parafoveal ratio 1.48 in phase 7). The ELM was first visible in phase 4 eyes anterior to the IS band 8. The RPE thickness (Figure 2) changes little except for subtle foveal thickening in phases 6–7 (foveal/parafoveal ratio 1.09 in phase 7).

Correlation Between SDOCT and Histology Images

Adult—This evaluation of the developing infant retina is based on the adult structures that are well documented by histopathologic comparison.^{4,5,9–11} Throughout this manuscript, we assign numbers to each of the reflective SDOCT bands and match these with histology. On SDOCT cross-sectional images (Figure 1, Top) of the adult fovea, we assign numbers to the hyperreflective layers as follows: NFL band 1; IPL band 3; OPL/PSL band 5; ELM (faint and thin) band 7; IS band 8; and RPE double band 10. These are interlaced with hyporeflexive GCL band 2, INL band 4, ONL+HFL band 6, and the photoreceptor OS band 9 (Figure 1, Top). GCL, IPL, INL, and OPL/PSL are displaced laterally from the foveal center, leaving a residual thin hyperreflective line at the foveal center. Photoreceptor-associated bands 6, 8, and 9 are thickest at the foveal center. A second hyperreflective RPE band appears between the OS band 9 and the main RPE reflex band 10, correlating with the location that OS tips interdigitate with RPE microvilli.³⁷ This band is variably visible depending on the quality and resolution of OCT scans. Histology sections demonstrate the anatomic layers in Figure 1 (Bottom). Here the Henle fiber layer is distinct from the ONL, in contrast to the SDOCT scan.

Phase 1: 30 to 32 weeks postmenstrual age (premature infants)—On the SDOCT cross-sectional images of the fovea (Figure 3, Top left), the contrast and borders between NFL band 1, GCL band 2, IPL band 3, and INL band 4 are more distinct than in the adult. A shallow foveal pit is present (Figure 3, Top left), the IRLs are thinned, and especially bands 3 and 4 persist across the fovea. The histology section from a 4-week-younger eye shows a GCL that is thicker at the fovea than on OCT of the older eye.

In prenatal retina, ONL+HFL, ELM, and IS and OS bands that are typically visible in the adult SDOCT appear to be missing. In this phase, below the thin OPL/PSL there is only a narrow hyporeflexive band that thins even further at the foveal center. Histology sections at PMA 27 weeks (Figure 3, Top right) show a distinct narrow OPL/PSL with cone pedicles and extremely short axons and short immature photoreceptors (ONL). In the fovea only a single layer of cones is present, which have minimal IS and nearly absent OS. Although IS and OS are slightly longer in the periphery,³⁴ we conclude that in phase 1 both IS and OS are usually too short and immature (immaturity might affect reflectivity) to form a distinct SDOCT band.

Phase 2: 33 to 36 weeks postmenstrual age (premature infants)—In this age group, overall retinal thickness increases outside the central 500 μm (Figure 2, Top left; Figure 3, Bottom left). By contrast, the GCL, IPL, and INL are thinner in the central fovea, causing deepening of the foveal pit. In SDOCT the outer narrow bright IS band 8 appears poorly defined in the periphery and absent in the fovea (Figure 3, Bottom left, band 8), corresponding to the elongation of peripheral cone and rod IS and OS (Figure 3, Bottom right³⁴).

Phase 3: 37 to 39 weeks (term infants)—The fovea appears more mature in SDOCT (Figure 4, Top left) and in histology all layers and neuronal types are present (Figure 4,

region c, Bottom right).¹⁸ The foveal pit deepens markedly by displacement of GCL, IPL, and INL out of the foveal center, causing the inner retinal thickness of the foveal shoulders to increase (Figure 2). The bright NFL band 1 is more reflective and distinct than in earlier phases, and will remain so into maturity. The fovea is thinnest in this phase because band 6 is thin and band 8 is nearly absent centrally (Figure 4, Top right) because of the single layer of foveal cones with <10 μm total height of IS plus OS (Figure 4, Top right, region a). Bands 6 and 8 are both thicker and band 8 is brighter outside the fovea where cone and rod nuclei are stacked into multiple layers and have elongated ellipsoidal IS and detectable OS (Figure 4, Center right, region b³⁴). In the periphery, band 8 is separated from the RPE by a narrow hyporeflective band 9 (Figure 4, Top left, arrow). We conclude that this dark line is caused by OS elongation, which has yet to occur in the fovea (Figure 3, Bottom).

In histology sections of phases 2–3, the INL has a gap filled with Müller cell processes called the transient layer of Chievitz (Figure 4, Bottom right, region c, TC¹⁹). Despite its prominence on histology sections, we have not been able to identify this distinctly in SDOCT at any age.

Phase 4: 40 to 42 weeks (term infants)—The foveal pit is deepest in this phase (Figure 4, Bottom left) as the IRL are further displaced and foveal ONL has not yet begun to thicken because of cone packing (Figure 4, Bottom right, region c). The ONL+HFL band 6 becomes thicker around the fovea from phase 2 to phase 4. Henle fibers (photoreceptor axons) are an identifiable layer on histology at this age, corresponding to the elongation and thickening of the layer of photoreceptor axons with neuronal displacements (Figure 4, Bottom right, Ax³⁴). The hyporeflective OCT band 6 now contains Henle fibers in its inner aspect, making the outermost component of band 6 the true ONL. In SDOCT, the GCL band 2 becomes less distinct from the IPL band 3 and will be so into adulthood.

For the first time in the foveal center, the bright IS band 8 is frequently detectable (Figure 4, Bottom left), signaling the onset of IS and OS elongation.³⁴

Phase 5: 43 weeks to 23 months (infants/children)—In the first months after birth, SDOCT images show that the foveal pit becomes wider and the base flatter over time (Figure 5, Left). GCL, IPL, and INL are fused in the pit center and are approximately 1 cell layer thick (Figure 5, Right). After birth, there is gradual thickening of the foveal ONL+HFL band 8 (Figure 2, and Figure 5, Left), attributable to central cone packing, which increases the foveal ONL from 1 to many nuclei thick over this phase and adds axons to HFL (Figure 5, Right).

Band 8 is more clearly separated from the RPE across the retina because of the increased height of the hyporeflective OS band 9. During phase 5 elongation of foveal IS and OS is rapid³⁴ so that, for the first time, foveal IS and OS length is similar to peripheral. A faint narrow outer hyperreflective band appears within the ONL+HFL, corresponding to ELM band 7 (Figure 5, Left, arrow), which is separated from the IS band (inner segment ellipsoid)¹⁴ by a hyporeflective band (Figure 5, Left, below arrow). The RPE band 10 is thicker than in earlier phases.

Phase 6: 24 months to 5 years (children)—In SDOCT images (Figure 6, Top left), the foveal pit is wider and flatter, with the inner layers fused in the foveal center and unchanged outside. The pit appears similar to the adult in the youngest eyes of this phase. This change is accompanied by a marked ONL+HFL thickening in the foveal center, attributable to cone packing and increased axon layer thickness. IS band 8 and OS band 9 increase further in height, especially in the fovea as foveal IS and OS become longer than peripheral (Figure 6, Top right, regions a and b³⁴). The ELM band 7 is clearly separated

from the IS band 8 as ELM (broad arrowhead, Figure 6, Top right and Center right) separates from packed IS. The second hyperreflective RPE band is now visible between the OS band 9 and band 10.

Phase 7: 6 years to 16 years (children)—At 13 to 16 years of age the alignment of layers between histology and OCT is comparable (Figure 6, Center left and right), and is similar to the adult (Figure 1). The retina has typical hyperreflective and hyporefective SDOCT bands. Histology demonstrates a thick layer of cone cell bodies in the foveal center with very long IS and OS (Figure 6, Center right). A thick layer of Henle axons is present on the foveal shoulders (Figure 6, Center right, Ax). Because of the angle of scanning, these fibers are visible as a wide hyperreflective band in SDOCT (Figure 6, Center left, Ax).

The change in photoreceptor IS and OS morphology and length is summarized for neonatal (Figure 6, Bottom left) and adult (Figure 6, Bottom right) retina near the fovea. Immunocytochemical labeling reveals the marked change in IS size and shape and OS length for both cones and rods.

Discussion

This is the first correlation of SDOCT images and histology during the normal prenatal and postnatal development of the human fovea (PubMed search terms: optical coherence tomography, human foveal development, prematurity). This correlation explains the sequence of appearance of hyperreflective and hyporefective bands in the SDOCT images and illustrates changes in cellular composition of layers during human foveal development. These are also the first normative data for total retinal thicknesses and sublayer thicknesses for the critical period of rapid foveal development from prematurity into young childhood.

SDOCT and histology are fundamentally different techniques that generate images of microscopic tissue elements. Despite the differences, both of these methods agree closely on the sequence of events during human foveal development. The most obvious finding is the extreme immaturity of the outer retina before and after birth, especially in the fovea. Peripheral IS/OS are longer until well after birth, and it is not until 13 to 15 months that foveal IS/OS length begins to overtake peripheral. This sequence explains the early absence of bands 8 and 9, and their appearance first in the midperiphery and last in the fovea. At approximately 5 years of age, foveal IS and OS are adult in both histology and SDOCT. The presence of IRL, especially IPL and INL, at the fovea and their shift out of the foveal center is also a striking finding that was correlated between the 2 techniques.

The light micrograph is the gold standard in this correlation, providing high-resolution images of cells and organelles and specialized staining of structures (Figure 6, Bottom left and right). Images from fixed tissue also have limitations. Even when tissue donors have no diseases that would adversely affect development, changes between death and fixation may be present. Variable shrinkage with dehydration and processing may add to potential artifact and make it difficult to compare layers (OS separation in Figure 6, Top right). Specimens may be scarce, and histology is at a single time point for each specimen.

In contrast, the SDOCT image is of lower resolution than histology, and quality of the image is affected by ocular media, movement, shadowing, and inability to separate adjacent tissues with similar relative reflectivity (HFL and ONL). SDOCT imaging does, however, provide an in situ, undisturbed image of retinal layers and structures and enable us to perform in vivo, large cohort studies and longitudinal studies of individual subjects. It is important to note that calculated retinal thicknesses may vary based on the location of segmentation of retinal layers through different algorithms or across different OCT systems and that the

investigational pediatric system used in this study has not been included in comparisons across OCT systems.³⁸

Despite the above limitations, the onset and development of retinal layers visible on histology sections, with concomitant demonstration of these changes on SDOCT images, validate the subcellular structures that contribute to defined OCT bands. For example, correlation of ellipsoid IS growth on histology³⁴ with the onset of band 8 on SDOCT validates its description as IS band on SDOCT image. There has been much debate in the current literature about the designation of the hyperreflective outer retinal bands seen in high-resolution SDOCT images. Recently, Spaide and Curcio¹⁴ provided anatomic correlates of these outer SDOCT retinal bands. They proposed that band 8 corresponds to the ellipsoid IS. They also defined a second hyperreflective band, as interdigitation of the cone OS and apical processes of the RPE. We detect these layers and show similar correlation in developing human retina. In phases 1–2, histologic images show that there is almost no space between the ELM and RPE because of the very short IS and OS (Figure 3, Top left and right), causing band 8 to not be present in SDOCT. In phases 3–5, first peripheral and later foveal IS and OS elongate, causing band 8 and later band 9 to appear, and 8 matches the height of the IS ellipsoid (Figures 4 and 5).

Although overall correlation was very good between SDOCT and histology, there were several discrepancies. While the GCL and IPL were distinctly different in histology, their intensity difference became much less so that bands 2 and 3 tended to have less sharp contrast with increasing age. The transient layer of Chievitz was prominent around birth as a layer of fibers in the INL,³⁴ but was not routinely obvious in SDOCT. The Henle fiber layer was distinct on histology, but was hyporeflective on SDOCT and not readily distinguished from the ONL. Reflectivity of Henle fiber layer is directionally dependent on SDOCT beam entrance and is increased with scan tilt,^{12,13} and this could be used to demonstrate the layer in children (Figure 6, Center left). The earliest hyperreflective OPL/PSL on SDOCT images appeared consistent with cone and rod synapses on histology and matched the location of these structures across the phases of the study. This confirms that the photoreceptor synapses are a prime component to this layer.¹²

We report the first correlation of SDOCT scans and histology images in normal human foveal development. As SDOCT becomes an established diagnostic tool in pediatric practice, it is critical to understand the marked changes taking place in foveal structures from midgestation to late childhood in order to recognize normal development and quantify disease states.

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Figure 1. SDOCT-histology comparison of normal adult retina. Normal adult retina (65 years) imaged by portable hand-held SDOCT (Top) and light micrograph of an adult macula (72 years) (Bottom). SDOCT bands 1–10 are shown on Top, and histology layers are shown on Bottom. 1 = nerve fiber layer (NFL); 2 = ganglion cell layer (GCL); 3 = inner plexiform layer (IPL); 4 = inner nuclear layer (INL); 5 = outer plexiform layer on OCT and includes photoreceptor synapses (OPL/PSL); however Henle fibers (Ax), which are part of histologic outer plexiform layer, are hyporeflexive^{12,13} and included in 6 = outer nuclear layer (ONL +HFL) on OCT; 7 = external limiting membrane (ELM); 8 = photoreceptor inner segments ellipsoid (ISE); 9 = photoreceptor outer segments (OS); 10 = retinal pigment epithelium (RPE), which is split into 2 hyperreflective bands. P = foveal pit. This and all other SDOCT imaging are summed to improve image resolution.

Figure 2.

Mean thickness of retinal layers during human foveal development and maturation. The mean thicknesses of neurosensory retina and segmented retinal layers are represented from 30 weeks postmenstrual age (PMA) to 16 years. Premature infants are in phases 1 and 2, phases 3 and 4 include term birth, and children are in phases 5–7. The youngest age group (phase 1) is represented by a pale dotted line with increasing color intensity up to the oldest (phase 7), a black line. Standard deviations are plotted as error bars.

Figure 3. SDOCT-histology comparison of normal premature infant retinas. (Top) Phase 1; (Bottom) phase 2. SDOCT numeric bands are indicated in Top left and Bottom left. Bands are matched as near as possible for a 31 weeks postmenstrual age (PMA) SDOCT (Top left) and 27 weeks PMA light micrograph (Top right, projected ~2:1 scale to match SDOCT, seen in the “tall” single row of nuclei in the ONL) through the fovea. In phases 1–2, the GCL+IPL band at the fovea is primarily IPL. In phase 1, the inner (IS) and outer (OS) segments are too short to form band 8. In phase 2 peripheral IS (Bottom left, box) are now long enough to be imaged as band 8 in SDOCT. In Phase 2 on histology (Bottom right) ELM is indicated by horizontal white arrowheads, but is not distinct on SDOCT (Bottom left). 1 = nerve fiber layer (NFL); 2 = ganglion cell layer (GCL); 3 = inner plexiform layer (IPL); 4 = inner nuclear layer (INL); 5 = outer plexiform layer on OCT and includes photoreceptor synapses (OPL/PSL); however Henle fibers (Ax), which are part of histologic outer plexiform layer, are hyporeflective^{12 13} and included in 6 = outer nuclear layer (ONLHFL) on OCT; 8 = photoreceptor inner segments ellipsoid (ISE); 10 = retinal pigment epithelium (RPE), which is split into 2 hyperreflective bands.

Figure 4.

SDOCT-histology comparison of normal-term infant retinas. (Top left) Phase 3, 39 weeks postmenstrual age (PMA) and (Right and Bottom left) phase 4, 40 weeks PMA. In contrast to earlier phases, in addition to SDOCT bands 1 and 2, bands 3 and 4 are now also thin at the fovea, more so at 40 weeks PMA. Photoreceptors at the fovea begin their rapid growth in these phases. In phase 3 (Top left) the IS band was generally not discernable at the foveal center, while in phase 4 (Bottom left) this band is commonly visible. The equivalent location of regions a, b, and c (Right) are indicated on the phase 4 SDOCT scan (Bottom left). On histology, lengths of IS+OS are notably shorter at the fovea than in the periphery (region a vs c, Right, white double-headed arrows). The elongation of OS in the periphery results in hyporeflective band 9 (horizontal white arrow in SDOCT Top and Bottom left) that separates IS from RPE. Henle axons (Ax, in regions a and c) would appear hyporeflective and contribute to thickness of band 6 outside the foveal center in SDOCT (Bottom left). The transient layer of Chievitz (TC, in region c) is not clearly defined on SDOCT band 4 (Bottom left). 1 = nerve fiber layer (NFL); 2 = ganglion cell layer (GCL); 3 = inner plexiform layer (IPL); 4 = inner nuclear layer (INL); 5 = outer plexiform layer on OCT and includes photoreceptor synapses (OPL/PSL); however Henle fibers (Ax), which are part of histologic outer plexiform layer, are hyporeflective^{12,13} and included in 6 = outer nuclear layer (ONLHFL) on OCT; 8 = photoreceptor inner segments ellipsoid (ISE); 10 = retinal pigment epithelium (RPE), which is split into 2 hyperreflective bands.

Figure 5.

SDOCT-histology comparison of the retinal layers during early childhood. Phase 5. (Left) SDOCT image at 9 months compared to (Right) a histology image at 15 months (projected at ~2:1 scale to match SDOCT). Most adult SDOCT bands can be recognized. In Left, note the thickening of the ONL in and around the fovea attributable to cone packing and elongation of Henle axons, both of which contribute to the thicker SDOCT band 6. White arrow represents SDOCT band 7 (ELM). 1 = nerve fiber layer (NFL); 2 = ganglion cell layer (GCL); 3 = inner plexiform layer (IPL); 4 = inner nuclear layer (INL); 5 = outer plexiform layer on OCT and includes photoreceptor synapses (OPL/PSL); however Henle fibers (Ax), which are part of histologic outer plexiform layer, are hyporeflective^{12,13} and included in 6 = outer nuclear layer (ONLHFL) on OCT; 7 = external limiting membrane (ELM); 8 = photoreceptor inner segments ellipsoid (ISE); 9 = photoreceptor outer segments (OS); 10 = retinal pigment epithelium (RPE), which is split into 2 hyperreflective bands.

Figure 6.

SDOCT-histology comparison of the retinal layers during childhood and inner/outer segment (IS/OS) growth from birth through adulthood. (Top) Phase 6 and (Center) phase 7. By phase 6, 5 years, the outer retina has clear SDOCT bands in fovea and periphery (Top left). The growth of foveal IS and OS is striking, and there is little difference between foveal (Top right, region a) and peripheral (Top right, region b) by phase 6. SDOCT bands are adult by phase 7 (Center left). The fovea is now mature, as can be seen by matching SDOCT (Center left) and histology (Center right, projected ~2:1 scale to match SDOCT). The thick layer of Henle axons (Ax) is demonstrated on the foveal slope (Center right) and is visible as a wide hyperreflective layer in SDOCT (Center left) because of the eccentric angle of scanning. White horizontal arrow on SDOCT (Top left) represents SDOCT band 7, and arrowheads on all right histology panels represent ELM. The significant growth in IS and OS length and change in photoreceptor morphology between birth (Bottom left) and adulthood (Bottom right) is demonstrated by immunolabeling for rod opsin (red) and cone arrestin (green). Bottom panels are aligned on the ELM (white arrowheads). 1 = nerve fiber layer (NFL); 2 = ganglion cell layer (GCL); 3 = inner plexiform layer (IPL); 4 = inner nuclear layer (INL); 5 = outer plexiform layer on OCT and includes photoreceptor synapses (OPL/PSL); however Henle fibers (Ax), which are part of histologic outer plexiform layer, are hyporeflexive^{12 13} and included in 6 = outer nuclear layer (ONLHFL) on OCT; 7 = external limiting membrane (ELM); 8 = photoreceptor inner segments ellipsoid (ISE); 9 = photoreceptor outer segments (OS); 10 = retinal pigment epithelium (RPE), which is split into 2 hyperreflective bands.

TABLE 1
Demographic Characteristics of Pediatric Subjects Imaged by Spectral-Domain Optical Coherence Tomography

Phase	Age Range	Median Age	Sex	Race
1 (n = 8)	30–32 wk	31 wk	4 M; 4 F	2 W; 5 B; 1 MR
2 (n = 14)	33–36 wk	35 wk	5 M; 9 F	5 W; 9 B
3 (n = 19)	37–39 wk	39 wk	9 M; 10 F	9 W; 8 B; 1 A; 1 H
4 (n = 11)	40–42 wk	41 wk	3 M; 8 F	7 W; 4 B
5 (n = 6)	43 wk–23 mo	7 mo	4 M; 2 F	4 W; 1 B; 1 A
6 (n = 5)	2–5 y	3y	5M	5W
7 (n = 5)	6–16 y	8y	2 M; 3 F	3 W; 2 A

A = Asian; B = black; F = female; H = Hispanic; M = male; mo = months; MR = multiracial; W = white; wk = week; y = year.

TABLE 2
Changes in Foveal/Parafoveal^a Ratios of Retina and Retinal Layers Over Time in Early Infancy

	Phase 130–32 wk PMA	Phase 233–36 wk PMA	Phase 337–39 wk PMA	Phase 440–42 wk PMA	Phase 543 wk–23 mo PMA	Phase 62–5 y	Phase 76–16 y
Neurosensory retina	0.66	0.56	0.56	0.36	0.48	0.57	0.64
NFL	0.43	0.33	0.17	0.23	0.27	0.15	0.29
GCL+IPL	0.48	0.41	0.16	0.13	0.05	0.17	0.07
INL	0.99	0.72	0.27	0.23	0.24	0.21	0.24
OPL-PSL	0.75	0.78	0.99	0.99	0.99	0.99	0.99
ONL+HFL	0.79	0.83	0.65	0.60	0.98	1.51	1.66
IS+OS	0.38	0.42	0.66	1.15	1.22	1.28	1.48
RPE	1.04	1.03	1.02	0.97	0.92	1.15	1.09

GCL+IPL = ganglion cell layer and inner plexiform layer; INL = inner nuclear layer; IS+OS = inner+outer segments; NFL = nerve fiber layer; ONL+HFL = outer nuclear layer and Henle fiber layer; OPL-PSL = outer plexiform layer or photoreceptor synapse layer; PMA = postmenstrual age; RPE = retinal pigment epithelium; wk = week; y = year.

^aParafoveal = 1000 μ m from fovea.