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Spectral Domain Optical Coherence Tomography in Mouse Models of Retinal Degeneration

Gesine Huber¹, Susanne C. Beck¹, Christian Grimm², Ayse Sahaboglu-Tekgoz³, Francois Paquet-Durand³, Andreas Wenzel², Peter Humphries⁴, T. Michael Redmond⁵, Mathias W. Seeliger^{1,§}, and M. Dominik Fischer^{1,§}

¹Division of Ocular Neurodegeneration, Institute for Ophthalmic Research, Centre for Ophthalmology, Tuebingen, Germany ²Lab of Retinal Cell Biology, Department of Ophthalmology, University of Zurich, Zurich, Switzerland ³Division for Experimental Ophthalmology, Institute for Ophthalmic Research, Centre for Ophthalmology, Tuebingen, Germany ⁴Ocular Genetics Unit, Trinity College Dublin, Ireland ⁵National Eye Institute, NIH, Washington, USA

Abstract

Purpose—Spectral domain optical coherence tomography (SD-OCT) allows cross-sectional visualization of retinal structures *in vivo*. Here, we report the efficacy of a commercially available SD-OCT device to study mouse models of retinal degeneration.

Methods—C57BL/6 and BALB/c wild type mice and three different mouse models of hereditary retinal degeneration (*Rho*^{-/-}, *rd1*, *RPE65*^{-/-}) were investigated using confocal scanning laser ophthalmoscopy (cSLO) for en face visualization and SD-OCT for cross-sectional imaging of retinal structures. Histology was performed to correlate structural findings in SD-OCT with light microscopic data.

Results—In C57BL/6 and BALB/c mice, cSLO and SD-OCT imaging provided structural details of frequently used control animals (central retinal thickness, CRT_{C57BL/6} = 237±2µm and CRT_{BALB/c} = 211±10µm). *RPE65*^{-/-} mice at 11 months of age showed a significant reduction of retinal thickness (CRT_{RPE65} = 193±2µm) with thinning of the outer nuclear layer. *Rho*^{-/-} mice at P28 demonstrated degenerative changes mainly in the outer retinal layers (CRT_{Rho} = 193±2µm). Examining *rd1* animals before and after the onset of retinal degeneration allowed to monitor disease progression (CRT_{rd1 P11} = 246±4µm, CRT_{rd1 P28} = 143±4µm). Correlation of CRT assessed by histology and SD-OCT was high (r² = 0.897).

Conclusion—We demonstrated cross sectional visualization of retinal structures in wild type mice and mouse models for retinal degeneration *in vivo* using a commercially available SD-OCT device. This method will help to reduce numbers of animals needed per study by allowing longitudinal study designs and facilitate characterization of disease dynamics and evaluation of putative therapeutic effects following experimental interventions.

Keywords

optical coherence tomography; retinal degeneration; imaging; mouse models

Corresponding Author: M. Dominik Fischer, MD Institute for Ophthalmic Research, Centre for Ophthalmology University of Tuebingen, 72076 Tuebingen, Germany Dominik.Fischer@med.uni-tuebingen.de.

[§]These authors contributed equally to the manuscript

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Conflict of Interest: None

Introduction

Optical coherence tomography (OCT) has evolved over the last two decades to become an important diagnostic tool in clinical ophthalmology and other medical specialties.¹ Widespread application of this powerful tool in animal research however, was restricted due to poor image quality in commercially available first and second generation time domain OCT devices.^{2, 3} Sufficiently high image quality could only be achieved using prototype devices with improved depth resolution that were specifically adapted for the respective animal visual system.⁴⁻⁷ Only recently, latest generation SD-OCT devices have become commercially available that provide scanning speed and depth resolution sufficient for small animal experimentation.⁸ Since SD-OCT is ideally suited to study changes of retinal integrity, wild type mice and mouse models of retinal degeneration were chosen to explore the efficacy of this method.

So far, animal models of retinal degeneration have been studied extensively on both functional and structural level. Morphological changes were either detected using *en face* imaging methods such as funduscopy⁹ and confocal scanning laser ophthalmoscopy (cSLO)¹⁰, or more commonly using *ex vivo* histological approaches. While light and electron microscopy provide (ultra-)high structural resolution, fixation procedures, dehydration preparatory to subsequent embedding and staining, even the process of cutting, flattening and mounting histological sections are potential sources for significant and variable alterations in dimensions.^{11, 12} Indeed, retinal thickness measures of rodent retina are reported to be 95 μm in one study¹³ and 170-217 μm in other studies.^{11, 14} Taken together, *ex vivo* analysis of retinal tissue has certain limitations and should be interpreted accordingly. *In vivo* analysis of the retina has significant benefits as delicate, but functionally important changes, such as retinal edema or focal neurosensory and/or pigment epithelial detachments can readily be detected by SD-OCT, while potentially remaining undetected or masked by handling procedures in histological analysis. Finally, monitoring of dynamic changes in individual animals requires a non-invasive study design. Here, SD-OCT has the potential to complement the existing *in vivo* methods in vision research by providing histology-analog structural details on retinal integrity. Since individual animals may be investigated at multiple time points, SD-OCT can also help to reduce the numbers of animals needed for such a study, which has both ethical as well as economic implications. To the best of our knowledge, this is the first study to demonstrate the efficacy of a commercially available SD-OCT device to obtain cross sectional images from wild type mice and mouse models of retinal degeneration.

Materials and Methods

Animals

Animals were housed under standard white cyclic lighting (200 lux), had free access to food and water, and were used irrespective of gender. *Rho*^{-/-}¹⁵ (n = 46), *RPE65*^{-/-}¹⁶ (n = 10), BALB/c (n = 15, Charles River Laboratories), C57/BL6/J (n = 37, Charles River Laboratories), C3H *rd1/rd1* (n = 6, *rd1*), and control C3H wild-type (n = 6, wt) mice¹⁷ were used for imaging. All procedures were performed in accordance with the local ethics committee, German laws governing the use of experimental animals, and the ARVO statement for the use of animals in ophthalmic and visual research. Because of the critical changes after post-natal day 11 (PN11) 18-19, imaging in *rd1* and corresponding WT were carried out at PN11 and PN28. Due to the later onset and slower course of degeneration in the *Rho*^{-/-} and *RPE65*^{-/-} mouse models, imaging was performed at 1 and 11 months respectively.

Confocal scanning laser ophthalmoscopy—For *en face* retinal imaging, we used the commercially available HRA 1 and HRA 2 (Heidelberg Engineering, Heidelberg, Germany) featuring up to two Argon wavelengths (488/514 nm) in the short wavelength range and two infrared diode lasers (HRA 1: 795/830 nm, HRA 2: 785/815 nm) in the long wavelength range.

A detailed protocol for anesthesia and imaging is described elsewhere.¹⁰ Briefly, mice were anaesthetized by subcutaneous injection of ketamine (66.7 mg/kg) and xylazine (11.7 mg/kg), their pupils dilated with tropicamide eye drops (Mydriaticum Stulln, Pharma Stulln, Germany) before image acquisition. By applying hydroxypropyl methylcellulose (Methocel 2%, OmniVision, Puchheim, Germany) on the eye, the refractive power of the air corneal interface was effectively negated. A custom-made contact lens (focal length = 10 mm) was used to reduce the risk of corneal dehydration and edema and act as collimator.

Spectral domain optical coherence tomography (SD-OCT)—SD-OCT imaging was done in the same session as cSLO, i.e. animals remained anaesthetized using identical preparatory steps. Mouse eyes were subjected to SD-OCT using the commercially available Spectralis™ HRA+OCT device from Heidelberg Engineering featuring a broadband superluminescent diode at $\lambda = 880$ nm as low coherent light source. Each two-dimensional B-Scan recorded at 30° field of view consists of 1536 A-Scans, which are acquired at a speed of 40,000 scans per second. Optical depth resolution is ca. 7 μm with digital resolution reaching 3.5 μm .⁸

To adapt for the optical qualities of the mouse eye, we mounted a commercially available 78D double aspheric fundus lens (Volk Optical, Inc., Mentor, OH) directly in front of the camera unit. Imaging was performed using the proprietary software package Eye Explorer version 3.2.1.0 from Heidelberg Engineering. Length of the reference pathway was adjusted manually according to manufacturers instructions using the “OCT debug window” (press Ctrl/Shift/Alt/O simultaneously to open window in the active, calibrated OCT mode) to adjust for the optical length of the scanning pathway. The combination of scanning laser retinal imaging and SD-OCT allows for real-time tracking of eye movements and real-time averaging of OCT scans, reducing speckle noise in the OCT images considerably.⁸ Resulting data were exported as 8 bit grey scale image files and processed in Adobe Photoshop CS2 (Adobe Systems, San Jose, CA). For quantification of central retinal thickness based on high resolution volume scans, we used the proprietary Eye Explorer. Briefly, each volume scan consisted of at least 70 B-Scans recorded at 30° field of view centered on the optic disc, which were used to calculate an interpolated retinal thickness map across the scanned retinal area. Central retinal thickness was quantified using the circular OCT grid subfield at 3 mm diameter with the center located on the optic disc.

Histology—Three animals from each mouse line (*Rho*^{-/-}, *RPE65*^{-/-}, BALB/c and C57/BL6) were sacrificed and enucleated for histological analysis. After orientation was marked, the eyes were fixed overnight in 2.5 % glutaraldehyde prepared in 0.1 M cacodylate buffer and processed as described previously.²⁰ Semi-thin sections (0.5 μm) of Epon-embedded tissue were prepared from the central retina, counterstained with methylene blue and analyzed using a light microscope (Axiovision, Zeiss, Jena, Germany).

For the timeline analysis of *rd1* mice (n = 3 for each group: rd1 P11, rd1 P28, wt P11 and wt P28), eyes were embedded unfixed in Jung tissue freezing medium (Leica Microsystems, Wetzlar, Germany), frozen and sectioned (14 μm) in a cryotome (HM560, Microm, Walldorf, Germany). Sections were then stained using Hematoxylin/Eosin staining. Morphological observations and light microscopy were performed on a Zeiss Imager.Z1 Apotome microscope equipped with a Zeiss AxioCam MrN digital camera. Images were captured using Zeiss Axiovision 4.6 software.

SD-OCT vs. Histology: For correlation of retinal thickness measurements, inner retina was defined as ranging from the inner limiting membrane to the outer plexiform layer (OPL) and outer retina ranging from the outer nuclear layer (ONL) to the retinal pigment epithelium (RPE). In case of morphometric assessment of histologic sections, all measurements were taken

at 1.4 mm eccentricity from the optic nerve head to mirror the SD-OCT based thickness measurement along a circular ring scan ($r = 1.4$ mm) centered on the optic nerve head. Respective thickness was quantified by computer assisted manual segmentation analysis using the proprietary Eye Explorer software in case of SD-OCT data and the calibrated “line-tool” in Adobe Photoshop CS2 in case of the histological micrographs.

Student's *t* test was used to analyze statistical significance between respective inner/outer retinal thickness measurements (C57/Bl6 vs. *Rho*^{-/-}, *RPE65*^{-/-} or BALB/c; C3H wild-type vs. C3H rd1/rd1 at P11 and P28). Correlation between thickness measurements by OCT vs. histology was assessed by Pearson's correlation coefficient.

Results

C57BL/6

A basic examination using cSLO en-face imaging was performed in four week old C57BL/6 mice to confirm the presence of regular retinal and vascular structures typical for normal wild type (wt) animals. The examination included native red free (513 nm), infrared (830 nm) and autofluorescence (AF) modes, as well as fluorescein angiography (FLA) and indocyanine green angiography (ICGA) (Fig. 1). The cross-sectional SD-OCT imaging in these animals provided detailed *in vivo* data on retinal layer composition (Fig. 1) and retinal thickness. In a central circular area of approx. 3 mm diameter, total retinal thickness was 237 ± 2 μ m (mean $\pm$ standard deviation). The laminar organization of the murine retina as determined *in vivo* by SD-OCT correlated well with *ex vivo* light microscopy studies (Fig. 1). Notably, some wt mice feature a Bergmeister's papilla, a structural remnant of the developmental hyaloid vascular system (HVS) at the optic disc.²¹ Indeed, angiography showed characteristic perfusion of the HVS in mice until approximately P10 (data not shown), after which the hyaloid artery usually obliterates and leaves behind the channel of Cloquet.²² In some animals however, this development remains incomplete leading to a Bergmeister's papilla as seen in Figure 1F.

BALB/c

En-face imaging in BALB/c mice (PW10) using cSLO showed non-pigmented retinal structures in the native red free (RF, 513 nm), infrared (IR, 830 nm) and autofluorescence (AF) modes (Fig. 2). As BALB/c mice are highly susceptible to light-induced retinal degeneration^{23, 24}, the hyperfluorescent dots in the AF mode may reflect lipid degradation products. Accordingly, overall retinal thickness in the central 3mm as determined by SD-OCT was 211 ± 10 μ m, considerably less than in the C57BL/6 strain, which would be hypothetically consistent with a mild light-induced photoreceptor loss. Virtual cross-sections displayed identical laminar organization in the inner retina compared to C57BL/6. However, as lack of pigmentation in BALB/c mice resulted in altered optical characteristics in the outer retina, signal composition of SD-OCT scans distal of the external limiting membrane differed considerably (Fig. 2). Specifically, instead of two highly reflective layers (HRLs) thought to represent the inner/outer segment border (IOS) and RPE (Fig. 1), SD-OCT scans in BALB/c mice demonstrated two additional HRLs possibly demarcating the non pigmented choriocapillary and choroidal structures (Fig. 2).

Rho^{-/-}

The visual pigment of the rod photoreceptors, rhodopsin, is an essential element of the phototransduction cascade and also serves as a structural protein of the discs in the rod outer segments (ROS). Hence, in rhodopsin deficient mice (*rho*^{-/-}) ROS are never formed and rod-derived ERG signals can not be generated.¹⁵ En-face imaging in *rho*^{-/-} mice (PW4) using cSLO showed characteristic signs of retinal degeneration with RPE irregularities visible in the native red free (513 nm, not shown), infrared (830 nm) and autofluorescence (AF) modes (Fig. 3).

SD-OCT imaging revealed a complete lack of ROS while the ONL appeared only marginally thinner compared to wt retina. Accordingly, central retinal thickness in *Rho*^{-/-} mice was reduced to $193 \pm 2 \mu\text{m}$. The entire retina showed absence of ROS, while the outer limiting membrane seemed not to be disturbed and could be distinguished from the RPE signal by a distance roughly equivalent to the extent of inner segment remnants (Fig. 3). Likewise, histological sections show a lack of ROS, while the outer nuclear layer appears essentially intact with about 6-8 rows of nuclei (Fig. 3).

Rpe65^{-/-}

Mutations in the gene encoding *RPE65* causes LCA2, a major form of Leber's congenital amaurosis²⁵⁻²⁷, which is targeted in current clinical gene therapy trials.^{28, 29} The protein RPE65 is expressed in the RPE, where it plays a pivotal role in maintaining normal vision by regenerating the visual pigment rhodopsin.¹⁶ In *Rpe65*^{-/-} mice, the blocked visual cycle causes accumulation of retinyl esters in RPE cells, where they form large lipid droplets (Fig. 4A). Cone photoreceptors degenerate rapidly, while the remaining rods are the exclusive source of electrophysiologic response and start to degenerate slowly only after approximately 6 months of age.^{30, 31} En-face imaging at PM11 showed a characteristic pattern of hyperfluorescent flecks in the AF mode, which may again indicate metabolic remnants of photoreceptor outer segments (Fig. 4). Image resolution of SD-OCT in *Rpe65*^{-/-} mice was currently not sufficient to resolve intracellular lipid accumulations *in vivo*. However, cross sectional images revealed a reduction of ONL size resulting in decreased total central retinal thickness of $193 \pm 2 \mu\text{m}$, which is more than the expected roughly 10% reduction with age, and would be consistent with the observed AF.³² Laminar organization in *Rpe65*^{-/-} mice was not as clearly delineated, which might be due to a generalized reaction to the ongoing photoreceptor loss. Indeed, it has been shown that the genetic response in *Rpe65*^{-/-} mice includes modified expression of cytoskeletal elements and components of the extracellular matrix.³³ Such a global response might reasonably affect the optical characteristics of the retinal sublayers.

rd1

The autosomal recessive retinal degeneration in the *rd1* mouse is caused by a loss-of-function mutation in the gene encoding the β subunit of the rod cGMP phosphodiesterase 6 (PDE6 β).³⁴ Numerous mutations in the catalytic domain of the human homolog, *PDE6 β* , have been found in human patients suffering from autosomal recessive retinitis pigmentosa (arRP).^{35, 36} Hence, the *rd1* mouse and other mouse strains harbouring PDE6 β mutant alleles are considered relevant animal models of Retinitis Pigmentosa.

First signs of rod photoreceptors cell loss in *rd1* mice become evident at about P10 when looking at TUNEL stainings while ONL reduction becomes evident at age P12-13 and the vast majority of rod photoreceptor nuclei have disappeared by P21.^{37, 38} En-face imaging in *rd1* mice at P11 failed to detect signs of retinal degeneration. In contrast, *rd1* mice at P28 revealed significant retinal degeneration both in the cSLO and SD-OCT imaging (Fig. 5). Cross sectional images showed an intact inner retina, while the inner nuclear layer seemed to border the RPE almost directly with outer plexiform/nuclear layers and photoreceptor segments virtually non existent. While central retinal thickness in *rd1* at P28 showed a marked reduction to only $145 \pm 5 \mu\text{m}$, *rd1* mice at P11 featured a central retinal thickness at $246 \pm 2 \mu\text{m}$. The rapid progression of retinal degeneration in the *rd1* model has been analyzed before using a custom made SD-OCT device.⁵ However, this study adds to the published data by presenting the onset of retinal degeneration in this model and resolving the retinal architecture prior to photoreceptor cell death (P11).

SD-OCT vs. Histology

Direct comparison of inner and outer retinal thickness assessed either by SD-OCT or histomorphometrical analysis revealed an excellent correlation (Fig. 6A-D). Consistent with existing data based largely on histological assessment,^{15, 31, 37} SD-OCT data revealed a significant reduction of the outer retinal thickness in *Rho*^{-/-}, *RPE65*^{-/-} and BALB/c mice when compared to C57/Bl6 animals (Fig 6A). Likewise, timeline analysis of C3H wild-type vs. C3H rd1/rd1 mice at P11 and P28 showed significant changes only in the rd1 mice at P28 (Fig. 6B). Traditional histo-morphometric assessment mirrored the findings gained by SD-OCT data analysis (Fig. 6C) and direct comparison between the two data sets revealed excellent correlation coefficients for the whole retinal thickness ($R^2 = 0.897$) and outer retinal thickness respectively ($R^2 = 0.978$). When analyzing the two data sets stemming from different protocols for histology separately (data not shown), the protocol using overnight fixation (*Rho*^{-/-}, *RPE65*^{-/-}, BALB/c and C57/Bl6) featured the lower correlation coefficient ($r^2 = 0.802$) compared to the protocol using direct embedding, freezing and sectioning (C3H wild-type and C3H rd1/rd1 mice at P11 and P28: $r^2 = 0.954$). This might arguably be due to differential shrinkage during processing for histology and highlights the benefit of non invasive assessment of retinal thickness by SD-OCT.

Discussion

OCT has emerged as a valuable tool to analyze and monitor structural changes in the retina. While non-invasive in character, the resolution of cross sectional images obtained by third generation OCT begins to approach the level of low power micrographs gained from light microscopy. This allows for detailed *in vivo* structural analysis of retinal disorders and bears importance for a refined genotype/phenotype correlation in clinical setting as well as for non-invasive longitudinal studies on animal models that mimic respective disease characteristics. Indeed, various prototype OCT setups have been reported to allow non invasive, high resolution imaging of rodent retina.^{6, 7} Srinivasan et al. used a custom build OCT setup to scan a normal C57/Bl6 mouse and to analyze the retinal thickness in a virtual cross section obtained from a Long-Evans rat. Ruggeri et al. demonstrated non invasive retinal imaging e. g. in a mouse model of retinal degeneration (*Rho*^{-/-}) using an experimental OCT system. Both studies compare single virtual cross sections to conventional histology without quantification, while others have used custom OCT systems to monitor the dynamic changes of natural disease progression in respective animal models.^{2, 3, 5} Yet, the potential to evaluate therapeutic and/or adverse effects of experimental interventions in time course experiments might prove to be even more important. Consequently, comparability between individual preclinical studies and preferably also between the preclinical and clinical setting will be of eminent importance. This is a challenge best addressed using commercially available equipment already approved for clinical use. In this study, we present data from frequently used wild type mice and models of hereditary retinal degeneration using a commercially available third generation OCT device approved for clinical diagnostic use.

The identity of signal composition in linear A- and two-dimensional B-scans acquired by OCT imaging has been subject to debate. However, it is generally thought^{1, 39, 40} that -from vitreous to sclera- the first thin dark band resembles the nerve fiber layer (NFL) followed by the lighter ganglion cell layer (GCL). The consecutive thick band of higher intensity illustrates the inner plexiform layer (IPL), approximated by a light band, the inner nuclear layer (INL). The OPL appears as a thin stripe with signal intensity comparable to that of the IPL. The outer retina is composed of a thick light band, the ONL with similar signal intensity as in the INL. Apparently, both nuclear layers share the low signal intensity because they scatter and/or reflect light to a much lesser extent than both plexiform layers, which appear much darker. Located just distal to the ONL is the external limiting membrane (ELM), which is formed by adherence junctions

between apical villi of Müller glia cells and distal photoreceptor cell bodies. Photoreceptor inner segments appear with the same signal intensity as the cell bodies, whereas the IOS generates a strong signal possibly due to the high mitochondrial content in the ellipsoid region of the outer segment³⁹. The photoreceptor outer segments are visible between the IOS border and the equally strong signal of the RPE/Choriocapillary complex (RPE/ChC). In pigmented retinæ, choroidal structures can not reliably be detected, as light is almost completely scattered and/or reflected as it travels through the more proximal layers. In non-pigmented retinæ, the signal composition is considerably different distal of the ELM (Fig. 2), which argues for scatter/reflection due to melanin granules to play the main part in this phenomenon.

Earlier work exploring the correlation between OCT and histology in mice was mainly performed using second generation time domain² or custom made high resolution OCT devices⁵ and reported a substantial overestimation of retinal thickness by OCT.³ In this study we used a commercially available SD-OCT device and obtained high overall correlation of total retinal thickness ($R^2 = 0.897$) with only minor overestimation (2.5%) of retinal thickness by OCT. Interestingly, correlation of outer retinal thickness representing the photoreceptor cell layer separately showed an even higher Pearson's correlation coefficient ($R^2 = 0.978$, Fig. 6D). Overall, the results obtained *in vivo* are in good agreement with earlier studies on the three different animal models for retinal degeneration.^{15, 31, 37}

Although conventional histology features higher resolution, OCT delivers the advantage of producing morphological data undistorted by handling, fixating and staining procedures, while being co-registered with en face topological information. Latter aspect is particularly useful when assessing retinal degeneration types that do not show a uniform progression in the entire retina. OCT based screening may also be useful for breeding purposes since it enables a non-invasive assessment of retinal health in mouse strains that are prone to develop sporadic retinal degeneration.

In analogy to electroretinography, where use of identical hardware in the clinical setting and laboratory led to new insights³¹, the application of a clinically approved OCT device for animal studies bears the potential to translate insights from bench to bedside in an efficient and timely manner. Here, we present evidence on the efficacy of a commercially available SD-OCT in small animal retinal imaging and provide *in vivo* structural data on mouse models of retinal degeneration. This should facilitate further studies on dynamic changes of retinal structure through the natural course of disease and help to monitor putative therapeutic effects of novel interventional strategies.

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Figure 1.

Retinal SLO imaging and OCT in C57BL/6 mice (PW4) with a regular retinal structure. (A)-(D): Representative example of en-face imaging using cSLO. (A) native infrared (830 nm), (B) red free (513 nm), and (C) autofluorescence (AF) mode. (D) Fluorescein angiography (FLA) confirming an intact retinal vasculature (arrowhead = artery, arrow = vein). (E)-(H): Corresponding OCT data. (E) Fundus picture with indicated orientation of cross-sectional SD-OCT scans. (F) Corresponding B-Scan at the optic nerve head displaying a Bergmeister's papilla characterized by a structural remnant of the developmental hyaloid vascular system (asterisk) including blow-up section. (G) Light microscopic data of an age-matched C57BL/6 animal. (H) Representative B-Scan with retinal layers labeled. GC/IPL: Ganglion cell / Inner plexiform layer, INL: Inner nuclear layer, OPL: Outer plexiform layer, ONL: Outer nuclear layer, OLM: Outer limiting membrane, I/OS: Inner-/outer segment border, RPE/CC: RPE/Choriocapillary complex.

Figure 2.

Retinal SLO imaging and OCT in BALB/c mice (PW10). Typical results of cSLO en-face imaging in the presence of non-pigmented retinal structures in (A) native infrared (830 nm), (B) red free (513 nm), and (C) autofluorescence (AF) modes. (D) Fluorescein angiography (FLA) in BALB/c mice displayed both retinal as well as choroidal vasculature (asterisk), as lack of pigment allows light at $\lambda = 488$ nm to better penetrate the RPE/Choriocapillary complex. (E) Fundus image with indicated orientation of (F/H) corresponding B-Scans. (G) Representative histological data of an age-matched BALB/c mouse. (H) Virtual cross section with designation of the different retinal layers. Note the altered signal composition in the outer retina due to lack of pigmentation in BALB/c mice ((F) blow-up). There are four highly reflective layers (HRLs) possibly demarcating the inner/outer segment border (IOS) and RPE, the non pigmented choriocapillary and choroidal structures. GC/IPL: Ganglion cell / Inner plexiform layer, INL: Inner nuclear layer, OPL: Outer plexiform layer, ONL: Outer nuclear layer, OLM: Outer limiting membrane, I/OS: Inner-/outer segment border, RPE/CC: RPE/Choriocapillary complex, Cho: Choroid.

Figure 3.

Retinal degeneration and RPE irregularities in the *rho*^{-/-} mouse model (PW4). (A) Native infrared (830 nm) and (B) autofluorescence (AF) mode cSLO data. (C) SD-OCT section and blow-up, revealing a complete lack of rod outer segments (ROS) together with an apparent thinning of the outer nuclear layer (ONL). (D) *Ex vivo* histology for comparison, confirming the observed reduction in ONL thickness and the lack of ROS. GC/IPL: Ganglion cell / Inner plexiform layer, INL: Inner nuclear layer, OPL: Outer plexiform layer, OLM: Outer limiting membrane, IS: Photoreceptor inner segments, RPE/CC: RPE/Choriocapillary complex.

Figure 4.

Retinal degeneration and RPE irregularities in the *Rpe65*^{-/-} mouse model (PM11). (A) Histology showing lipid droplets from stored retinyl esters (arrows) in the RPE, together with a reduced retinal thickness. (B) Spots of hyperfluorescence in the AF mode, suggesting the presence of photoreceptor debris. (C) OCT cross sectional image confirming a reduction of ONL thickness, while laminar organization (blow-up) was not as clearly delineated as in wt mice. GC/IPL: Ganglion cell / Inner plexiform layer, INL: Inner nuclear layer, OPL: Outer plexiform layer, ONL: Outer nuclear layer, OLM: Outer limiting membrane, I/OS: Inner-/outer segment border, RPE/CC: RPE/Choriocapillary complex.

Figure 5.

En-face and SD-OCT imaging in *rd1* and corresponding wild type control mice at P11 and P28. At P11, no signs of retinal degeneration were evident in (A/D) cSLO or (B/E) SD-OCT imaging. (C/F) Histology confirmed normal outer nuclear layer thickness. In contrast, *rd1* mice at P28 revealed significant retinal degeneration (G). This pattern was also evident in the SD-OCT cross sectional images (H) and corresponding histology (I), where inner retinal layers appeared intact, while the inner nuclear layer seemed to border the RPE almost directly. Outer plexiform/nuclear layers and photoreceptor segments were virtually non-existent. (J-L) Wild type controls at P28 showed no signs of retinal degeneration. All scale bars = 100μm.

Figure 6.

Evaluation of inner and outer retinal thickness in wildtype mice and animal models of hereditary retinal degeneration using either SD-OCT *in vivo* imaging or conventional morphometric assessment by histology. (A-C) Bar graphs indicate inner (dark) and outer (light) retinal thickness. (A) Note statistically significant reduction of outer but not inner retinal thickness in BALB/c, *Rho*^{-/-} and *RPE65*^{-/-} compared to C57/BL6 mice. (B) There is no statistical significant difference between *rd1* and *wt* mice at P11. While outer retina remained unchanged in the *wt* mice at P28, there is complete loss of outer retina in the age matched *rd1* mice. (C) Conventional morphometric assessment of retinal thickness in histological sections with similar changes as reported by SD-OCT (A-B). Significance was calculated using Student's T-test. Abbreviations: ns = not significant ($p > 0.01$), * = $p \leq 0.01$, ** = $p \leq 0.001$, *** = $p \leq 0.0001$. (D) Scatter plot shows the correlation of histological vs. OCT data on total (dots) and outer retinal thickness (error bars only) from C57/BL6, BALB/c, *Rho*^{-/-}, *RPE65*^{-/-}, *rd1*-wt P11, *rd1* P11, *rd1*-wt P28 and *rd1* P28 mice. Pearson's correlation coefficients (r^2) are displayed for total retinal thickness ($y = 0.975x$) and outer retinal thickness ($y = 1.018x$) separately. All data are reported as mean values $\pm$ standard deviation (error bars).