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Foveal Fine Structure in Retinopathy of Prematurity: An Adaptive Optics Fourier Domain Optical Coherence Tomography Study

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Abstract

Purpose—To describe the fine structure of the fovea in subjects with a history of mild retinopathy of prematurity (ROP) using adaptive optics–Fourier domain optical coherence tomography (AO-FDOCT).

Methods—High-speed, high-resolution AO-FDOCT videos were recorded in subjects with a history of ROP ($n = 5$; age range, 14–26 years) and in control subjects ($n = 5$; age range, 18–25 years). Custom software was used to extract foveal pit depth and volume from three-dimensional (3-D) retinal maps. The thickness of retinal layers as a function of retinal eccentricity was measured manually. The retinal vasculature in the parafoveal region was assessed.

Results—The foveal pit was wider and shallower in ROP than in control subjects. Mean pit depth, defined from the base to the level at which the pit reaches a lateral radius of $728 \mu\text{m}$, was $121 \mu\text{m}$ compared with $53 \mu\text{m}$. Intact, contiguous inner retinal layers overlay the fovea in ROP subjects but were absent in the control subjects. Mean full retinal thickness at the fovea was greater in the subjects with ROP ($279.0 \mu\text{m}$ vs. $190.2 \mu\text{m}$). The photoreceptor layer thickness did not differ between ROP and control subjects. An avascular zone was not identified in the subjects with ROP but was present in all the control subjects.

Conclusions—The foveas of subjects with a history of mild ROP have significant structural abnormalities that are probably a consequence of perturbations of neurovascular development.

The fovea, which mediates the excellent visual acuity enjoyed by healthy adults, is characterized by an absence of retinal vasculature, a high density of elongated cone inner and outer segments, and a pit without overlying ganglion cell or inner nuclear layers. The fovea is the last retinal region to reach maturity.¹ During the development of the central retina, the fovea forms in a rod-free zone that decreases in diameter as the cone inner segments become more slender, outer segments elongate, and the cones pack tightly together.^{2,3} A ring of parafoveal vasculature defines a central avascular zone (AZ) and the developing foveal dimple.^{4,5} The protracted course of central retinal and foveal development continues after birth into early childhood as neural and vascular elements take their proper places.^{6,7} Knowledge of development of the normal central retina and foveal

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structure has depended heavily on anatomic studies of the simian retina^{6,8,9} with fewer observations on the human fovea.^{2,3} Retinopathy of prematurity (ROP) is known to alter development of the central retina and, even if mild, may be associated with deficits in acuity and visual sensitivity.^{10–12} However, foveal fine structure in ROP has yet to be studied. So far, imaging the living macula in human subjects with a history of ROP has yielded only coarse information about foveal structure.¹³

The advent of high-speed, high-resolution retinal imaging enables investigation of the central retina in the living human eye with nearly the same fidelity as traditional histology. New technologies include optical coherence tomography (OCT), in which optical cross-sections with high axial resolution are generated¹⁴; Fourier domain OCT, a high-speed, multiplexed version of OCT^{15,16}; and adaptive optics (AO), which overcomes a fundamental limitation on lateral resolution imposed by ocular aberrations.^{17–19} In combination, these technologies provide the ability to visualize microstructures in the living eye.^{20,21} We used adaptive optics–Fourier domain optical coherence tomography (AO-FDOCT) to investigate the fine structure of the central retina in subjects with ROP.

Methods

Subjects

Five ROP and five healthy control subjects participated (Table 1). All ROP subjects have been observed at Children's Hospital Boston since infancy and participated in a study of multifocal electroretinography (mfERG) responses.¹¹ Birth was at 24 to 28 weeks' gestation, and birth weight was 600 to 1077 g. Term is at 40 weeks' gestation. All had a documented history of mild ROP that resolved spontaneously, leaving no visible residua on ophthalmoscopy except for the right eye of subject 7, which had a dragged macula. All others were judged to have a normal macula with a foveal dimple identifiable on ophthalmoscopy. The right eye of ROP subject 8 had refractive amblyopia. Although all but one (ROP subject 6) had become myopic by the time of this imaging study, only the eye with the dragged macula had a spherical equivalent outside the 99% prediction interval for normal²² during early childhood. All control subjects were myopic and had excellent corrected letter acuity (Table 1). For the imaging study, all subjects had pupils dilated with ophthalmic phenylephrine 2.5% (Mydrin; Alcon Laboratories, Inc., Fort Worth, TX) and cyclopentolate (Cyclogyl 1%; Alcon Laboratories, Inc.). Written informed consent was obtained from all subjects 18 years of age or older and from the parents of those younger. The study conformed to the Declaration of Helsinki and was approved by the Children's Hospital Committee on Clinical Investigation.

The AO-FDOCT Instrument

The AO-FDOCT system (Fig. 1) has been described more completely elsewhere.²¹ The system combines AO, FDOCT, and a wide-field confocal line-scanning laser ophthalmoscope (LSLO) into a compact platform. The AO component uses a Hartmann-Shack wavefront sensor (HS-WS) and a deformable mirror (DM; Boston Micromachines Inc., Watertown, MA) with 141 actuators and $\sim 4\text{-}\mu\text{m}$ stroke. The OCT source is a superluminescent diode (SLD; Superlum Diodes Ltd., Moscow, Russia) with 60-nm bandwidth that achieves a theoretical axial resolution of $4\text{ }\mu\text{m}$. The FDOCT spectrometer uses a transmission grating (1200 lp/mm, $830\text{-}\mu\text{m}$ λ_c) and a $10\text{-}\mu\text{m}$, 2048-pixel linear array detector (Atmel Inc., San Jose, CA). The LSLO images provide an en face retinal view that facilitates alignment of the OCT scan. Custom-designed objectives are used in the AO relay, spectrometer optics, and LSLO.^{21,23,24} An integrated 8×8 LED array controlled from the serial port provides a fixation target. Two computer-controlled motors adjust OCT and DM stages for range gate alignment and focus.

Procedure

The sequence of OCT scans used is summarized in Table 2. A line scan is a single cross-sectional scan through the fovea in the x or y dimension. A raster scan is swept through both x and y dimensions to build three dimensional (3-D) retinal maps. A radial scan is multiple lines that are rotated through 180° . Larger line ($1455\ \mu\text{m}$) and raster ($1455 \times 1455\ \mu\text{m}$) scans were used, sometimes without adaptive optics, to image the entire fovea. Smaller line ($873\ \mu\text{m}$), raster ($873 \times 873\ \mu\text{m}$), and radial ($873 \times 873\ \mu\text{m}$) scans were used with adaptive optics to map the ultrastructure of the fovea, including the retinal vasculature. The radial scans were used to confirm the raster results and augment the data when the raster scans were corrupted by motion. Two or three scans of each type were acquired.

A bite bar mounted on a three-axis stage was used to assist in alignment of the subject and to control head motion. Trial lenses dropped into the optical path at a pupil conjugate in front of the DM removed most low-order defocus and astigmatism and preserved DM stroke for high-order ocular aberrations. After the subject's pupil was aligned in the instrument, any residual defocus was removed with the front focus adjustment, which controlled the front lens relay, although observing both the OCT signal intensity and the sharpness of the HS-WS spots. The OCT range gate was then adjusted to the length of the subject's eye, whereupon a retinal image appeared in the software display. Once the pupil was aligned and the OCT focus optimized, AO was initiated, and the FDOCT scan acquired. The imaging session was limited to approximately 1 hour, to minimize subject fatigue.

Because the system was not equipped with retinal tracking, eye motion limited image quality, especially for raster scans of ROP subjects. High-quality, high-density scans of the fovea (256×256 -pixel raster acquired in 4.3 seconds) are difficult to obtain because the subject tends to fixate on and follow the line as it passes over the fovea. This voluntary drift does not occur for radial scans in which the center of rotation creates a fixation point. Radial scans are composed of evenly spaced line scans that produce nonuniform 3-D mapping. We developed custom software to interpolate the maps generated by the radial scans.

Measurements and Analyses

The characteristics of the fovea in ROP and control subjects were compared. On cross-sectional line scans through the fovea, the thickness of retinal layers was measured at selected eccentricities along the horizontal meridian. Foveal pit depth and volume were measured on 3-D maps generated from the raster and radial scans. The retinal vasculature was assessed at the level of the inner and outer plexiform layers.

Processing of Images—During acquisition, raw, unprocessed 12-bit gray-scale pixel values from the linear detector of the FDOCT spectrometer were saved to binary files. This spectrum was converted into an OCT image with processing steps including background subtraction, high-pass filtering, interpolation, and dispersion compensation.²¹ The image scans were then flattened to the retinal pigment epithelial (RPE) layer and aligned to a single-depth plane, in preparation for composite (coaddition) or en face presentation. In addition to standard image processing and input–output routines, such as saving frames and videos, the software contains extensive routines for analysis of multiple 2-D cross-sectional and 3-D volumetric retinal maps.

Thickness of Retinal Layers—The thickness of retinal layers was measured manually on the axial profiles of line scans through the fovea (Fig. 2). The fovea was located by indicators including foveal reflex, increased brightness, and continuity of the external limiting membrane (ELM), increased outer segment layer thickness (i.e., increased distance between connecting cilium [CC] and RPE layers), increased thickness of the outer nuclear

layer [ONL], and in the control subjects, absence of overlying outer retinal layers (nerve fiber layer [NFL] to the outer plexiform layer [OPL]).

Foveal Pit Depth and Volume—3-D representations of the fovea were created from the raster and radial scans. The cross-sectional area of the pit for successive axial slices was used to calculate pit depth and volume. On a decent raster scan, free of significant corruption by eye and head motion, the pit area can be readily identified by the “hole” in each en face slice above the foveal reflex (Fig. 3a), whereas the hole is not seen if there is significant transverse motion (Fig. 3c). Although the data could be corrected for some of the transverse motion, it is impossible to compensate for the out-of-plane motion.

Custom software was written for automatic identification of edges of the hole for pit volume measurement. First the en face image was inverted and then thresholded. The individual particles in the thresholded image were processed with morphologic operators, open then closed, and then particle filter and hole-filling routines were used to remove small particles and holes. The convex hull of the largest particle was found and an edge-detection routine applied to the resultant image. The edge was added to the image overlay, and particle statistics including area and center of mass were calculated. The pit area was calculated by two methods. The first simply summed the pixels in the particle. The second used the average of the length (superior-inferior) and width (nasal-temporal) of the box that bounds the particle. The latter method assumes a circular area (accounting for any asymmetry), which appears a reasonable approximation (Fig. 3a). Information about pit volume was also extracted from radial scans displayed in a manner similar to en face raster scans (Fig. 4).

Results

Figure 5 shows the pit volume results for control subject 3. In general, the pit depth and volume measurements from both eyes of the same subject were similar. Control subject 3 had the largest right-left difference, with the exception of ROP subject 7 who had a dragged macula in the right eye (Table 1). The average difference between the two methods of pit area measurement for the rasters shown in Figure 5 was 5.4%. A slight asymmetry (pit width/pit height ≈ 1.1) was found in both control and ROP subjects, but this is accounted for in both measurement methods.

Figure 6 shows composite images of one eye of all subjects. The foveal pit is present although quite shallow in the ROP subjects. Vessels were identified overlying the fovea in all ROP subjects except subject 9. In the control subjects, the inner retinal layers (NFL, GCL, inner plexiform layer [IPL], inner nuclear layer [INL], and OPL) at 500 to 750 μm eccentricity were identifiable. With decreasing eccentricity, these inner layers tapered and were absent in the central 100 to 200 μm of the fovea. In contrast, all inner retinal layers were present and contiguous across the central fovea in the ROP subjects, except subject 9. In both control and ROP subjects, the ONL thickness increased in the fovea. The thickness of photoreceptor inner (ELM-CC) and outer (CC-RPE) segment layers was similar in ROP and control subjects.

Retinal layer thickness is quantified in Figure 7. The measurements include all except subject 7, whose right eye, which had high myopia and a dragged macula, and subject 9, whose left eye was not imaged. Although individual retinal layers of the right eye of subject 7 could not be resolved, we estimated the full retinal thickness from cross-sectional scans to be $\sim 522 \mu\text{m}$, which exceeded that in any other subject with ROP.

The full retinal thickness was greater in subjects with ROP than in control subjects at all eccentricities (Fig. 7a). The difference was greatest in the fovea where the full retinal

thickness was 47% higher in subjects with ROP (279.0 μm vs. 190.2 μm) and the difference decreased to 17% at 437 μm eccentricity (302.0 μm vs. 257.6 μm ; average of -437 and $+437$ μm). In the subjects with ROP, the total retinal thickness did not vary significantly with eccentricity ($P = 0.65$), further indicating a shallow pit. The inner retinal layers were also significantly thicker in the subjects with ROP (Fig. 7b), and the difference varied little with eccentricity. The difference between ROP and control for the total thickness (Fig. 7a) and inner retinal thickness (Fig. 7b) was significant ($P < 10^{-8}$). The thickness of the inner retinal layers in subjects with ROP is slightly greater nasally than temporally ($P = 0.013$ at 437 μm). The difference in ONL thickness (Fig. 7c) between ROP and control subjects was greatest at the fovea (31.5 μm , $P < 0.00005$) and negligible for eccentricities ≥ 250 μm . There was very little difference (< 4 μm , $P = 0.184$) found in the photo-receptor layer thickness between ROP and control subjects (Fig. 7d). For the control subjects, the photoreceptor layer increased from 62 μm at 437 μm eccentricity to 75 μm in the fovea.

The pit depth for ROP and control subjects is shown in Table 3. The foveal pit depth was measured from the 3-D maps and cross-sectional images and defined as the distance from the bright reflex at the base of the pit to the arbitrarily chosen point where the pit reaches a radius of 728 μm . The pit depth is smaller by more than a factor of two in ROP subjects.

The results of the pit volume measurement are shown in Figure 8. Pit area as a function of depth extracted from raster and lines scans of five control (10 eyes) and five ROP (8 eyes) subjects is plotted. If volumetric raster scans were corrupted by eye movements (Fig. 3c), line scans were used and radially isotropic pits were assumed. The second-order polynomial fit and equation are also shown on the graph. The foveal pit in ROP subjects was wider and shallower than that in the control subjects, except for ROP subject 9.

En face images (Fig. 9) compare the vasculature in two distinct layers (IPL and OPL) from an ROP and a control subject. In the ROP subject, vessels overlay the entire foveal region (Figs. 9e, 9g). The capillaries that surrounded the AZ were not clearly resolved in the control subject, although a vessel within 250 μm of the fovea center that may be part of the ring is indicated by an arrow in Figure 9a. The average diameter of 10 vessels in the OPL for the subject with ROP and the control subject is compared in Figure 10. The average FWHM diameter of the capillaries was 35% lower in the subject with ROP.

Discussion

In seven of eight ROP eyes analyzed, the foveal pit was broad and shallow, with inner retinal layers of neural cells extending across the fovea (Table 3; Figs. 6, 8). Normally, a foveal pit without overlying layers of neural cells is produced by centrifugal movement of the neural cells during development.^{1–5,25} In the ROP subjects, retinal capillaries extended across the fovea (Figs. 9, 10), which is normally an AZ, and became intertwined with the neural cells that overlay the fovea (Fig. 6). In these ROP eyes, there was no history of adverse events beyond the mild ROP that resolved spontaneously by term. Thus, ROP, which was an active disease at preterm ages when the fovea is quite immature, probably accounts for the neurovascular abnormality that is documented by these OCT data. Myopia alone, which was frequent among the subjects with ROP, is not a suspected cause, because neurovascular abnormalities were not observed in the control subjects, all of whom were myopic. One ROP eye (subject 9, right eye) had exceptional results including foveal cross-sections and pit dimensions that were indistinguishable from those in the control subjects. Acuity, clinical appearance of the macula, ROP history, and multifocal ERG topography¹¹ in subject 9 and the other ROP eyes were similar. ROP may not universally produce the neurovascular abnormality seen in the seven other ROP eyes.

The neurovascular abnormality in the central retina may have slightly degraded the best corrected acuity in some of the subjects with ROP (Table 1) by causing mild optical aberration or metabolic effects on neural cells that are sensitive to contrast. Loss of foveal cones, or increased cone–cone spacing could also degrade acuity. The OCT data offer no evidence of loss of cones in the central retina of the subjects with ROP. The ROP photoreceptors had inner and outer segment lengths identical with those in the control subjects and slightly greater thickness of the layer of photoreceptor nuclei, the ONL (Fig. 7). Thus, even in the absence of a well-formed foveal pit, there was no apparent paucity of foveal cones, even though formation of the pit and packing of the foveal cones are companion events in normal foveal development.^{1–3,26–28}

In accord with these new OCT observations were other lines of evidence for long-lasting effects of mild ROP on the neurovascular characteristics of the macula, even if the active disease did not directly affect the macula. Fluorescein angiography showed that the foveal AZ is small or absent in children with a history of ROP.²⁹ Central retinal ERG responses to multifocal stimulation are significantly attenuated in ROP subjects¹¹; the bipolar cells are the main contributors to the mfERG responses. The topography of the ROP mfERG responses suggest higher bipolar cell density of the centralmost retina, perhaps due to impaired centrifugal movement of the inner retinal neurons during development of the ROP fovea. The OCT results support this idea of impaired centrifugal movement of the bipolar cells and also the cone nuclei, as the ONL was slightly thickened in the ROP retina (Fig. 7c). In addition, OCT evidence of capillary encroachment leads us to suspect that altered neurovascular interactions attenuate ERG responses of the inner retinal neurons.³⁰

Neurovascular interactions are involved in foveal pit formation and cone–cone packing. Anatomic studies^{1–4} document formation of a parafoveal AZ by midgestation, a period that coincides with the birth of subjects with ROP. Later in gestation, widening of the pit, elongation of cone inner and outer segments, and closer cone–cone packing occurs and continues after birth and into early childhood. A mechanical model of pit formation and expansion^{26–28} proposes that the AZ generates a gradient of elasticity that is acted on first by intraocular pressure and then by shearing forces exerted by the expanding peripheral retina in the growing eye. As the pit forms, centripetal forces pack the developing cones with elongating inner and outer segments closer and closer together. In formation of the ROP fovea, the centrifugal forces that lead to pit widening may not be intimately linked to the centripetal forces that lead to cone packing. This said, we acknowledge that we cannot specify the cone packing in ROP subjects, because we have yet to use adaptive optics to study the ROP cone mosaic. Furthermore, growth factors, including VEGF, neuropilin, and semaphorin, cooperatively guide normal development of both retinal neurons and vasculature³¹ and are suspected of a role in abnormal neurovascular development in ROP.³² The mechanical and trophic models are not necessarily mutually exclusive. Because the preterm formation of the foveal pit is mediated by the elasticity of the parafoveal vasculature that is altered in ROP, it is unlikely that the abnormal neurovascular features of the ROP fovea are a healing reaction in response to acute ROP.

The AO-FDOCT data documented foveal abnormalities in subjects in whom mild ROP had resolved spontaneously, many years before the imaging session. The images had nearly the same resolution of the retinal layers as those achieved by light microscopy, and had the great advantage of showing the neurovascular relationships in the living human eye. Technical advances, including adaptive optics and noninvasive retinal tracking strategies,²³ offer realistic hope of adding new knowledge, not only about pediatric retinal diseases, but also about the structural and functional details of normal development of the human fovea.

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References

1. Hendrickson, AE. The morphologic development of human and monkey retina. In: Albert, DM.; Jakobiec, FA., editors. *Principles and Practice of Ophthalmology: Basic Sciences*. Philadelphia: WB Saunders; 1994. p. 561-577.
2. Hendrickson AE, Yuodelis C. The morphological development of the human fovea. *Ophthalmology* 1984;91:603–612. [PubMed: 6462623]
3. Yuodelis C, Hendrickson A. A qualitative and quantitative analysis of the human fovea during development. *Vision Res* 1986;26:847–855. [PubMed: 3750868]
4. Provis JM, Sandercoe T, Hendrickson AE. Astrocytes and blood vessels define the foveal rim during primate retinal development. *Invest Ophthalmol Vis Sci* 2000;41:2827–2836. [PubMed: 10967034]
5. Springer AD. New role for the primate fovea: a retinal excavation determines photoreceptor deployment and shape. *Vis Neurosci* 1999;16:629–636. [PubMed: 10431912]
6. Dorn EM, Hendrickson L, Hendrickson AE. The appearance of rod opsin during monkey retinal development. *Invest Ophthalmol Vis Sci* 1995;36:2634–2651. [PubMed: 7499086]
7. Hansen RM, Fulton AB. Dark adapted thresholds at 10 deg and 30 deg eccentricities in 10-week old infants. *Vis Neurosci* 1995;12:509–512. [PubMed: 7654607]
8. Hendrickson A, Kupfer C. The histogenesis of the fovea in the macaque monkey. *Invest Ophthalmol Vis Sci* 1976;15:746–756. [PubMed: 822712]
9. Hendrickson A. A morphological comparison of foveal development in man and monkey. *Eye* 1992;6:136–144. [PubMed: 1624035]
10. Isenberg SJ. Macular development in the premature infant. *Am J Ophthalmol* 1986;101:74–80. [PubMed: 3753633]
11. Fulton AB, Hansen RM, Moskowitz A, Barnaby AM. Multifocal ERG in subjects with a history of retinopathy of prematurity. *Doc Ophthalmol* 2005;111:7–13. [PubMed: 16502302]
12. Barnaby AM, Hansen RM, Moskowitz A, Fulton AB. Development of scotopic visual thresholds in retinopathy of prematurity. *Invest Ophthalmol Vis Sci* 2007;48:4854–4860. [PubMed: 17898313]
13. do Lago A, Matieli L, Gomes M, et al. Stratus optical coherence tomography findings in patients with retinopathy of prematurity? *Arq Bras Oftalmol* 2007;70:19–21. [PubMed: 17505713]
14. Drexler W, Morgner U, Ghanta RK, Kärtner FX, Schuman JS, Fujimoto JG. Ultrahigh-resolution ophthalmic optical coherence tomography. *Nat Med* 2001;7:502–507. [PubMed: 11283681]
15. Wojtkowski M, Leitgeb R, Kowalczyk A, Bajraszewski T, Fercher AF. In vivo human retinal imaging by Fourier domain optical coherence tomography. *J Biomedical Opt* 2002;7:457–463.
16. Nassif N, Cense B, Park BH, et al. In vivo human retinal imaging by ultrahigh-speed spectral domain optical coherence tomography. *Opt Lett* 2004;29:480–482. [PubMed: 15005199]
17. Liang J, Williams D, Miller D. Supernormal vision and high-resolution retinal imaging through adaptive optics. *J Opt Soc Am A* 1997;14:2884–2892.
18. Roorda A, Williams DR. The arrangement of the three cone classes in the living human eye. *Nature* 1999;397:520–522. [PubMed: 10028967]
19. Roorda A, Romero-Borja F, Donnelly W III, Queener H, Hebert T, Campbell M. Adaptive optics scanning laser ophthalmoscopy. *Opt Express* 2002;10:405–412. [PubMed: 19436374]

20. Zawadzki R, Jones S, Olivier S, et al. Adaptive-optics optical coherence tomography for high-resolution and high-speed 3D retinal in vivo imaging. *Opt Express* 2005;13:8532–8546. [PubMed: 19096728]
21. Bigelow CE, Iftimia NV, Ferguson RD, Ustun TE, Bloom B, Hammer DX. Compact multimodal adaptive optics spectral domain optical coherence tomography instrument for retinal imaging. *J Opt Soc Am A* 2007;24:1327–1336.
22. Mayer DL, Hansen RM, Moore BD, Kim S, Fulton AB. Cycloplegic refractions in healthy children aged 1 through 48 months. *Arch Ophthalmol* 2001;119:1625–1628. [PubMed: 11709012]
23. Hammer DX, Ferguson RD, Bigelow CE, Iftimia NV, Ustun TE, Burns SA. Adaptive optics scanning laser ophthalmoscope for stabilized retinal imaging. *Opt Express* 2006;14:3354–3367. [PubMed: 19516480]
24. Hammer DX, Ferguson RD, Ustun TE, Bigelow CE, Iftimia NV, Webb RH. Line-scanning laser ophthalmoscope. *J Biomed Opt* 2006;11:041126. [PubMed: 16965154]
25. Hendrickson A, Troilo D, Possin D, Springer A. Development of the neural retina and its vasculature in the marmoset *Callithrix jacchus*. *J Comp Neurol* 2006;497:270–286. [PubMed: 16705674]
26. Springer AD, Hendrickson AE. Development of the primate area of high acuity. 1. Use of finite-element analysis models to identify mechanical variables affecting pit formation. *Vis Neurosci* 2004;21:53–62. [PubMed: 15137581]
27. Springer AD, Hendrickson AE. Development of the primate area of high acuity. 2. Quantitative morphological changes associated with retina and pars plana growth. *Vis Neurosci* 2004;21:775–790. [PubMed: 15683563]
28. Springer AD, Hendrickson AE. Development of the primate area of high acuity, 3: Temporal relationships between pit formation, retinal elongation and cone packing. *Vis Neurosci* 2005;2:171–185. [PubMed: 15935110]
29. Mintz-Hittner HA, Knight-Nanan DM, Satriano DR, Kretzer FL. A small foveal avascular zone may be an historic mark of prematurity. *Ophthalmology* 1999;106:1409–1413. [PubMed: 10406630]
30. Fulton AB, Hansen RM, Moskowitz A. The cone electroretinogram in retinopathy of prematurity. *Invest Ophthalmol Vis Sci* 2008;49:814–819. [PubMed: 18235032]
31. Gariano RF, Hu D, Helms J. Expression of angiogenesis-related genes during retinal development. *Gene Expr Patterns* 2006;6:187–192. [PubMed: 16330258]
32. Akula JD, Hansen RM, Martinez-Perez ME, Fulton AB. Rod photoreceptor function predicts blood vessel abnormality in retinopathy of prematurity. *Invest Ophthalmol Vis Sci* 2007;48:4351–4359. [PubMed: 17724227]

Figure 1.

Simplified schematic of the AO-FDOCT system. LSLO, line-scanning laser ophthalmoscope; FT, fixation target; D, dichroic beamsplitter; DM, microelectromechanical system (MEMS)-based deformable mirror; HS-WS, Hartmann-Shack wavefront sensor; BS, pellicle beamsplitter; ODL, optical delay line; FC, fiber coupler; SLD, superluminescent diode; RT, real-time controller.

Figure 2.

Retinal layer thickness analysis (ROP subject 6). (a) Profiles were averaged from 20 adjacent A-scans at seven retinal eccentricities (denoted by boxes). ILM, inner limiting membrane (vitreal–retinal interface); NFL, nerve fiber layer; GCL, ganglion cell layer; IPL, inner plexiform layer; INL, inner nuclear layer; OPL, outer plexiform layer; ONL, outer nuclear layer; ELM, external limiting membrane; CC, connecting cilium; RPE, retinal pigment epithelium; C, choroid; IS, inner segment layer; OS, outer segment layer; t, temporal; n, nasal. (b) The layers for each eccentricity ($\pm 437 \mu\text{m}$, $0 \mu\text{m}$ represents the fovea) were measured from the edges and peaks in the profiles, which are shifted horizontally for clarity. On the vertical axis, $0 \mu\text{m}$ represents the RPE layer. Symbols represent different eccentricities. (c) The final calculated thickness measurements are shown as a function of eccentricity. The thickness is measured from the RPE for the retina and photoreceptor layers, between the ILM and OPL for the inner retina, and between the OPL and ELM for the ONL.

Figure 3.

Pit volume analysis for raster scans. En face axial slices (**a**, **c**) were taken at the depth indicated by the *horizontal line* in the cross-sectional scans (**b**, **d**). (**a**) Pit area, automatically delineated by custom software, for control subject 3. (**c**) An example of a raster scan corrupted by eye motion for ROP subject 7. Raster scan size: $873 \times 873 \mu\text{m}$.

Figure 4.

Pit volume analysis for radial scans (control subject 3). En face axial slices (**a**, **c**, **e**) were taken at the depth indicated by the horizontal line in the cross-sectional scans (**b**, **d**, **f**). (**a**) The unprocessed axial slice at the base of the foveal pit. (**c**) The same image as (**a**) adjusted for transverse eye movement. (**e**) An axial slice with pit area automatically delineated by software. Radial scan size: $873 \times 873 \mu\text{m}$.

Figure 5.

Pit volume data for control subject 3 comparing raster and radial scans, raster area calculation methods, and left and right eyes. *Squares*: left eye data; *diamonds*: right eye data. *Filled symbols*: calculation method that uses the particle-bounding box; *open symbols*: the method that counts the pixel area of the particle. Also shown is a second order polynomial fit (with intercept set to 0) through the raster data for each eye and each method. The highest order polynomial fit coefficients for the two methods were within ~4%, indicating the degree to which the circular assumption is valid. The radial data are collected over a smaller scan region (873 μm vs. 1455 μm). The raster algorithm tends to underestimate the area near the base of the pit by incorrect delineation of pit margins near the foveal reflex.

Figure 6.

Montage of cross-sectional composite images from one eye of all subjects. (a–e) Control subjects; (f–j) subjects with ROP. Composite images were created from 4 to 11 frames. Scan length is 1455 μm except for those in (e) and (j) which are 1164 μm . (d) Scale bar for (a–d, f–i). (e) Scale bar for (e, f). Cross-sectional videos for subjects 1 and 6 (Movies 1, 2) can be viewed online at <http://www.iovs.org/cgi/content/full/49/5/2061/DC1>.

Figure 7. Thickness measurements (mean $\pm$ SE) of retinal layers at several eccentricities in ROP (8 eyes, all except subjects 7, OD, and 9, OS) and control subjects (10 eyes). *Top*: layers measured in (a–d). (a) Full retinal thickness from ILM to RPE. (b) Inner retinal layer thickness (ILM to ONL). (c) ONL thickness. (d) Photo-receptor thickness.

Figure 8.

Pit area as a function of depth measured from the base of the pit for ROP (8 eyes) and control subjects (10 eyes). Equation for second-order polynomial fit (with intercept set to 0) and correlation coefficient is shown for each data set. Fit for ROP subjects does not include subject 9.

Figure 9.

Retinal vasculature in control subject 3 and ROP subject 8. (**a–d**) Control and (**e–h**) ROP subjects. En face views (**a, c, e, g**) were created by averaging the axial slices shown between the *horizontal lines* in the corresponding cross-sectional images (**b, d, f, h**). (**a, e**) Vessels in the IPL; (**c, h**) vessels in the OPL. (**a, arrow**) Capillary surrounding the AZ; (**e, arrow**) center of the fovea. Raster scan size: $873 \times 873 \mu\text{m}$. En face fly-through videos (Movies 3, 4) can be viewed online at <http://www.iovs.org/cgi/content/full/49/5/2061/DC1>.

Figure 10.

OPL vessel diameter measurement in **(a)** control subject 3 and **(b)** ROP subject 8 (from the same en face images as shown in Fig. 9). Ten vessels were randomly chosen and their cross-sectional profiles measured, aligned, and averaged. The profile locations are indicated by *numbered lines* across the vessel. **(a, b)** Indicate and number the coordinates of the vessel profile. **(c)** The profile (mean $\pm$ SE) and the full width at half maximum (FWHM) diameter.

Table 1

ROP and Control Subjects

Subject	GA (wk)	BW (g)	Age (y)	Refraction (D)		Acuity	
				Right	Left	Right	Left
Control							
1			24	-2.75S	-3.75S	20/20	20/20
2			21	-5.75S	-6.25S	20/20	20/20
3			25	-1.25S	-1.50S	20/16	20/16
4			25	-3.75S	-3.50S	20/25	20/20
5			18	-6.75S	-6.00S	20/15	20/15
ROP							
6	26	800	14	+1.00S	+1.00S	20/16	20/20
7	26	780	14	-10.0S	-5.50S	20/400	20/20
8	24	600	16	-2.00S	Plano	20/40	20/25
9	26	860	15	-0.75S	-0.75S	20/20	20/20
10	28	1077	26	-6.00S	-4.00S	20/20	20/25

GA, gestational age; BW, birth weight.

Table 2

Sequence of OCT Scans

Scan Type	Size		Frame Rate		Frames	Acquisition Time (s)
	(μm)	(Pixels)	(Frames/s)			
Line	1455	1024	18	100	5.6	
Raster	1455 $\times$ 1455	128 $\times$ 128	120	128	1.1	
Line	873	1024	18	100	5.6	
Raster	873 $\times$ 873	256 $\times$ 256	60	256	4.3	
Radial	873 $\times$ 873	256 $\times$ 256	60	256	4.3	

Table 3

Depth of the Foveal Pit

Subject	Depth (μm)	
	Right	Left
Control		
1	116	102
2	111	119
3	117	125
4	128	129
5	145	—
Mean $\pm$ SE	121 $\pm$ 4.3	
ROP		
6	55	61
7	—	60
8	40	69
9	98	—
10	16	23
Mean $\pm$ SE	53 $\pm$ 9.9	