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Interpretation of OCT and OCTA images from a histological approach: clinical and experimental implications.

Nicolás Cuenca, Isabel Ortuño-Lizarán, Xavier Sánchez-Sáez, Oksana Kutsyr, Henar Albertos-Arranz, Laura Fernández-Sánchez, Natalia Martínez-Gil, Agustina Noailles, José Antonio López-Garrido, Maribel López-Gálvez, Pedro Lax, Victoria Maneu, Isabel Pinilla

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Title: Interpretation of OCT and OCTA images from a histological approach: clinical and experimental implications.

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Abstract:

Optical coherence tomography (OCT) and OCT angiography (OCTA) have been a technological breakthrough in the diagnosis, treatment, and follow-up of many retinal diseases, thanks to its resolution and its ability to inform of the retinal state in seconds, which gives relevant information about retinal degeneration.

In this review, we present an immunohistochemical description of the human and mice retina and we correlate it with the OCT bands in health and pathological conditions. Here, we propose an interpretation of the four outer hyperreflective OCT bands with a correspondence to retinal histology: the first and innermost band as the external limiting membrane (ELM), the second band as the cone ellipsoid zone (EZ), the third band as the outer segment tips phagocytosed by the pigment epithelium (PhaZ), and the fourth band as the mitochondria in the basal portion of the RPE (RPEmitZ). The integrity of these bands would reflect the health of photoreceptors and retinal pigment epithelium.

Moreover, we describe how the vascular plexuses vary in different regions of the healthy human and mice retina, using OCTA and immunohistochemistry. In humans, four, three, two or one plexuses can be observed depending on the distance from the fovea. Also, specific structures such as vascular loops in the intermediate capillary plexus, or spider-like structures of interconnected capillaries in the deep capillary plexus are found. In mice, three vascular plexuses occupy the whole retina, except in the most peripheral retina where only two plexuses are found. These morphological issues should be considered when assessing a pathology, as some retinal diseases are associated with structural changes in blood vessels.

Therefore, the analysis of OCT bands and OCTA vascular plexuses may be complementary for the diagnosis and prognosis of retinal degenerative processes, useful to assess therapeutic approaches, and it is usually correlated to visual acuity.

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Keywords: OCT, OCTA, Retina, Animal models, Clinical implication, Pathology, Immunohistochemistry, Human fovea, Retinal human vascularization.

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List of abbreviations:

AMD, age-related macular degeneration; AOOCT, adaptative-optics OCT; AOSLO, adaptive optics scanning laser ophthalmoscope; COST, cone outer segment tip; DCP, deep capillary plexus; ELM, external limiting membrane; ERG, electroretinogram; FAZ, foveal avascular zone; GA, geographic atrophy; GCL, ganglion cell layer; HFL, Henle

fiber layer; ICP, intermediate capillary plexus; ILM, inner limiting membrane; INL, inner nuclear layer; IPL, inner plexiform layer; IS, inner segment; EZ, ellipsoids zone; MNU, N-methyl-N-nitrosourea; OCT, optical coherence tomography; OCTA, OCT angiography; ONL, outer nuclear layer; OPL, outer plexiform layer; OS, outer segment; PhaZ, phagosomes zone; RCS, Royal College of Surgeons rats; RD, retinal detachment; RNFL, retinal nerve fiber layer; RP, retinitis pigmentosa; RPCN, radial peripapillary capillary network; RPE, retinal pigment epithelium; RPEmitZ, retinal pigment epithelium mitochondria zone; SD-OCT, spectral-domain OCT; SS-OCT, swept-source OCT; SVP, superficial vascular plexus; TD-OCT, time-domain OCT; VA, visual acuity.

1. Introduction

Since its development in 1991 (Huang et al., 1991), optical coherence tomography (OCT) has brought a revolution to the diagnosis and treatment of human retinal diseases, as well as to the study of diseases in animal models of retinal degeneration. For a long time, ophthalmological diagnosis relied on the observation of the eye fundus with biomicroscopy, ophthalmoscopy, and fluorescein or indocyanine green angiographies, but it was impossible to visualize the structure of the retinal layers or determine the health of the different cell types. Newer technologies like fluorescence

lifetime imaging ophthalmoscopy (FLIO) (Dysli et al., 2017), which measures the time that endogenous retinal fluorophores remain, have emerged and may provide alternative information about retinal metabolic processes and their specific changes in retinal diseases. Also, since the development of OCT, it has become possible to investigate the retinal health *in vivo* at a level closer to histology and to identify alterations in retinal morphology during pathological states. Thus, to evaluate and interpret the cellular changes caused by different diseases, clinicians and researchers must have detailed knowledge of the retinal structure, the regular arrangement of the different cell types that constitute each layer, and the synaptic connections in a healthy retina. This knowledge would facilitate the interpretation of OCT images by studying retinal layers thickness, band disruptions, or generation of new structures, resulting from cell changes associated with a pathological process.

OCT has also become an important tool to analyze the structure of the retina in animal studies. Quantification of retinal thickness in different species and animal models is a useful approach to study retinal degeneration and the neuroprotective effects of several compounds without sacrificing the animals. In this case, the exact correlation of

the hyper and hyporeflective bands seen in an OCT profile with the retinal histology is also essential for the accurate interpretation of the images.

Research and improvement of the OCT techniques allowed the development of OCT angiography (OCTA) (Makita et al., 2006) to visualize retinal blood vessels in patients or animal models. When using this technique in the diagnosis or evaluation of pathology, it is important to have proper knowledge about the histology of retinal vasculature in health and disease.

In this review, we aim to correlate retinal histology and morphology with OCT and OCTA images and to describe and analyze the changes observed during retinal degeneration using both OCT and histological techniques.

2. The use of OCT and OCTA in the study of the human retina

2.1. OCT technology

OCT is a non-invasive imaging technique based on low coherence interferometry that makes it possible to view *in vivo* high-resolution cross-sectional images of the retina (Sakata et al., 2009). In the ophthalmology field, OCT is widely used to detect early stages of diseases and track the progression and results after treatment of retinal

pathologies, such as age-related macular degeneration (AMD), diabetic retinopathy and glaucoma (van Velthoven et al., 2007). In this system, light from a source of low coherence is divided by a beam splitter into two paths: one directed towards a reference mirror and the other towards the retina. The light waves that are backscattered by the retina converge with the returning light from the optical reference mirror and produce an interference pattern, which is recorded by the detector. This interference pattern, composed by the reflectivity of each retinal layer, offers information about the depth of the tissue (Adhi and Duker, 2013; Popescu et al., 2011; van Velthoven et al., 2007). Based on this technology, time-domain OCT (TD-OCT) assesses the reflectivity of the retinal layers in depth through a moving mirror using a photodetector. On the other hand, spectral-Domain OCT (SD-OCT), which uses a broadband light source; and Swept-Source OCT (SS-OCT), which employs a tunable swept laser, are both constituted by a fixed mirror that enables the images acquisition through an interference pattern (Gabriele et al., 2011; Miller et al., 2017).

OCT systems generate cross-sectional images (B-scans) of the retina that shows the reflectivity information captured in depth by means of whiter or darker bands. They also permit to obtain *en-face* images that are formed by C-scans measured on the XY

or coronal plane, with an axial resolution of approximately 10 μm (van Velthoven et al., 2007). This method allows studying a specific area or layer "*en-face*" instead of only visualizing the depth in cross-sectional OCT images (Kiernan et al., 2012) or the traditional fundus by ophthalmoscopy.

2.2. Histology of the human macula

In humans, other primates and raptors, there is a specially organized region in the central retina called the fovea. It is located temporally to the optic nerve head and has an approximate size of 1.5 mm. *In vivo*, the fovea can be distinguished by its yellow coloration, provided by the lutein pigment, which is thought to act as a protector against blue light damage. The total yellow-colored region is called *macula lutea* and it comprises the perifoveal, parafoveal and foveal areas. Within the fovea, other three structures can be differentiated: the foveal avascular zone, the foveola, and the umbo. The sizes and locations of each area are schematically shown in a flat retina (Fig.1 A-C), in a histological section (Fig. 1D) and correlated in an OCT image (Fig. 1E)

Since its first description by Santiago Ramón y Cajal (Ramón y Cajal, 1893), the retina has been classically described as a tissue containing ten layers, named from the inner to the outer layer as follows: inner limiting membrane (ILM), retinal nerve fiber layer

(RNFL), ganglion cell layer (GCL), inner plexiform layer (IPL), inner nuclear layer (INL), outer plexiform layer (OPL), outer nuclear layer (ONL), external limiting membrane (ELM), photoreceptor segments (PS), and retinal pigment epithelium (RPE). Although this is the structure generally followed in the retina, the fovea is an anatomically specialized region lacking most of these layers.

The umbo is composed only of tightly and densely packed cones (Fig. 2A, B), containing more than half of all the retinal cones. Close to it, at the foveola and the foveal slope (or *clivus*), rods start to appear (Fig. 2A, B). Both the foveola and the foveal slope lack blood vessels and, together, they compose the foveal avascular zone (FAZ) (Fig. 1C-E). Consequently, the fovea is the retinal area with the highest visual acuity (VA), which allows detection of the finest details, and it is responsible for our ability to read, distinguish colors and for our spatial resolution capacity.

Adjacent to the foveola, an additional layer appears: the Henle fiber layer (HFL), composed by cone, rod and Müller processes (Fig. 2A, B). In this region, central cones and rods have long axons (2 to 400 μm) that make synaptic contacts with bipolar and horizontal cells located far from the foveola. These long axons are always surrounded by equally long processes of Müller cells, which change their usual columnar

morphology and become elongated and oblique (Fig. 2B) constituting, together with cone and rod axons, the HFL. In a histological section of the fovea, the HFL can be differentially observed from the ONL (Fig. 2A). The diagram in Fig. 2B shows the morphological features of photoreceptors and Müller cells in the central retina, and also how Müller cell processes travel together with photoreceptor axons generating the HFL. OCT measurement of HFL thickness could reflect retinal abnormalities associated with cones, rods and Müller cells.

Fig. 2C displays the different parts of a typical cone: the outer segment (OS), where membranous discs containing photopigments are located; the inner segment (IS), where the ellipsoid, full of mitochondria and responsible for generating the cell energy, and the myoid can be identified; the cell body containing the nucleus and located in the ONL; the axon; and the pedicle, where synapses are mainly established, in the OPL. The various parts of a cone differ morphologically at different retinal eccentricities. At the umbo (Fig. 2D), cones have very thin and long inner and outer segments and a short axon that usually connects with midget bipolar cells. At the foveola and fovea (Fig. 2E), the cone inner and outer segments are still thin, but a bit thicker than at the umbo, and rods start to appear. Axons of both photoreceptors are very long and travel

obliquely, contributing to the HFL. At locations further from the fovea, inner and outer segments of cones become shorter and thicker, and their axons are shortened. At the peripheral retina (Fig. 2F) their axons are straight and photoreceptors connect to their second-order neurons directly distal to them. A schematic representation of the gradual changes in cone and rod morphology is displayed in Fig. 2G, showing the shortening of cone and rod axons towards the periphery. Mosaics of cones and rods at different retinal eccentricities are visible in *en-face* adaptive optics OCT (AOOCT) images and they show different cell thickness and distribution in the different locations (Dubra et al., 2011; Merino et al., 2011; Wells-Gray et al., 2016).

2.3. Correlation of OCT bands and histology in the human retina

Knowledge of retinal and foveal histology is essential to understanding OCT images, as the retinal layers identified in histological sections can be correlated to the hyper and hyporeflective bands of OCT images. Since the development of OCT imaging, many authors have proposed their interpretation of the correlation between OCT bands and histological layers. Some of the OCT bands/histology correlations are widely accepted, while others still generate lively discussions and controversy. In Fig. 3, the most commonly accepted correlations between histological and OCT layers are shown.

2.3.1. Nerve fiber layer and ganglion cell layer

The innermost hyperreflective band is considered to be the RNFL, composed of ganglion cell axons that travel in bundles until the optic nerve, through which they leave the retina and transmit the visual information to the visual centers of the brain. Nevertheless, the RNFL is not constant throughout the entire retina and its thickness varies: it only has the structure of a layer itself in the central-nasal region, around the optic nerve head, where axon density is higher (Fig. 4). As a result, this hyperreflective band would only correspond to the RNFL in those OCT images taken nasally from the fovea, between the fovea and the optic nerve (Fig. 4E, G). At temporal locations from the fovea, especially at the temporal raphe, axons are not arranged with such a high density, and they usually run among ganglion cells, not constituting an independent layer (Fig. 4D, F). While in the nasal region the RNFL may measure up to 65-70 μm , bundles in the temporal region near the temporal raphe measure a maximum of 5-7 μm , and they are not arranged as a continuous layer. As a result, it would be more appropriate to identify and measure the RNFL in OCT images taken at nasal locations. At temporal locations, the thin hyperreflective band (Fig. 4C) observed would probably correspond to the ILM, the interconnected Müller end-feet and/or the astrocytes. The

presence of these cells and structures should be also taken into consideration when measuring RNFL at the nasal regions, as it would also contribute to the generation of this OCT band. Advances in the visualization of this band, thanks to the AOOCT technology, already allow to identify and measure bundles of axons in the RNFL (Kocaoglu et al., 2011).

The next band observed in OCT is hyporeflective and is considered to correspond to the GCL, which is composed of the nuclei of ganglion and displaced amacrine cells. At the umbo, this layer is absent, as shown in Fig. 4A, B. At the foveola, one first row of ganglion cells appears, and its density increases until it is composed of 5 or 6 rows of nuclei at 1.25 mm from the fovea (Fig. 4). After this point, the number of ganglion cells decreases again, having only one row from 4 mm onwards. This variation in the number of rows is reflected in the varying thickness of this layer in both histological sections and OCT images. Thus, when comparing GCL thickness in OCT images, it is essential to make sure that measurements are taken at the same location in all subjects studied, as small variations in eccentricity may result in large variations in GCL thickness. Also, some ganglion cell bodies are located at the INL. Notice that the

proportion of these displaced ganglion cells is very high at the foveola, as compared to more peripheral locations (Fig. 4B).

Although traditional OCT systems are only able to show ganglion cells as a hyporeflective band, high-resolution AOOCT technology allows imaging and quantifying individual ganglion cells in the living eye, in a resolution close to histology (Liu et al., 2017). Advantages in imaging systems to observe nerve bundles and ganglion cells will be extremely useful for diagnosis and follow up of inner retinal pathologies, such as glaucoma or other optic neuropathies.

2.3.2. Inner plexiform layer and inner nuclear layer

The following hyperreflective band in OCT is thought to be originated by the IPL (Fig. 3, Fig. 4). The IPL is formed by the synaptic connections between ganglion cells, bipolar cells, and amacrine cells, and it is subdivided into five strata (S1-S5), corresponding to the OFF (S1-S2) and ON (S3-S5) pathways. The synaptic structures composing the IPL contain many membranes, a high density of vesicles, and lots of mitochondria. All these components may be the origin of the hyperreflectivity seen in OCT. As seen in Fig. 4B, the thickness of IPL is very thin at the fovea and gradually becomes thicker.

The maximum thickness of the IPL is reached at the point of maximum thickness of the GCL, and it decreases again towards more peripheral regions.

Moving towards the outer retina, the next layer is the INL, which is the source of a hyporeflective band at the OCT (Fig. 3, Fig. 4B-C). The INL comprises the cell bodies of amacrine, bipolar and horizontal cells, as well as some displaced ganglion cells. Müller cell bodies are also located in this layer, and from here, they extend two processes: one to the inner retina that makes up the ILM and another to the outer retina, which forms the ELM (Fig. 3).

2.3.3. Outer plexiform layer, Henle fiber layer, and outer nuclear layer

Synaptic contacts between horizontal cells, bipolar cells, and photoreceptors compose the OPL: a thin layer (approximately between 6-8 μm) seen as a hyperreflective band in OCT images (Fig. 3). As in the IPL, the synaptic structures with many folded membranes and the large mitochondria located at cone pedicles and rod spherules may be responsible for the hyperreflectivity of the band. Due to its thin size, a thin hyperreflective band is expected in OCT. Nevertheless, in OCT images at the fovea, it sometimes appears as a thicker band where an additional layer is included: the HFL (Fig. 3). Depending on the eye orientation when taking OCT images, and thus on the

light angle incidence, this HFL may appear either hyporeflective, hyperreflective or both. When this layer is imaged as hyperreflective, its signal is added to the OPL band, which makes it seem thicker than it is. Thus, special care should be taken when measuring OPL thickness, and images from different patients must be taken with the same eye orientation. Otherwise, measurement differences may only reflect different image acquisition parameters and different contributions of the HFL to OPL thickness.

When this band appears as hyporeflective, the HFL signal would be added to the ONL. The ONL represents the cell bodies of photoreceptors, which are only cones at the umbo. Notice that, in all cases, nuclear layers correspond to hyporeflective bands. The high density of cones in the foveal pit, with up to five or six rows, generates the triangle-like shape of the ONL at this location, while it soon decreases to two or three rows of both cones and rods. In the "triangle-shaped" region of the foveal pit, the entire hyporeflective signal corresponds to the cone nuclei (the ONL), but at the rest of the fovea and foveola, rod nuclei appear, and part of the signal may also correspond to hyporeflective HFL. Therefore, in order to compare subjects, it would be better to measure ONL only at the foveal pit or, if measured at the foveola, consider the HFL contribution and be sure to take all images with the same eye orientation. Another

possible solution would be to measure the complex OPL+HFL+ONL altogether as a single unit, to avoid segmentation errors derived from differential HFL contributions.

2.3.4. Outer hyperreflective bands

Concerning the four outer hyperreflective bands, a consensus has not been reached in terms of their nomenclature and interpretation. One of the best known anatomical correlation of these bands was proposed by Spaide and Curcio in 2011 (Spaide and Curcio, 2011), based on a literature review and subsequent interpretation. They interpreted the first hyperreflective band as the ELM, the second as the ellipsoid zone of the photoreceptor inner segments, the third as the contact cylinder of the RPE around the photoreceptor outer segments, and the fourth as the RPE. Nevertheless, this nomenclature has not been exempted from debate, and other different interpretations have been proposed to date. In 2014, at a consensus meeting, an attempt was made at reaching an agreement on the naming of the OCT bands, and it was decided to interpret the third hyperreflective band as the RPE interdigitations (interdigitation zone; IZ) (Staurenghi et al., 2014). Also in 2014, using AOOCT, Jonnal *et al.* provided evidence supporting the hypothesis that the structure responsible for hyperreflectivity at band 2 was the inner segment/outer segment union (IS/OS) (Jonnal

et al., 2014), an interpretation also used in the early 2000s (Drexler et al., 2001). In this and other research using AOOCT, they also propose the interpretation of band 3 as the cone outer segment tips (COST) (Jonnal et al., 2017; Jonnal et al., 2014; Zhang et al., 2006). Finally, in a recent study comparing histological microscopy images and OCT images, we proposed the RPE phagosomes as the structure generating hyperreflectivity at band 3 (Cuenca et al., 2018). In this section, we will provide a description and interpretation of the four outer OCT bands from a histology-based point of view.

2.3.4.1. First outer hyperreflective band

The first outer hyperreflective band is the thinnest and the least intense of the four outer OCT bands. It is generated by a thin structure called the ELM, composed of the outer Müller cell terminal processes and microvilli (Fig. 5). As can be seen in Fig. 5A-C, Müller cell apical processes extend towards the outer retina, surrounding and surpassing photoreceptor cell bodies, and they reach the beginning of the photoreceptor myoids (arrowheads). At this point, where the Müller processes end, cellular junctions, such as *adherens* junctions and desmosomes, are formed between Müller cells and between Müller cells and photoreceptors. As a result, the Müller apical processes are connected by continuous heterotypic *adherens* junctions to other Müller

apical processes and to photoreceptors, generating the so-called ELM. In Fig. 5A-D, Müller cells are stained red, and their apical end processes can be observed forming an almost continuous layer at the level of the photoreceptor nuclei/myoid junction: the ELM.

Although the ELM has not traditionally been considered as a part of the blood-retinal barrier, it plays an important role in maintaining the retinal structure, metabolism, and homeostasis. Alterations in Müller cells during different retinal pathologies may affect the ELM state and involve hyperreflectivity changes in the first outer OCT band.

Some diseases are associated with Müller cell gliosis involving hypertrophy, proliferation or migration. In early-onset Stargardt disease, a transitory thickening of the ELM band can be observed in OCT, which is thought to be associated with the gliotic response of Müller cells (Bringmann et al., 2006; Lee et al., 2014). A similar process occurs in retinal detachment (RD) when Müller cells become reactive as a response to the photoreceptor hypoxia generated by the separation to the RPE. After two days, they start to proliferate, show outgrowing cell processes, and increase the expression of gliosis markers like GFAP (Bringmann et al., 2006). These changes may be reflected as increased hyperreflectivity of the first outer OCT band and thus some

authors propose that they may contribute to the formation of outer retinal folds found during recovery after rhegmatogenous RD surgery (dell'Omo et al., 2012).

Other common alterations of Müller cells in pathological conditions are related to their ability to maintain retinal homeostasis where, among others, they are unable to keep the water equilibrium. Therefore, changes in the global osmotic gradient of the retina would promote water entrance and accumulation into Müller cells, contributing to the development of intracellular edema (Bringmann et al., 2006). In other cases, reactive Müller glia releases different factors like metalloproteinases that affect the endothelial tight junctions and provoke vascular leakage. The continued vascular leakage could accumulate and end up developing extracellular edema (Bringmann et al., 2006). Fluid-filled cysts and edema are common signs in diverse retinal pathologies such as diabetic macular edema, retinal vein occlusion or posterior uveitis (Catier et al., 2005; Daruich et al., 2018). In these diseases, the first outer band disruption or loss is sometimes observed in OCT in the regions associated with edema (Muftuoglu et al., 2017). Nevertheless, it might be that depending on the disease stage (early or advanced) and on the edema location within the retina (inner or outer), this band might not be affected.

Some other retinal pathologies such as macular hole, retinoschisis, CRALBP-mutation retinitis pigmentosa, or hepatic retinopathy have a great involvement of Müller cells in the physiopathology of the disease (Bringmann et al., 2006).

In most of the described pathologies, the recovery of the first outer retinal band after treatment is correlated with better VA recovery and, as more damaged is the band, worst VA recovery is expected (Muftuoglu et al., 2017; Padron-Perez et al., 2018). Nevertheless, the reconstitution of the band may not always involve a total recovery of the VA, as happens in rhegmatogenous RD (dell'Omo et al., 2015). A possible explanation for this fact is that even if the retinal structure is recovered, Müller cell function may be still partially impaired and affect photoreceptors' function. For example, as they are involved in 11-cis retinol recycling for cones, in neurotransmitters uptake or in glucose metabolism (Bringmann et al., 2006), a partial failure in some of these processes may affect the visual process and avoid the total recovery of the VA.

2.3.4.2. Second outer hyperreflective band

The interpretation of the second hyperreflective band has been a matter of greater controversy. The first interpretation, and currently the one most supported by AOOCT results, suggests that it is the IS/OS junction that generates the second hyperreflective

band (Jonnal et al., 2014). Other interpretations based on anatomical correlations suggested this second hyperreflective band represents the photoreceptor inner segment ellipsoids (EZ) (Spaide and Curcio, 2011; Staurenghi et al., 2014), a cellular compartment full of mitochondria. In Fig. 5, cone ellipsoids can be observed in blue, stained with cytochrome C to show mitochondria, while the IS/OS junction cannot be stained, as it is not a histological structure as such. Supporting the idea of ellipsoids as the source of band 2 are some studies that reveal mitochondria as the most light-scattering organelles (Wilson et al., 2007). It would then seem logical to argue that ellipsoids, a compartment full of the most light-scattering organelles with intricate, refolded membranes, could generate a hyperreflective band at OCT (Fig. 5C, D, E, F). On the contrary, some AOOCT studies suggest that the second band thickness and position (closer to band 3 than to band 1) are inconsistent with ellipsoids, which are histologically thicker and closer to the ELM. They thus propose that the only photoreceptor region that meets both requirements of the band measurements is the IS/OS junction (Jonnal et al., 2014).

Nevertheless, more recent histological correlations (Cuenca et al., 2018) and other studies evaluating this band in pathological states of human and non-human primates

again advocate for the ellipsoid interpretation. For example, in the mature phase of AMD, there is evidence that outer segments are lost while inner segments are maintained, which would make inner segment ellipsoids responsible for band 2 (Litts et al., 2018) as the loss of outer segments means there is no IS/OS junction that could generate hyperreflectivity. In addition, in late stages of AMD, outer retinal tubulation occurs, and photoreceptor mitochondria undergo processes of translocation towards the nuclei and fission. Some researches claim that the hyperreflectivity observed in the outer retinal tubulation regions is the result of mitochondria translocation in these areas, again supporting the mitochondria in ellipsoids as the source of reflectivity in the second band (Litts et al., 2015; Litts et al., 2017; Litts et al., 2018). In another study evaluating induced photoreceptor damage in primates, Walters *et al.* histologically identified a loss of outer segments in some retinal regions. In the same areas, the second band was still present, even when outer segments were lost and there was no IS/OS junction (Walters et al., 2019), supporting the ellipsoid interpretation of the hyperreflective band 2.

Considering this band as the EZ, changes in photoreceptor ellipsoids and thus in their mitochondria would be observed as changes in the second outer OCT band. As

mitochondria are responsible for generating cellular energy, their integrity is related to photoreceptor function and health. Mitochondrial loss or morphological variations would indicate an energetic failure of photoreceptors and may affect the visual function. An example of this is found in retinitis pigmentosa (RP). RP is usually characterized by a progressive loss of rods, followed by the subsequent degeneration of cones in advanced disease stages. As a result, degeneration starts and is predominant in the peripheral retina, affecting more central regions as the disease progresses (Milam et al., 1998). This fact can be observed in OCT, where the thickness of the second band is progressively reduced, or even lost, from the periphery towards the central retina as the disease advances (Mitamura et al., 2013). Analyzing the state of the second band (normal, abnormal, or absent) in RP, authors have been able to classify patients in different grades of disease and, in all cases, the presence of the band correlated with a preserved foveal function (Aizawa et al., 2009).

Besides, this band may be gradually recovered after treatment in different conditions. For example, after surgery in macular hole (Ooka et al., 2011), in resolved central serous chorioretinopathy (Nakamura et al., 2016), and in RD (dell'Omo et al., 2015), a progressive recovery of the second band is found with time. As mitochondria are able

to divide and generate new ones, they might explain the restoration of the second OCT band after treatment and, with it, the recovery of photoreceptors' health.

Finally, if the first band is interpreted as the ELM and the second band as the EZ, the hyporeflective band in between could be explained as the myoid, the photoreceptor compartment of the inner segment closest to the nuclei (Fig. 5E-F). Myoid components are mainly cytoplasm and cellular organelles, such as the endoplasmic reticulum and the Golgi apparatus, and it rarely has mitochondria. None of their components have a great ability to scatter light, which agrees with the representation of a hyporeflective band in the OCT.

2.3.4.3. Third outer hyperreflective band

The first description of this band was a "contact cylinder" of RPE around the photoreceptor outer segment (Spaide and Curcio, 2011), but this interpretation soon changed. Currently, the third band is mainly interpreted as RPE interdigitations (Staurenghi et al., 2014) or as COST (Jonnal et al., 2017; Jonnal et al., 2014). Also, another interpretation suggesting that the third band is generated by the phagosomes zone (PhaZ) inside the RPE was recently proposed (Cuenca et al., 2018).

Histologically, RPE interdigitations extend from the RPE cell all along the entire length of the outer segment, until the end of the ellipsoids at the IS/OS junction, as shown in Fig. 5C, F, G and H, where interdigitations are stained in red. Considering that OCT images show a hyporeflective band between hyperreflective bands 2 and 3, if band 3 is the interdigitation zone and band 2 is the IS/OS junction, there is no other photoreceptor structure between them that could generate the hyporeflective band. We, therefore, believe that "interdigitation zone" is not an accurate interpretation of the third band, and other proposals should be considered. Also, the mentioned hyporeflective band between the second and third outer bands would correspond to the photoreceptor outer segments (Fig. 5E-F).

A less accepted theory is that band 3 is generated from melanin in the RPE. But OCT studies performed in patients with albinism still reveal the four layers seen in normal OCT images (Wilk et al., 2017; Wilk et al., 2014), suggesting that melanin may not play a crucial role in the hyperreflective bands.

Using high-resolution AOOCT to analyze the properties of band 3, Jonnal *et al.* suggested that the cellular origin of the third band should be the same as for the second band. They propose outer segment tips as the structure within photoreceptors

that meets this criterion (Jonnal et al., 2017; Jonnal et al., 2014; Zhang et al., 2006).

Nevertheless, they also state that, based on their results, this band “may originate at the posterior tips of the OS, or originate more distally (in the RPE, for instance), but consist of light that is scattered from distal tissue and recoupled into the OS waveguide” (Jonnal et al., 2014).

Lastly, phagosomes have been also proposed to be the cellular structure that causes the hyperreflectivity of this band (Cuenca et al., 2018). Phagosomes can be observed in Fig. 6 as green or blue oval irregular structures inside the RPE cell (red). At the umbo, where only cones (green) are present, all phagosomes are formed from cone outer segment tips shedding (Fig. 6A, green). At the foveola, first rods (blue) emerge and few of the phagosomes belong to the rods shedding process (Fig. 6A, blue). When looking at locations gradually further from the umbo (parafovea, perifovea, periphery), the density of rods and rod phagosomes increases (Fig. 6B, C, blue). At the same time, cones are fewer and their phagosomes are less present in peripheral regions (Fig. 6B, C, green).

Phagosomes display an ultrastructure very similar to that of mitochondria, with a high density of non-parallel membranes, and are thus supposed to have similar light-

scattering properties. Their histological location would also fit with the location of the third outer hyperreflective band, and it is separated enough from the second band to provide a space for a hyporeflective band in between: the photoreceptor outer segments (Fig. 5E, F). They also meet some of the band criteria established by AOOCT: they are located at the same axial location as ellipsoids or IS/OS, and as COST, and they are inside the RPE, a possible origin of band 3 as stated by Jonnal *et al.* (Jonnal *et al.*, 2014).

In general, phagosomes are structures resultant from phagocytosis of outer segment tips by the RPE during the continuous photoreceptors disc shedding. In the fovea, where cones are as long as rods and both reach the RPE, this process consists of direct phagocytosis of the outer segment posterior tips, and phagosomes are directly formed at the RPE cytoplasm. Nevertheless, in the perifovea and periphery, where cones are shorter than rods, RPE interdigitations reach cone tips, enfold and transport them towards the RPE cytoplasm, where they will be digested. In this region, rods still reach RPE and their shedding process is direct phagocytosis, as in the fovea.

The interpretation of phagosomes as the third band is, in fact, very similar to the interpretation of this band as outer segment tips and both are involved in the

shedding process: phagosomes are the outer segment tips of cones and rods engulfed by the RPE (Fig. 6), even if they are still located nearer the photoreceptor (in the first steps of short cones shedding) than to the RPE. Both the early phagosomes and the ones completely phagocytized would origin the PhaZ band in OCT. In AOOCT studies of the shedding in the living human eye (Kocaoglu et al., 2016), researchers detect 745 cones while only 175 "COST events". If we interpret those signals as COST or ROST, every cone and every rod (not only 175) should have a tip: the outer end of the outer segment. On the contrary, if we interpret them as phagosomes, a hyperreflective signal will only be observed in those photoreceptors undertaking OS disc shedding. Considering this fact, the interpretation of these "COST events" is more probably to be the engulfed cone tip, in the formation of the early phagosomes.

This third band is also peculiar because it changes over time. Using scanning light ophthalmoscopy-OCT (SLO-OCT), Pircher *et al.* have described bright reflection spots within the band that move towards the RPE in the function of time. They interpret these reflections as outer segment abnormalities. Considering the phagosomes' interpretation, these bright spots could be phagosomes from shorter cones moving towards the RPE (Pircher et al., 2010). Kocaoglu *et al.* have also described variations in

the location and intensity of the third band over time, and they relate these changes to the temporal photoreceptor disc shedding process (Kocaoglu et al., 2016). In their study, the third band disappears for some minutes. When considering this band as COST, it is hard to figure out how to explain the disappearance and reappearance of the band since, even when the COST is phagocytized, photoreceptor would have always a tip. By contrast, if phagosomes were the source of the third band, band disappearance could be explained by phagosome digestion or maturation, with a subsequent change in structure and optic properties. In fact, this 3rd band may reflect the whole process of outer segment discs shedding, from the formation of the phagosomes near the outer segment tip, until their digestion in the RPE cytoplasm. Although being similar, the biological interpretation and the clinical implications of both names drastically change. When interpreting a pathology, if this 3rd band is considered as phagosomes, the disruption of the band would involve RPE affection and shedding impairment; while if being COST, it would involve a problem at the photoreceptor outer segments.

For example, in RP, the first histopathological change is the alteration of rod outer segments, affecting the shedding process and, in the end, the global integrity of

photoreceptors (energetic failure, ellipsoid degeneration, etc.) (Milam et al., 1998; Mitamura et al., 2013). This degeneration of outer segments would be first translated into a shortening of the outer segment band; followed by a progressive loss of the third band and, later, of the second band in OCT images (Mitamura et al., 2013; Sandberg et al., 2005). In this case, as the outer segments start to degenerate, their shedding process will be impaired, no phagosomes will be made, and thus, disruptions will be observed in the third outer hyperreflective band.

In other retinal pathologies such as RD or macular hole, one of the main alterations that occur is the physical separation of RPE and photoreceptors, which, among others, hampers the shedding process and the formation of phagosomes. This fact may be involved in the loss of the third OCT outer band. An interesting aspect of these diseases is that this band is usually progressively recovered after surgery (dell'Omo et al., 2015; Mitamura et al., 2013). Nevertheless, this recovery is gradual: it starts with the restoration of the first band, then the second band is reconstituted and, it is not until both are completely evident that the third band becomes discernable (dell'Omo et al., 2015; Mitamura et al., 2013). From a cellular interpretation, the recovery of the global integrity of photoreceptors (and thus of their inner and outer segments) would allow

restoring the shedding process and the formation of phagosomes, which may be responsible for the reappearance of the third outer band in OCT. In addition, this recovery would mean that interactions between RPE and photoreceptors are reestablished, being an indicator of photoreceptor-RPE health, and it is significantly correlated with an improvement of VA after treatment (Mitamura et al., 2013).

Some authors have also described that a new band appears between the third and fourth bands approximately from 4° eccentricity onwards, and they attribute it to the rod outer segment tips (ROST) (Liu et al., 2016; Srinivasan et al., 2008). Phagosomes of rods and cones inside the RPE will be always found at the same location but, further from the fovea where cones become shorter and separated from the RPE, a discontinuous line reflecting recently formed cone phagosomes close to cone outer segment tips may appear. This fact may be responsible for the duplicity of bands distal to band 4 found with some devices.

2.3.4.4. Fourth outer hyperreflective band

The origin of this band is widely thought to be the RPE or the RPE/Bruch's membrane complex. But the interpretation of this band as the whole RPE cell could not explain the hyporefective band between third and fourth bands. Considering the third band

either as interdigitations, phagosomes or COST, if band 4 is the entire RPE cell (which is adjacent to these three structures), what cellular origin could explain the hyporeflective band between them? In a recent study, a more localized origin of the fourth band was proposed: the basal mitochondria inside the RPE (RPEmitZ) (Cuenca et al., 2018). In Fig. 6D, a mitochondria-dense region in the basal portion of the RPE can be observed in blue, stained with cytochrome C, displaying the appearance of a continuous layer. As stated before, mitochondria are one of the most light-scattering organelles in the cell (Wilson et al., 2007), which suggests that this basal RPE region would be very light-scattering. The contribution of Bruch's membrane to this fourth band hyperreflectivity cannot be rejected, and it would probably be added to the mitochondria reflectivity to generate the band. If the fourth band contains basal mitochondria and Bruch's membrane and considering the third band as phagosomes or COST, the OCT hyporeflective band between them would correspond to the rest of RPE cell body: the central region, where the nucleus, cytoplasm, and melanosomes are located (Fig. 6 D). This interpretation is in accordance with most of the other hyporeflective bands, whose origins are mainly the cell bodies at the nuclear layers,

and with the limited effect of melanin in OCT bands, as revealed by studies in albino subjects (Wilk et al., 2017; Wilk et al., 2014).

The alteration of this band during a disease would involve the affection of the RPE cells. In these cases, RPE may lose or restructure their morphological features, suffer energetic failure, or have their physiological functions (like outer segment phagocytosis, recycling of visual pigments, or factors secretion) impaired. Also, it is one of the main photoreceptor support systems, RPE degeneration would lead to photoreceptor degeneration and vision impairment (Sparrow et al., 2010; Strauss, 2005). An example of this can be observed in geographic atrophy (GA), where histological studies reveal dramatic changes in RPE cells near and within the atrophic region. As they approach this region, some epithelial cells become dissociated and located over the basal laminar deposit, some undergo apoptosis death, and others seem to transdifferentiate into a phagocytic phenotype and migrate into the retina (Zanzottera et al., 2016). These RPE changes have been described in band 4 OCT images as hyperreflective material deposits, irregular elevation, thickening of the band or hyperreflective spikes (Fleckenstein et al., 2008). Besides, subsequent to RPE

degeneration, the first, second and third bands are also altered, indicating the impairment of photoreceptors.

In this section, we offer a broad explanation of the current controversy surrounding the four outer retinal bands and give our interpretations in the most histological-based way, summarized in Fig. 7. Nevertheless, it is still difficult to reach a consensus and we will probably only figure out the exact interpretation of these bands with the continuous development of image techniques and OCT devices, with higher resolution images and with continuous research on band changes in both healthy and pathological conditions.

2.4. Correlation of OCTA and histology in the human retina

Traditionally, retinal and choroidal blood vessels have been visualized *in vivo*, using techniques like fluorescein angiography and indocyanine green angiography. Fluorescein angiography has been the gold standard technique to visualize retinal vessels, but it has a limited resolution to show deeper plexus and choroids, which appear diffuse and shadowy (Spaide et al., 2015). More recently, thanks to the development and improvement of OCT technology, a new imaging technique to observe retinal vessels has emerged: OCTA (Makita et al., 2006). Other techniques, such

as adaptive optics scanning light ophthalmoscope fluorescein angiography (AOSLOFA) can also provide a good visualization of retinal capillary structures, but nowadays, they present more technological barriers for use in clinical practice. OCTA is a new, non-invasive imaging technique, capable of acquiring high-resolution images of the vascular layers of the retina and choriocapillaris based on blood flow movements (Kashani et al., 2017; Spaide et al., 2018).

This technique is based on motion detection of the blood flow, which means that it detects the variation caused by moving particles (blood cells) in time-successive OCT signals and generates the resultant contrast images of retinal vasculature (Kashani et al., 2017). It performs multiple B-scans in the same location over time and compares these images pixel by pixel to detect the changes in the signal associated with the flow of erythrocytes. It allows not only the visualization of superficial plexus, but also the radial peripapillary capillary network, the intermediate and deep capillary plexuses, the choriocapillaris and, partially, large choroidal vessels (Spaide et al., 2018). Besides, OCTA reduces image acquisition time and allows the visualization of retinal vessels without the need to use a dye, thus avoiding any kind of adverse effect

(Balaratnasingam et al., 2018; Mo et al., 2016). For this reason, it is becoming increasingly used in ophthalmology clinics and research (Spaide et al., 2018).

Despite the improvement in the visualization of retinal microvasculature, the usefulness of OCTA depends mostly on the correct interpretation, treatment, and analysis of the images generated. For that reason, it is crucial to know and understand the normal basic histology of retinal vasculature. In this section, a histological description of retinal vessels will be provided and compared to OCTA images in order to make their interpretation easier and more accurate.

2.4.1. Histological description of retinal vessels

The retina is one of the most metabolically active tissues and thus it has a high demand for blood supply to fulfill its nutritional requirements. This is achieved through two types of vasculatures: (1) intraretinal arteries, veins, and capillaries, which supply the inner two-thirds of the retina, up to the outer region of the INL; and (2) the choroidal vasculature, which supplies the outer third of the retina, where the photoreceptors are located (Bill, 1984; Chang-Ling, 2010; Provis, 2001). In this section, we will mainly focus on intraretinal microvasculature.

The central retinal artery and vein enter and exit the retina through the optic disc.

Once in the retina, they divide, anastomose and form different plexuses, generating a complex network that covers almost the entire retinal area (Hogan et al., 1971; Provis, 2001; Snodderly et al., 1992). Only two regions completely lack retinal vasculature: the most peripheral 1-1.5 mm and the FAZ (Hayreh, 2010; Provis, 2001). In the rest of the retina, depending on the eccentricity, one, two, three or four vascular plexuses can be identified (Campbell et al., 2017; Hayreh, 2010) responding to different metabolic needs and tissue thicknesses in the different locations. The four existing plexuses are (1) the radial peripapillary capillary network (RPCN), (2) the superficial vascular plexus (SVP), (3) the intermediate capillary plexus (ICP), and (4) the deep capillary plexus (DCP).

The radial peripapillary capillary network is the most superficial vascular plexus, located within the RNFL between the nerve fiber bundles. The RNFL is composed of unmyelinated axons, which have a considerable metabolic demand and need large quantities of energy to maintain ion pumping and ion concentrations (Snodderly et al., 1992; Spaide et al., 2018). Radial peripapillary capillaries are only present in those retinal regions where the RNFL constitutes an independent layer and they are

responsible for providing the RNFL with the blood supply it needs for its high energy requirements. Vessels in this layer display a particular morphology: they rarely anastomose and they are very long and highly oriented, running parallel to the fibers (Campbell et al., 2017; Hogan et al., 1971). Their appearance differs from the other three layers, which show a more lobular morphology (Kashani et al., 2017).

The superficial vascular plexus is located within the GCL, running among retinal ganglion cells. This plexus is the only one with larger arteries, arterioles, veins, and venules (Spaide et al., 2018). Arteries and veins of this superficial plexus give rise to smaller capillaries, some of which run in the same plane, while others run obliquely or vertically into the other vascular layers (both the INL and the RNFL) (Chan et al., 2012; Tan et al., 2012). This layer has a more three-dimensional structure, as compared to the single-layer appearance of deeper plexuses (Yu et al., 2010).

The intermediate capillary plexus is found in the innermost part of the INL. Morphologically, it has a planar configuration and is composed of vascular loops that are small capillaries and vertical and oblique segments that give it a lobular appearance and great tortuosity (Campbell et al., 2017; Chan et al., 2012).

The deep capillary plexus is located in the outermost region of the INL. It is a laminar planar plexus made up of small interconnected capillaries (Chan et al., 2012; Spaide et al., 2018).

2.4.2. Correlation between histology of retinal vasculature and OCTA images

In this section, we compare the vasculature of human retinas immunostained with specific antibodies with images obtained using OCTA, in order to establish its correlation. Fig. 8A-J shows cross-sectional OCT and histological images of a human retina at various eccentricity points, to observe the different number and disposition of the vascular plexuses in different retinal regions (schematically represented in Fig. 8K-N). Blood vessels in the histological sections are stained with collagen IV and can be seen as blue hollow structures with an oval morphology, resulting from the transverse cut. Amacrine and some bipolar cells can be observed in red, and cones in green, both helping to locate the microvasculature in the different retinal layers (Fig. 8B, D, F, H, J).

2.4.2.1. Optic nerve

In the vicinity of the optic nerve (Fig. 8C, D, K), the four plexuses can be observed. In this region, the RNFL has its maximum thickness, reaching up to 65-70 μm , and thus it needs a high blood supply by the radial peripapillary capillary network. Capillaries in

this layer are long and in close relationship with nerve fiber bundles, traveling in parallel among them. The density of capillaries in this layer diminishes with increasing distances to the optic nerve and with RNFL thinning (Kashani et al., 2017). Just below, in the inner part of the GCL, almost in the border with the RNFL, the superficial vascular plexus appears. In this layer, the vasculature has larger diameters in the proximity of the optic nerve (Fig. 8C, D). The two other plexuses are located at the two ends of the INL. The intermediate capillary plexus can be observed in the innermost INL, among the amacrine cells, and the deep capillary plexus appears in the outermost INL, among the horizontal cells (Fig. 8C, D). Capillaries of both plexuses have a constant density, are small in size, and display a very small z-plane variation, which gives both of them the aspect of a single-layered laminar structure.

These retinal vascular plexuses can be also visualized in *en-face* OCTA images and in retinal whole-mount histological preparations. Representative images of the plexuses obtained with both techniques are shown in Fig. 9. Near the optic nerve, the same four plexuses can be distinguished using both OCTA and histology (Fig. 9A-H). Notice that the morphological features previously described can be seen in both images: the long and parallel capillaries of the radial peripapillary capillary network, the larger arteries

and veins in the superficial vascular plexus, and the two lobular and tortuous deeper plexuses. Inserts in Fig. 9C, G are higher magnification images of the intermediate capillary plexus, where the morphology of this plexus can be observed in detail. In both OCT and confocal images, the presence of the characteristic vascular loops can be observed. Inserts in Fig. 9D, H display high magnification images of the deep capillary plexus. In them, the "spider-like" structures, typical of this plexus and abundant in this retinal location, are shown. These structures, which have a central node that ramifies into several branches, can be easily detected both in OCT and confocal images.

2.4.2.2. Central retina and fovea

In a central area of the retina, close to the fovea, but away from the optic nerve, only three vascular plexuses can be identified (Fig. 8E, F, L). In this region, there are fewer axons and they travel around ganglion cells: in this region, the RNFL is not identified as a layer itself. As a result, the most superficial vascular layer here is the superficial vascular plexus, located in the GCL (Fig. 8E, F, L). In the central retina, the GCL contains two to six ganglion cell rows, which makes it thicker than in other areas. Thus, the superficial vascular plexus has a tridimensional appearance, as it is located in varying z-

planes within the GCL. The intermediate and deep capillary plexuses maintain their location and density.

This distribution is maintained throughout the central retina except in one specific region: the foveal avascular zone (FAZ) (Fig. 8G, H, N). The FAZ occupies about 400-500 μm of the most central fovea and is a zone free of vasculature, which enables a direct path of the light towards the photoreceptors. When the three central plexuses reach a point near the *clivus*, they terminate and converge into one plexus (Fig. 8N) that forms the perifoveal capillary ring around the FAZ (Campbell et al., 2017; Spaide et al., 2018). The FAZ can be clearly observed in histological and OCTA images (Fig. 9I-P) as a central area lacking vasculature. The morphological features of each plexus are maintained in this region, where the intermediate capillary plexus has the appearance of loops (Fig. 9K, O) and the deep vascular plexus presents "spider-like" structures (Fig. 9L, P arrowheads). Nevertheless, although the three plexuses described are observable in different *en-face* OCTA images, the OCTA segmentation in this layer is problematic and many times not very accurate, as the software does not generally take into account the different anatomy of this retinal region. Not considering the anatomical variations in the area, OCTA software usually considers layers of uniform thickness,

which results in part of the deep vascular plexuses possibly being added to the superficial vascular plexus (Spaide and Curcio, 2017; Spaide et al., 2018). On the contrary, in histological analysis using confocal microscopy, the three plexuses can be easily identified and segmented. The perifoveal capillary ring is a fusion of the three plexuses, but it is located in the same plane as the intermediate capillary plexus (Fig. 8N).

2.4.2.3. Peripheral retina

When looking at more eccentric regions (approximately 10-12 mm of eccentricity) (Fig. 8I, J, M), where the retina is thinner and has lower metabolic requirements, only two plexuses are distinguished: the superficial vascular plexus and the deep vascular plexus (Campbell et al., 2017). Small segments vertically or obliquely traveling from one plexus to another can be also detected in histological cross-sections, but they do not constitute a plexus itself. In whole-mount retinas, these two plexuses can be clearly differentiated (Fig. 9Q-T), but they cannot be well separated in OCTA. Because of the thinness of the retina in this area, the motion of the large vessels from the superficial vascular plexus projects towards the outer retina, and consequently they are inaccurately represented and visualized in the OCTA images of the deep vascular

plexus (Fig. 9Q, S). In addition, the typical morphological characteristics of the deep vascular plexus change: it loses the "spider-like" structures and is mainly formed by loops (Fig. 9S, T). Spider-like structures are more abundant as closer to the central retina, while they start to turn into lobular structures with increasing eccentricities.

Finally, more peripherally, (around 15 mm of eccentricity), the deep capillary plexus disappears and only the superficial vascular plexus remains, generating a vascular border end (Fig. 9U). Nevertheless, current OCTA instruments are not designed for visualizing such peripheral regions, and for now, they can only be anatomically studied using histological techniques. Beyond this superficial vascular end, there is still 1-1.5 mm of avascular retinal tissue (Fig. 9U arrow) that corresponds to the non-laminar retina (Gariano et al., 1994; Tan et al., 2012).

2.4.3. OCTA strengths and limitations

As seen in Fig. 9, the vascular representation in OCTA is very similar to the retinal vascular anatomy and may be a reliable technique to study both healthy and pathological retinal blood vessels *in vivo* (Mammo et al., 2015). But for doing so, OCTA limitations must be taken into account when analyzing the resulting images. One of the most important limitations is that many manufacturers provide an incorrect

segmentation of the vascular plexus, especially near the fovea or in pathologies (Spaide and Curcio, 2017). In these cases, it is crucial that users have good knowledge of retinal vascular anatomy and OCT images interpretation, in order to perform manual segmentation. For that, it is useful to combine the cross-sectional OCT view with the OCTA overlay images. In addition, different users may consider different criteria for segmentation, as there is not a global consensus on the layer definitions (Kashani et al., 2017). This fact hampers the comparison between different studies and points to the urgent need for a standardized and reliable segmentation method.

Other artifacts may also alter the final result of OCTA images. For example, the projection of vessels in erroneous locations: in some cases, vessels from the superficial vascular plexus may be seen in *en-face* images of deeper plexuses. Also, if different vessels are in the same location at a different depth, in the resulting *en-face* image, both plexuses may overlap and appear as one. These kinds of image artifacts are very common and may be identified by analyzing sequential *en-face* images at different depths. Motion artifacts as the result of blinking or saccadic eye movements may also appear in OCTA images (Spaide et al., 2015), but this phenomenon is easily identified,

and it is being continuously improved in newer OCTA instruments or software packages.

Concerning capillary density analysis, OCTA users should bear in mind that, while OCTA is generally reliable, it may overestimate the capillary density in comparison to normal retinal vascular histology, mainly due to imaging artifacts (Kashani et al., 2017; Matsunaga et al., 2014; Weinhaus et al., 1995). Also, for the performance of vascular leakage or permeability analysis, fluorescein or indocyanine green should be used, because OCTA is unable to detect leakage.

Now that the histological interpretation and limitations of OCT and OCTA have been established, their use in disease diagnosis and recovery can be discussed.

3. The use of OCT and OCTA in the study of animal models

The use of animal models of retinal diseases is a key tool to understand the molecular mechanisms associated with pathology, or to test and develop new molecules with therapeutic potential. Non-human primates are a highly relevant animal model for studying retinal diseases due to the structural and functional similarities of human and other primate retinas, including the presence of the fovea. Other animal models, such

as rodents, pigs, dogs, and cats, are also widely used in the field thanks to the possibility of studying disease progression in shorter periods of time, or with an elevated sample size due to their usual numerous progenies. Moreover, the use of OCT imaging in these animal models allows the study of disease progression *in vivo* and its correlation with histological images and functional analysis.

The first studies using TD-OCT were only able to reveal a small number of hyperreflective bands, due to the poor resolution of the devices. The outer retinal layers were difficult to distinguish, and the combination of IS, OS, and the RPE was usually identified as a single layer (Gekeler et al., 2007). OCT images in mice showed three outer hyperreflective bands that authors correlated either with the ELM, the IS/OS junction and the RPE (Pennesi et al., 2012; Yamauchi et al., 2011) or with the IS/OS junction, the RPE and the choroid (Gadjanski et al., 2011). The continuous evolution of the OCT techniques has made it possible to obtain sharper images and to resolve an increased number of bands. Nowadays, using OCT devices, either SD-OCT or SS-OCT, with higher resolution power, it is possible to identify four hyperreflective layers in the outermost retina of different animal models, the characterization of which is still subject to debate.

3.1. Correlation of OCT bands and retinal histology in animal models

Experimental animal models are widely used to study human retinal pathologies and to evaluate the responses of therapeutic interventions. For the correct extrapolation of the results, an accurate interpretation of OCT images is required for each animal model, due to the histological variability among different species (Linberg et al., 2001). OCT has been extensively used to analyze the retina of different animal species such as mice, rats, rabbits, pigs, dogs or monkeys (Table 1). A plethora of studies shows how OCT can be useful to evaluate retinal health with a good correlation with histological findings. However, each animal species presents its own challenges and needs unique image settings to obtain reproducible OCT images (McLellan and Rasmussen, 2012). Even among individuals of the same species, differences can be observed due to the pigmentation or the age of the animals. This is the case for the intensity differences of the hyperreflective bands observed between albino and pigmented mice (Berger et al., 2014; Huber et al., 2009), the significant band visibility differences in primates due to different ages (Fan et al., 2019), or postnatal maturation (Patel et al., 2017). The different interpretation of the retinal bands in distinct animal species is summarized in

Table 1 and extensively described in the following sections, mainly based on histological knowledge of the mouse retina. In order to establish a correlation between OCT and histology, mouse retinal sections and OCT images showed from now on were always performed in the same retinal locations and, whenever possible, in the same eye.

3.1.1. Retinal nuclear layers

There is a broad consensus concerning the correlation between OCT bands and their equivalent histological layers in the inner retina (Bartuma et al., 2015; Cheng et al., 2018; Pinilla et al., 2016; Yiu et al., 2018). As can be seen in Fig. 10, there is a clear correspondence between the hyporeflective bands in OCT and the outer and inner nuclear layers stained with TO-PRO in the retina of mice (Fig. 10A, B). The other nuclear layer, the GCL, is often quite difficult to distinguish from the RNFL in OCT images. Thus, the innermost hyperreflective band in OCT (Fig. 10D, red line) is usually identified indistinctly as RNFL, as GCL, or as RNFL plus GCL (Berger et al., 2014; Garcia Garrido et al., 2014; Pennesi et al., 2012; Pinilla et al., 2016; Ryals et al., 2017; Yi et al., 2016; Zeng et al., 2016). In fact, most authors prefer to name these sections together, in an unsegmented manner, as RNFL plus GCL plus IPL (Huber et al., 2009; Ohno et al.,

2013). Pennesi et al. noticed that, in some mice, a thin hyporeflective band between the RNFL and could be observed (Pennesi et al., 2012), probably corresponding to the GCL. This fact is in agreement with the GCL hyporeflective band found near the optic nerve in other animal species (Xie et al., 2018; Yiu et al., 2018). In our results, a unique hyperreflective band, corresponding to the ganglion cells and the nerve fibers, can be observed in mice (Fig. 10D, line red), whose hyperreflectivity is probably due to the high density of mitochondria contained within the ganglion cell axons. Double immunostaining of retinal ganglion cells (with Brn3a) and ganglion cell axons (with Tuj-1) (Fig. 10E) shows the close relationship of these two structures, making it difficult to separate them into two independent layers in most OCT images. In addition, astrocytes and the end feet of Müller cells are also located in the same zone (Fernandez-Sanchez et al., 2015) and can contribute to this hyperreflective OCT band (Fig. 10F). Furthermore, it must be noted that amacrine cells represent about 60% of the total cells in the GCL in different mammals (Aboelela and Robinson, 2004; Jeon et al., 1998; Muniz et al., 2014; Perez De Sevilla Muller et al., 2007). Fig. 10G shows ganglion cells and displaced amacrine cells in the GLC, immunostained against Pax6, and their correlation with the first hyperreflective band. All these anatomical features of the RNFL

and the GCL evidence that quantitative changes in this OCT band should be carefully interpreted, considering the different structures that may be responsible for these changes.

The next nuclear layer, the INL, correlates with the innermost hyporeflective band. In histological sections, this layer can be observed with double immunostaining against Chx10 and Pax6, markers of bipolar cells and amacrine and ganglion cells, respectively (Hitchcock et al., 1996) (Fig. 10D-G). Finally, the ONL corresponds with the thickest hyporeflective band (Fig. 10D, E), formed by the nuclei of photoreceptors.

3.1.2. Retinal plexiform layers

The retinal plexiform layers generate two hyperreflective bands in OCT (Fig. 10H; yellow and light blue lines). The origin of the hyperreflectivity within these plexiform layers might be related to the high density of mitochondria, vesicles, and membranes found in the cellular structures making synaptic connections (Wilson et al., 2007).

Using a specific marker of synaptic connections, bassoon (Fig. 10I, K), and a marker of bipolar cells, PKC- γ (Fig. 10J, K), the two plexiform layers can be observed in a histological section and correlated with OCT images (Fig. 10H, yellow and light blue line). The first plexiform band, the IPL (yellow), presents lower hyperreflectivity than the

RNFL-GCL band and is the layer where the synaptic connections between bipolar, amacrine and ganglion cells are located (Fig. 10H-K, yellow line). The second plexiform layer, the OPL, is thinner than the IPL, and is formed by the connections between photoreceptors and their post-synaptic cells: bipolar and horizontal cells (Fig. 10H light blue line; I-K).

3.1.3. Outer hyperreflective bands

In the outer retina of mice, OCT reveals four hyperreflective bands in the region spanning from the ONL to the RPE (Fig. 10, 11 A box, and B, magnification). Although the mice retina is structurally different to the human retina described in previous sections (lower cone to rod ratio or absence of fovea), the proposed origin of the outer hyperreflective OCT bands is based on subcellular structures and organelles that are present in cones and rods in both species. Thus, their interpretation in mice will be similar to the one proposed in humans.

3.1.3.1. First outer hyperreflective band

Based on previous studies done in different species (Adachi et al., 2016; Anger et al., 2004; Aplin et al., 2014; Bartuma et al., 2015; Berger et al., 2014; Cuenca et al., 2014b; Fan et al., 2019; Garcia Garrido et al., 2014; Gloesmann et al., 2003; Hernandez-Merino

et al., 2011; Huber et al., 2009; Koinzer et al., 2013b; Occelli et al., 2016; Pinilla et al., 2016; Ryals et al., 2017; Tian et al., 2017; Xie et al., 2018; Yiu et al., 2018; Zeng et al., 2016), it is accepted that the first hyperreflective band in the outer part of the ONL corresponds to the ELM (Fig. 11B, C arrowheads). This thin continuous layer, formed by the junctions between the end processes of Müller cells and the photoreceptors, can be identified using anti-CRALBP antibodies (Fig. 10F, Fig. 11C, F green).

3.1.3.2. Second outer hyperreflective band

There is a lack of agreement referring to the nature of the next hyperreflective OCT band, although it is accepted that its origin resides within photoreceptors. Photoreceptors are highly organized and complex cells, divided into several defined compartments composed of different organelles whose arrangement depends on their functional needs. On the one hand, the second outer hyperreflective OCT band has been referred to as the ellipsoid zone (EZ) in monkeys (Anger et al., 2004; Fan et al., 2019), pigs (Cheng et al., 2018; Gloesmann et al., 2003; Xie et al., 2018), rabbits (Bartuma et al., 2015; Tian et al., 2017) and cats (Minella et al., 2018; Occelli et al., 2016). However, other authors have classified this band as the IS/OS junction in monkeys (Garcia Garrido et al., 2014; Yiu et al., 2018), rabbits (Koinzer et al., 2013b)

and cats (Aplin et al., 2014), revealing the current controversy in the interpretation of the band.

Histological sections of mice retina are shown in Fig. 11, obtained using specific markers of photoreceptors and their cellular components. As has been previously described in the human retina (section 2.2.), the photoreceptor inner segment is divided into two areas: the ellipsoid and the myoid. Histological retinal sections show the photoreceptor outer segments in red, using antibodies against rhodopsin, and inner segments in blue, using mitochondria cytochrome C immunostaining (Fig. 11D, F). Bearing in mind that mitochondria are one the most light-scattering organelles, these long ellipsoids, which are completely full of mitochondria, are thought to correlate with the second hyperreflective band.

Therefore, in agreement with previous reports by other groups on different species, such as pigs (Gloesmann et al., 2003; Xie et al., 2018), rabbits (Bartuma et al., 2015), monkey (Anger et al., 2004) and rodents (Berger et al., 2014; Pinilla et al., 2016; Zeng et al., 2016), the hyporeflexive band between the first (ELM) and the second (EZ) hyperreflective bands should be the myoid zone in the inner segment of

photoreceptors, represented by the non-stained structures between ellipsoids and ELM (Fig. 11F).

3.1.3.3. Third outer hyperreflective band

Also controversial is the identification of the next hyperreflective band. While some authors have identified it as the RPE in monkeys (Anger et al., 2004; Garcia Garrido et al., 2014; Yiu et al., 2018), pigs (Cheng et al., 2018; Gloesmann et al., 2003), cats (Aplin et al., 2014) and rodents (Berger et al., 2014; Huber et al., 2009), others defend the idea that it belongs to the interdigitation zone (IZ), based on studies conducted on rabbits (Bartuma et al., 2015; Tian et al., 2017), cats (Minella et al., 2018; Occelli et al., 2016), and adult (but not infant) monkeys (Fan et al., 2019) and rodents (Adachi et al., 2016; Berger et al., 2014; Monai et al., 2018; Ryals et al., 2017). Additionally, some studies define this band as the cone OS tips (COST) in monkeys (Garcia Garrido et al., 2014) and others interpret the band as the OS/RPE/Bruch's membrane in rabbits (Koinzer et al., 2013b).

Triple immunostaining using antibodies against cytochrome C, CRALBP and rhodopsin can help to elucidate the histological structures responsible for the third band in mice. In Fig. 11F, it is possible to see the photoreceptor outer segments (red) extending from

the ellipsoids (blue) up to the apical zone of the RPE (green) (Fig. 11F, G square bracket). Anti-CRALBP immunostaining shows the entire RPE cells, allowing us to visualize their interdigitations covering the entire photoreceptor outer segments (Fig. 11G arrowheads). Taking into account these results, the third band cannot be the interdigitation zone: the fact that RPE interdigitations are completely surrounding the entire OS and reaching the ellipsoid zone (second band) could not explain the formation of a hyporeflective band between these two hyperreflective.

In addition, rhodopsin staining permits to visualize the phagosomes inside the RPE cells, formed as a consequence of the phagocytosis of the photoreceptor OS (Fig. 11G-J, arrows). In higher magnification images, it is possible to see how the phagosomes, stained with antibodies against rhodopsin in red (Fig. 11H arrows), are distributed within the most apical part of the RPE cells (Fig. 11I, J arrows). These phagosomes are arranged in an almost continuous line along the retinal section and may be able to form a light-scattering layer. Thus, as previously proposed in humans (Cuenca et al., 2018), the origin of the third hyperreflective band in mice could be the phagosomes within the apical region of the RPE. Moreover, as proposed in section 2.3.4.3., the phagosome location is separated enough from the second hyperreflective band leaving

the space for a hyporeflective band in between, which then could correspond to the photoreceptor outer segments.

3.1.3.4. Fourth outer hyperreflective band

Concerning the last hyperreflective band, different works have identified it as the RPE, the choroid or the choriocapillaris. Among the studies supporting the RPE as its origin source, there are several works done in monkeys (Fan et al., 2019; Garcia Garrido et al., 2014), rabbits (Bartuma et al., 2015; Tian et al., 2017), and rodents (Adachi et al., 2016; Berger et al., 2014; Cuenca et al., 2014b; Monai et al., 2018; Pinilla et al., 2016; Ryals et al., 2017; Zeng et al., 2016). Other works in pigs (Xie et al., 2018), rabbits (Koinzer et al., 2013b), dogs (Hernandez-Merino et al., 2011) and cats (Aplin et al., 2014) support the hypothesis that it corresponds to the choroid. Yet, based on their results in pigs (Cheng et al., 2018; Gloesmann et al., 2003) and monkeys (Yiu et al., 2018), other authors have suggested that the source of this zone could be the choriocapillaris.

The RPE is formed by highly polarized cells with two main specialized regions: the apical and the basal region. At the apical portion the interdigitations and the OS phagosomes are located, while on the basal side, close to the Bruch's membrane, there is a high density of mitochondria (Fig. 11D, F arrows). According to the light-scattering

properties of mitochondria, we propose that this specific region of the RPE is which corresponds to the fourth outer hyperreflective band observed in OCT images. Finally, the hyporeflective band between the third and the fourth hyperreflective bands could correspond to the space located between the apical and the basal region of the RPE, where the melanosomes and the nucleus are located as described in section 2.3.4.4.

In summary (Fig. 11E), we propose that the OCT bands in the outer retina of mice correspond to: the ELM, as the first hyperreflective band; the myoid zone, as the first hyporeflective band; ellipsoids, as the second hyperreflective band; RPE phagosomes at the apical zone, as the third hyperreflective band and the basal side of RPE, as the fourth hyperreflective band.

Taking into account the histological features described, the measurements obtained from OCT images must be interpreted carefully, considering, among others, the limited image resolution or the fact that many of the OCT bands are formed by several cell types, as in the case of the RNFL and GCL, in which any changes detected could be due to nerve fibers, ganglion cells, displaced amacrine cells or astrocytes.

3.2. OCT in the evaluation of retinal degeneration

Histological studies allow for detailed characterization of the morphological state of the retina, detecting degenerative processes that affect specific cellular types, such as morphological cell changes, neuronal remodeling events, or glia and microglia activation (Noailles et al., 2016). Currently, a wide variety of animal models are used for investigating retinal degenerative diseases at these histological levels. These different models can be divided in two groups: animal models with retinal degeneration induced by external damage, and animal models of degenerative retinal diseases caused by genetic mutations. In addition to histology, OCT provides an easy and harmless method for longitudinally evaluating the retina of different animal models, considering parameters such as retinal thickness, or thickness and reflectivity changes of each band. The use of OCT allows the observation of the same animal over time and a reduction in the number of animals per study (Alex, 2019; Drexler and Fujimoto, 2008; Fischer et al., 2009). To date, multiple examples show that the OCT technique has been successfully applied to different procedures, and different works have used this technique to analyze induced injury and genetic models of retinal degeneration, as well as to assess the efficacy of different treatments.

3.2.1. OCT in the evaluation of induced retinal injury

The thickness measurement of retinal layers has been successfully used to evaluate retinal injury. For example, OCT has been used to study inner retinal degeneration in rodent models of total or partial optic nerve crush (Rovere et al., 2016; Yi et al., 2016), optic nerve transection (Rovere et al., 2015), anterior ischemic optic neuropathy (Ho et al., 2013; Shariati et al., 2015). Even with the first OCT devices, which offered poor resolution images, some authors were able to detect changes in the total retina thickness in an ischemia/reperfusion model (Sho et al., 2005). It is important to note that the earliest changes detected in OCT images of these models were those measuring total retina thickness or that of the ganglion cell complex, which include RNFL+GCL+IPL (Ho et al., 2013; Nakano et al., 2011; Ohno et al., 2013; Rovere et al., 2016; Sho et al., 2005). These studies were able to detect a very early increase in retinal thickness immediately after the retinal injury, presumably due to edema. Interestingly, these thickness differences were not statistically significant when only the RNFL+GCL were measured (Rovere et al., 2016). In other example, OCT scans of a rabbit model of bleb-induced outer retinal damage by injection of isotonic saline solutions showed the same degenerative changes in the outer retina as in the histological analysis. Both

techniques allowed to visualize a time-dependent thinning of the outer layers during the progression of degeneration (Bartuma et al., 2015). In addition, comparative studies of OCT and histological images (Koinzer et al., 2013a; Koinzer et al., 2013b) after photocoagulation in rabbits, OCT showed general tissue lesions, identifying the largest structural changes that were also visible in histology. However, some small alterations visible in histology could not be detected by OCT. Moreover, OCT has been used to monitor light-induced retinal degeneration in murine models where there is a thinning of the ONL band (Tullis et al., 2015).

Besides evaluating mechanical retinal damage, OCT has been also used to assess the changes in chemically-induced retinal degeneration models. In this line, the ganglion cell degeneration after intravitreal injection of N-methyl-D-aspartate (NMDA) can be observed by OCT as a reduction of the complex that includes the RNFL, GCL, and IPL bands (Nakano et al., 2011; Ohno et al., 2013). Also, with this technique, it is possible to monitor the photoreceptor degeneration induced after a N-methyl-N-nitrosourea (MNU) injection in rabbit, mouse and rat models (Ahn et al., 2019; Rösch et al., 2014a; Xiong et al., 2016).

In addition, OCT has been successfully used to monitor retinal changes and degeneration in experimental models of induced autoimmune disease, associated to an inflammatory process. As an example, in a rat experimental model of autoimmune uveoretinitis, generated by the injection of pathogen microorganisms and interphotoreceptor retinoid-binding protein, (Gadjanski et al., 2011) OCT made it possible to analyze the course of the disease and to observe retinal folds and detachments in the photoreceptor layer, which correlated with histological findings. In other example, in a mice model of autoimmune encephalomyelitis generated by the injection of a toxin and myelin, (Nishioka et al., 2019), OCT revealed ganglion cell complex thinning over time. This model is widely used as an animal model of multiple sclerosis.

Generally, a good correlation between retinal thickness assessed by both OCT and histology could be established in animal models, albeit correlating small and precise histological changes is not always possible with OCT.

3.2.2. OCT in the study of genetic models of retinal degenerative diseases

OCT has proven to be useful for the longitudinal analysis of the progression of genetic models of retinal degenerative diseases and the characterization of their dynamics. The

measurement of the thickness of the entire retina or some of its layers allows tracking the progress of the disease throughout the course of retinal degeneration. One of the pathologies that has been studied in depth using genetic models is retinitis pigmentosa (RP), for which different murine models are commonly used. Relevant examples of these models are the rd10, rd1 and P23H models, carrying mutations that affect photoreceptors; and the RCS and rd12 models, in which the retinal pigmentary epithelium is affected. In the study of these animal models, OCT imaging could be a useful technique to analyze the specific bands that change depending on the different degenerative processes.

3.2.2.1. Rd10 model

The rd10 mouse is a model of autosomal recessive RP with a spontaneous mutation in the rod specific phosphodiesterase 6B gene (*Pde6b*) (Chang et al., 2002). Several authors have described the progression of degeneration in the rd10 model using OCT (Hasegawa et al., 2014; Kim et al., 2018; Pennesi et al., 2012; Rösch et al., 2014b) and have performed comparative studies with histology and electroretinograms (ERG) (Hasegawa et al., 2016; Rösch et al., 2014b). In this model, until post-natal day 14 (P14), only a slight decrease in retinal thickness can be observed with OCT in some animals

compared to their corresponding control mice (Kim et al., 2018; Pennesi et al., 2012).

At later ages, significant degeneration is observed with OCT in rd10 mice around P25, when there is a decrease in total retinal thickness mainly due to photoreceptor death (Barhoum et al., 2008; Hasegawa et al., 2016; Kim et al., 2018; Pennesi et al., 2012; Rösch et al., 2014b). This correlates with the degeneration of photoreceptors observed through immunohistochemistry and hematoxylin-eosin staining, and the ERG a-wave depletion at these time points (Barhoum et al., 2008; Hasegawa et al., 2016; Rösch et al., 2014b). Fig. 12 shows the correlation between OCT, immunohistochemistry and ERG in a rd10 mouse. A decrease in overall retinal thickness is observed by OCT in the retinas of rd10 animals between P20 and P60, which is also observed in immunohistochemistry images (Fig. 12A-D). Nevertheless, although OCT can give an indicative value of the retina thinning, histology allows for visualizing the detailed changes in the different cell types and the morphological changes that the cells undergo with the progression of RP. In Fig. 12E-H, the degeneration of cones and ON rod bipolar cells is easy to evaluate using immunohistochemistry, as compared to OCT (Fig. 12A-D). Globally, OCT makes it possible to visualize the progressive thinning of the ONL observed from P20 to P30, however, its resolution limit precludes the accurate

measurement of the ONL thickness from P30 onwards, when only one or two photoreceptor rows are left (Fig. 12C, D, G, H, K, L). Correlating OCT with histology, it has been reported that the rd10 ONL consists of only one row of somata at P49, which completely disappears at 6 months (Rösch et al., 2014b). In addition, changes that occur in the outer bands of the retina are observed with the progression of the disease. In this model, the OCT hyperreflective bands of the outer retina are seen only at an early age (Fig. 12A), since they are already lost at P25 (Fig. 12B) due to the degeneration and the abnormal morphological structure of the photoreceptors. Yet, the detailed morphological changes that photoreceptors undergo in this animal model can be traced by immunohistochemistry. Cones suffer a progressive size reduction and each compartment of the cell, from the pedicle, through the axon and to the outer segment, can be evaluated with this technique until they are barely noticeable at P60 (Fig. 12I-L). Likewise, despite there being no apparent changes in the inner retina in OCT images, immunohistochemistry reveals a shortening of bipolar cells at P60 (Fig. 12H). On the other hand, functional studies are required to evaluate the functional state of the cells in the retina. In this sense, ERGs evince a drastic reduction of the a-

and b-wave during RP degeneration (Fig. 12M-P), which correlates with the loss of photoreceptors (Fig. 12I-L).

Other event described in the rd10 model that is visible under OCT is retinal detachment. This retinal separation from the RPE, already detectable in some rd10 animals at P25, is evident in most animals starting from P60 (Hasegawa et al., 2014; Pennesi et al., 2012). OCT images from animals are usually taken in the central areas near the optic nerve. However, as can be seen in Fig. 13, OCT images not only allow for detecting the presence of retinal detachment, they also make it possible to locate the extension of detached tissue by moving the transverse line along the fundus (Fig. 13A-C). This detachment cannot be identified using histology, because of the artifacts generated during the sample manipulation.

To date, OCT images from rodents can only be obtained within a central window around the optic nerve, but not from the peripheral retina, which could be relevant for the correct evaluation of this tissue. Fig. 13A shows a mouse fundus, where a window of tissue of only approximately 2.5 mm x 2.5 mm can be observed. Likewise, the corresponding OCT image that the device is capable of providing is 2.5 mm long. However, a mouse retina measures around 5 mm long. In Fig. 13D, the bracket at the

top of the image indicates the OCT capture area, revealing that OCT allows only the observation of a small central portion of the retina, and leaving a relevant portion of the retina unable to be analyzed. Furthermore, it must be taken into account that OCT is usually performed in the area surrounding the optic nerve, but the degeneration of the retina in rd10 mice is not uniform (Fig. 13 D): it varies along a cross-section, presenting faster degeneration near the optic nerve and slower degeneration in the periphery, near the *ora serrata*. Fig. 13F-H shows the differences in ONL thickness in three zones along the retina: center, middle and periphery, as we move away from the optic nerve. In addition, the photoreceptors of the central zone (Fig. 13F) have a more degenerated morphology as compared to the peripheral zone (Fig. 13 H), where they have longer axons and outer segments.

3.2.2.2. *Rd1* model

Similar results have been described in rd1 mice, which also carry a mutation on the *Pde6b* gene that affect rods and have a more severe and faster degeneration than rd10 mice. Several studies describe the degeneration of this strain using OCT and histology (Hanna et al., 2018; Huber et al., 2009; Kim et al., 2008; Pennesi et al., 2012). OCT images show that the outer retina is drastically reduced at eye opening (Kim et al.,

2008), and that at around P30 there is a reduction of more than one-half of total retina. At this age, the outer retina is lost, but an intact inner retina can still be seen, which correlates with histological findings (Hanna et al., 2018; Huber et al., 2009; Kim et al., 2008; Pennesi et al., 2012).

3.2.2.3. P23H model

The P23H rat is a model of RP that carries several copies of the mouse rhodopsin-encoding gene with a point mutation, and its promoter (Orhan et al., 2015). Two P23H rat lines have been studied by OCT and histology: line 1, with fast-retinal degeneration (Cuenca et al., 2014b; Orhan et al., 2015; Pinilla et al., 2016) and line 2, with very slow retinal degeneration (Monai et al., 2018). It should also be mentioned that this model has been studied both with homozygous and hemizygous lines.

Our group evaluated homozygous P23H line 1 rat degeneration by means of OCT and histology (Cuenca et al., 2014b; Pinilla et al., 2016). Fig. 14 shows the correlation between OCT and histology in aging P23H retinas. TO-PRO, a nuclear marker, and antibodies against PKC- α and bassoon were used in order to track the changes in the nuclear and plexiform layers during retinal degeneration. As described in previous studies from our group, there is a decrease in total retinal thickness and in the

ONL+RPE complex in aging P23H retinas (Pinilla et al., 2016). OCT images from control and P23H at P18 display normal hyperreflectivity profiles (Fig. 14A, D). In contrast, a decrease in ONL thickness was already evidenced with immunohistochemistry in P23H rats at P22, with a slight loss of photoreceptor rows (Fig. 14E, F) as compared to control retinas (Fig. 14B, C). Photoreceptor loss became visible by OCT at 2 months, with an evident reduction of the ONL+RPE complex (Pinilla et al., 2016), and it was impossible to identify the ONL layer at 6 months in OCT images (Fig. 14G-I). This coincides with the ERG scotopic a-wave depletion at this time point (Cuenca et al., 2014b). Some authors have described histologic disorganization and glia activation in the photoreceptor layer in models with retinal degeneration (Fernandez-Sanchez et al., 2015; Hasegawa et al., 2016; Jones and Marc, 2005; Marc and Jones, 2003; Marc et al., 2003; Pennesi et al., 2012). As part of this remodeling, Jones and Marc described a migration of the remaining retinal neurons, glial cells and RPE cells to ectopic sites forming migration columns (Jones and Marc, 2005). Once the ONL have been completely lost, the rat P23H model undergoes a remodeling of the surviving retinal cells (Pinilla et al., 2016). From 18 months old, this event can be detected by immunohistochemistry as a column that invades the neural retina from the RPE

penetrating into the inner retinal layers (Fig. 14K, L, N, O). Also, by OCT it can be observed as a hyperreflective septum advancing from the band corresponding to the RPE that disrupts the INL and IPL bands (Fig. 14J, M, arrows). At advanced ages, these structures hypertrophy and become larger (Fig. 14M-O). Considering these facts, in this model, OCT imaging is useful for monitoring retinal degeneration by measuring retinal layers until 6 months and for studying the retinal remodeling after 11 months (Pinilla et al., 2016). With the current resolution of OCT, in order to achieve the maximum information about the degenerative process of an animal model and to have a good knowledge about the changes that occur at the cellular level, it is essential to combine OCT with immunohistochemistry.

On the other hand, the hemizygous P23H line 1 rat, which has half of the mutant rhodopsin gene copies and slower degeneration compared to homozygous line, has also been described by both OCT and histology. In these P23H rats, the inner retina is thinner than in controls at 1 month. From 3 months on, there is also progressive ONL thinning until 6 months of age, when the degeneration starts to be more severe (Orhan et al., 2015).

Finally, the homozygous P23H line 2 rat has been characterized by OCT and hematoxylin-eosin. This line experiences a very slow degeneration, and there are no relevant changes in retinal thickness until around two months, when they suffer a rapid thinning of the OPL and ONL that persists with aging (Monai et al., 2018). These results show that OCT can be used to describe the slowly-degenerating P23H lines.

OCT monitoring of retinal degeneration was not only performed on small rodent models. P23H retinal degeneration in pigs has been described using OCT scans *in vivo* and *ex vivo* (after enucleation). These measurements generally correlate with histology, but the individual ONL, INL and RNFL layers are thicker when using OCT (Cheng et al., 2018). These results agree with our previous results in the P23H rat, in which the same difference between OCT and histology in the ONL was found (Cuenca et al., 2014b).

3.2.2.4. RCS model

In Royal College of Surgeons (RCS) rat, another murine model for RP, some recent studies have shown the progression of the degeneration, comparing OCT, histology and ERG (Bubis et al., 2019; Rösch et al., 2017; Ryals et al., 2017). The RCS rat presents a mutation in the *Mertk* gene that impairs the phagocytic function of the RPE. In this model, a hyperreflective layer becomes visible from P30 to P90 that corresponds to a

subretinal debris zone (DZ) observed by histology (Rösch et al., 2017; Ryals et al., 2017). There is also a reduction in total retinal thickness. The ONL layer is not detectable at P56 (Bubis et al., 2019; Rösch et al., 2017; Ryals et al., 2017), coinciding with the photoreceptor degeneration observed by histology and with the reduction of the ERG a-wave (Rösch et al., 2017). Moreover, there are no differences in INL or RPE thickness (Ryals et al., 2017).

3.2.2.5. Rd12 model

The rd12 mouse is also a model of RP degeneration, in which the dysfunction affects the RPE. Specifically, it has a nonsense mutation in the exon 3 of the Rpe65 gene, producing a defective visual cycling with no ERG response, but maintaining photoreceptors with normal morphology in the early stages. There is a progressive degeneration of the photoreceptor layer that is detectable using OCT, where this layer gradually becomes thinner starting at P20 and until 13 months of age (Hasegawa et al., 2016).

3.2.2.6. Other animal models

A decrease in retinal thickness has been described for other models of retinal degeneration, such as RP rabbits (Muraoka et al., 2012) and Brn3b knockout mice

(Camp et al., 2011). It has also been found in progressive retinal atrophy in whippet dogs (Somma et al., 2017) and in RPGRIP1-deficient dogs (Lheriteau et al., 2009). OCT has also allowed to identify a thinning and loss of definition in the ONL band in dogs with sudden acquired retinal degeneration syndrome (SARDS), which correlates with the histopathological findings (Grozdanic et al., 2018; Osinchuk et al., 2018).

3.2.3. OCT strengths and limitations in the evaluation of animal models

With all the data known to date, it could be concluded that OCT offers many possibilities for an easy evaluation and monitoring of different models of retinal degenerative diseases, as longitudinal studies can be performed without sacrificing those animals, in contrast to immunohistochemical analysis. Also, other limitations of histological techniques are due to tissue processing such as tissue shrinkage, expansion, or artifacts appearance (mechanical retinal detachment).

Specifically, OCT can be helpful for evaluating the thickness of the entire retina during degeneration, as well as studying the progression of degeneration in different layers. Among others, the health of photoreceptors can be determined by analyzing the four external bands of the OCT, from the ELM, ellipsoids, phagosomes and to the RPE. In addition, OCT can be used to study other bands, such as the thickness of the INL, the

appearance of new structures, or the presence of retinal detachment. Even if the retina is degenerated and all the bands cannot be distinguished, in advanced ages, OCT allows to observe the remodeling of the retina: it makes it possible to *in vivo* study the appearance of migratory columns of blood vessels associated with the RPE penetrating into the retina and the consequent remodeling of the retinal cells. It should however be remembered that, for a correct interpretation of OCT images, histological knowledge of the retina is necessary in both healthy conditions and during retinal degeneration and remodeling.

3.3. Use of OCT in experimental neuroprotection

Animal models are a valuable tool, not only to study the progression of neurodegenerative diseases, but also to assess the effectiveness of different treatments. Traditionally, retinal neuroprotection success has been mainly evaluated by morphological and/or functional parameters using histological, physiological and behavioral tests. These experiments allowed to identify, for example, the therapeutic potential of TUDCA (Fernandez-Sanchez et al., 2017; Fernandez-Sanchez et al., 2011; Gomez-Vicente et al., 2015), safranin (Fernandez-Sanchez et al., 2012a), or norgestrel (Roche et al., 2016). More recently, some studies have also started to include OCT

analysis in the evaluation of neuroprotective strategies for retinal degeneration. Still, its use for this purpose is not very extended. Among the few published researches that employ OCT to determine the effectiveness of neuroprotection treatments, there are some done in different experimental models of retinal pathologies, such as retinal detachment (Meyer et al., 2002) or retinitis pigmentosa (Pang et al., 2011).

One of the reasons of the scarce use of OCT in neuroprotection assays may reside in its limited resolution power, between five to 7 μm , and in its limited ability to reflect slight morphological changes. Both facts mean that neuroprotective strategies that only reflect small retinal changes may be hardly identified by OCT. In this line, depending on how fast the degenerative process is and the therapy used, the effect of therapies for preserving ONL thickness in different experimental models with photoreceptor loss can be difficult to quantify by OCT.

Moreover, it is important to consider that small changes in cell survival, which are difficult to appreciate by OCT, may be translated into big improvements in retinal function (Fernandez-Sanchez et al., 2012b; Lax et al., 2014; Lax et al., 2019). For example, systemic administration of TUDCA in rd10 mice delayed the apoptosis process and slowed the reduction of ERG amplitudes (Drack et al., 2012). But these

changes could not have been clearly identified by OCT, as the ONL thickness preservation measured by hematoxylin-eosin staining was only around 2 μm (Drack et al., 2012). Another issue to ponder is that, in those animal models where the degenerative process is not homogeneous throughout the retina (Fig. 13), different neuroprotection strategies can be useful at different retinal locations. When using OCT this information may be missed by only looking at the central retina, for that reason complementary techniques would be required.

In contrast, the measurement of retinal thickness by OCT can be an appropriate method to detect neuroprotection in those cases when retinal preservation has reached values that are above the resolution threshold. Among others, the use of gene therapy involving PDE β gene replacement with an adeno-associated virus in rd10 mice attained a significant improvement in retinal thickness (around 30 μm thicker as compared to non-treated mice), measured by OCT (Pang et al., 2011). Similar results were evidenced by OCT in a mouse model of retinitis pigmentosa induced by MNU. Intravitreal injections of αB -crystalline reduced apoptosis and oxidative stress in the retina of these mice, alleviating the MNU-induced morphological impairment of the retinas (Tao et al., 2018).

Focusing on the inner retina, ganglion cells could be considered as a part of a complex which include NFL, GCL and IPL. As previously discussed in section 4.1. and as can be seen in Fig. 10, it is difficult to distinguish which portion of the innermost hyperreflective line belongs to each of the complex layers by OCT images during degeneration (Ohno et al., 2013). For this reason, neuroprotection studies focused on preserve any of these retinal layers need to be complemented with other techniques.

One of the advantages of OCT is related to its use for monitoring interventions *in vivo*, such as therapies aimed at retinal reattachment. In this sense, in the BEST1-mutant dog model, which develops retinal microdetachments, OCT allowed to effectively monitor the success of gene therapy, confirming the disappearance of subretinal fluid and retinal reattachment to the underlying RPE layer (Guziewicz et al., 2018). On the contrary, in those studies in which OCT has been employed to monitor the success of cell therapy, the resolution threshold of this technique continues to be a limiting factor.

Intravitreal injections of organoid-derived photoreceptors (Santos-Ferreira et al., 2016) and human CD34+ bone marrow-derived cells (Moisseiev et al., 2016) have demonstrated effective integration after transplantation. Notably, this integration could be observed not only by immunohistochemistry (Santos-Ferreira et al., 2016) but also

by OCT (Moisseiev et al., 2016). In contrast, the OCT images acquired did not show significant differences between the control and treated groups in the regenerative potential (Moisseiev et al., 2016; Santos-Ferreira et al., 2016). Probably, the resolution of current OCT equipment makes it difficult to determine small cellular changes, even when these small changes are relevant to developing new therapies for clinical application.

Thus, depending on the retinal degeneration models used and on the neuroprotective strategies employed, OCT could be an indispensable tool to analyze retinal structure and thickness or, in contrast, only a complementary technique to validate the results obtained. As an example, in a light-induced photoreceptor degeneration model, 15 mg/kg of sarpogrelate treatment (a selective 5-HT_{2A} receptor antagonist) was enough to obtain significant improvement of retinal function in ERG recordings that cannot be observed by OCT. Nevertheless, after a higher dose (40 mg/kg) OCT imaging was able to evidence an increase in ONL thickness (Ku et al., 2018; Tullis et al., 2015). On the other hand, OCT was useful to show the attenuation of neuroprotection when same treatment is combined with ERK1/2 inhibitor, while ERG recordings were not able to show these differences (Ku et al., 2018).

In addition to measure the thickness of retinal layers, OCT is useful to identify changes in band reflectivity or the restoration of certain bands, and to visualize new structures that may appear in retinal degeneration models. For this reason, the non-detection of retinal remodeling or migration columns (Fig. 14) could be also understood as a successful neuroprotective therapy in animal models at ages where these events usually appear (Jones and Marc, 2005; Pinilla et al., 2016).

Accordingly, damaged RPE or photoreceptors outer segment loss will be associated with changes in the outer retinal bands (Fig. 11). As an example, OCT images of retinal degeneration in a mouse model of X-linked retinoschisis (Rs1-knockout mouse) shows a retinal restructuration with a single wider hyperreflective band that encompasses the second and the third bands (Zeng et al., 2016). After gene therapy replacement there is a restoration of the second and third outer retinal hyperreflective bands and the authors suggest that it is due to the resumption of normal mitochondria distribution in the ellipsoids of photoreceptor inner segments. However, the hematoxylin-eosin stained sections showed major degeneration of the outer segments as compared to the inner segments. Following our hypothesis that the third outer hyperreflective OCT band corresponds to phagosomes, the previous results can be interpreted as that the

restoration of the Rs1 gene improves the morphology of the inner and outer photoreceptor segments and enhances the RPE shedding function. The recovery of the outer segments and of the RPE shedding would result in the accumulation and recovery of phagosomes and in the restoration of the third hyperreflective band. In other mice model of retinal degeneration (miR-182-knockout), the second and third hyperreflective bands also appeared as a single larger band (Wu et al., 2019), which would be explained by the progressive outer segment loss. In agreement with this, the inhibition of microRNAs 182 and 183 in non-human primates revealed an alteration in the second and third outer retinal bands by OCT (Walters et al., 2019). This fact could be explained by the RPE degeneration (with drastic decrease in RPE pigmentation and all-trans-retinol production) and the outer segment loss observed in histology sections (Walters et al., 2019). The fact that the inner segments and cell bodies of photoreceptors remained intact in these images, reinforces our hypothesis about the third band interpretation involving outer segment shedding by RPE.

In addition to ONL thickness measurement by OCT, the study of the four outer hyperreflective bands could be useful to analyze the integrity of photoreceptors and their relationship to the RPE. If the use of a specific therapy is able to preserve or

reestablish these four outer hyperreflective OCT bands, it would indicate a restoration of mitochondria health in the photoreceptor ellipsoids and of outer segment phagocytosis by the RPE, both indicating the state of photoreceptors and RPE cells.

Hence, from our point of view, OCT can be considered a good complementary tool in animal studies of retinal neuroprotection, but it still does not have enough resolution power to detect small changes in photoreceptor cell rows, or in retinal bands that are difficult to discern, such as the GCL. Additional studies are also needed to determine how to interpret changes in the reflectivity intensity of the OCT bands. Nowadays, OCT cannot replace the high resolution of histological images, or other functional and behavioral analysis, all of them needed to determine the effectiveness of neuroprotective therapies. However, OCT has an important advantage compared to immunohistochemistry technique allowing to monitor along the time the success of the therapy strategies.

3.4. Correlation of OCTA and histology in animal models

The development of the OCTA tool is an important advance for research and clinical ophthalmology, as it provides a high-resolution view of the vascular structures in the

retina and optic nerve head and allows monitoring vascular changes *in vivo*. This technique is useful to study vascular impairment in retinal degenerative diseases, as the progression of the degeneration is usually accompanied by dramatic changes in the vascular network. These changes are relevant, because the disturbed balance between oxygen supply and oxygen consumption can trigger retinal diseases and can be the cause of exacerbation in many retinal pathological conditions (reviewed by Cuenca et al., 2014a). Furthermore, OCTA makes it possible to conduct longitudinal monitoring of vascular degeneration, which in turn leads to the possibility of analyzing the effects of pharmacological treatments (Alnawaiseh et al., 2017).

3.4.1. Correlation between histology of retinal vasculature and OCTA images in mice

A correct interpretation of OCTA images requires in-depth knowledge of the normal vascular architecture of the retina. There are differences in retinal vasculature between humans and rodents, which can be explained by specific retinal structure differences that determine the vascular distribution. As an example, the FAZ described in humans does not exist in rodents, because of the absence of the fovea. In addition, there is not an equal number of vascular plexuses, and these plexuses are not located in exactly

the same retinal layer. As previously mentioned in section 2.4., in human retinas, we can find one, two, three or four vascular plexuses, depending of the zone. In rodents, the retinal vascular structure is organized into three connected plexuses except at the most peripheral retina where there are only two. Fig. 15 shows the three vascular plexuses evidenced by immunohistochemistry and OCTA in mice, which are the superficial vascular plexus (SVP), the intermediate capillary plexus (ICP) and the deep capillary plexus (DCP). Blood vessels enter the retina through the central retinal artery, which branches out and forms the SVP in the RGC layer (Fig. 15A, B, G). From this plexus, some branches sprout vertically, forming the ICP at the IPL/INL margin (Fig. 15C, D, H), which is a plexus of communication between the SVP and the DCP. The DCP is composed of capillary loops that can be seen using OCTA and immunohistochemistry in the OPL level (Fig. 15E, F, I arrowheads). These DCP loops can be quantified using both techniques, and may be indicative of degenerative processes (Fernandez-Sanchez et al., 2018). Finally, vessels leave the tissue through the central retinal vein from the SVP (revised in Cuenca et al., 2014a). Fig. 15G-I shows the different distribution of the three plexuses throughout the entire retina.

4. Summary

OCT and OCTA have become essential tools in biomedical imaging and diagnostics, widely used to monitor a broad spectrum of eye diseases, providing *in vivo* images of the retina. Their use has become indispensable in ophthalmology and they are increasingly used in basic, clinic and translational research of the retina.

This review shows a good correlation between the retinal bands observed in OCT and the histological layers of the retina. Here, we propose an interpretation of the four outer hyperreflective OCT bands with a correspondence to retinal histology, whose integrity determines the photoreceptor and retinal pigment epithelium health. Impairment or recovery of these bands and their relationship with visual acuity should be analyzed to achieve the adequate diagnosis and prognosis of retinal degenerative processes and their therapeutic approaches. Thus, the state of these outer bands should be always monitored in order to know the progression or recovery of retinal function; in addition to the common evaluations that consider total retinal thickness, detection of cysts, edema, retinal detachment, or other phenomena.

The interpretation of OCT images must be done carefully, considering OCT intrinsic limitations. For example, differences in OCT measures that are below the limit of

resolution of the device, even if they are statistically significant, should not be taken into consideration: they are within the margin of instrument error and they may not imply the existence of significant changes at a cellular level. Furthermore, a careful interpretation is also important because OCT bands correspond to retinal layers formed by several cell types, as in the case of ganglion cell layer, in which changes detected could be not only due to retinal ganglion cells but also to displaced amacrine cells and astrocytes. In addition, it must be considered that the automatic segmentation performed by the device is not always correct, especially during retinal degeneration. A deep histological knowledge of the retina in healthy and pathological conditions would allow the users to identify segmentation errors and to manually segment the obtained images when necessary.

OCT images are usually obtained in central retina, losing information of the peripheral area, which is anatomically different and may be affected by distinct degenerative processes. In humans the most studied region by OCT is the fovea, a specialized structure with specific cellular distribution, localized in the central retina. Correlation studies between OCT and immunohistochemistry images in this area allow the correct cellular interpretation of each OCT band in health and disease. In this context Henle

fibers layer, which is formed by Müller cells and axons of cones, are a relevant structure that should be analyzed by OCT in order to know the pathological condition in retinal diseases.

The morphological features and the number of vascular plexuses varies in the different regions of the healthy human retina observed by OCTA and immunohistochemistry. In this line, it can be observed four, three, two or one plexus depending of the distance from the fovea. Also, specific structures are found in the different plexuses, as is the case of the vascular loops found in the intermediate capillary plexus or the small interconnected capillaries with spider-like appearance in the deep capillary plexus. This should be considered in clinics because changes in these structural characteristics could be impaired during the retinal pathologies that are associated with a vessel degeneration.

OCT has become a useful tool in retinal pathology research with animal models allowing to measure retinal layers easily and harmless, without the sacrifice of animals, and performing precise longitudinal studies in a way not allowed by immunohistochemically analysis. Therefore, the use of both techniques is strongly recommended in the study of retinal degeneration, since they complement each other.

Moreover, the different animal species used as models in vision science have different retinal structures, with different morphology, thickness and cellular types. These variations need to be known using immunohistochemistry to explain the different band pattern found in OCT images.

Retinal thickness measurement and changes in the reflectivity of the outer bands by OCT give information of the retinal state during different degenerative processes in animal models with a photoreceptor loss. Once the peak of degeneration occurs, OCT can still monitor other processes of the disease like retinal detachment or the migration columns that appears as retinal remodeling events.

In regard to the development of therapeutic strategies in retinal degenerative models, it may be concluded that only a sufficiently large change of the retinal thickness can be detected *in vivo* by OCT. Moreover, at this moment OCT cannot replace morphological and physiological studies of retinal functionality, which are essential to analyze the maintenance or restoration of function of any therapy.

5. Future directions

Nowadays, OCT imaging is a widely used technology that allows the *in vivo* visualization and analysis of the retina in a non-invasive and reliable way. Although its current utility is undeniable, the technological improvement, the development of new uses or the clarification of band interpretations, will make OCT even more useful in the ophthalmological clinic and research.

First, OCT devices with increasingly better resolution power will allow, in a near future, the accurate identification of cellular structures and layers, being more similar to histological images and facilitating the correct interpretation of OCT images. An example of higher resolution equipment is adaptive optics (AO), which is being developed in this direction. Doing more studies with current and future OCT instruments both in health and disease, in patients and animal models, would help, among other things, to clarify the interpretation of the four outer OCT bands and to reach an accurate consensus. Since the different interpretations of each OCT band would have different clinical implications, clarifying this issue would improve the clinical ophthalmological practice in the diagnosis, treatment, and follow-up of retinal pathologies.

In addition, the development of new analysis tools and software for both OCT and OCTA would improve the amount of information that users are able to obtain from images. In their development, standardization of analysis and interpretation criteria should be taken into account in order to make comparable the studies done by different instruments and users, not yet totally comparable today. Also, the development of OCT and OCTA technology or software capable of analyzing not only the central region but also more peripheral areas would help in the analysis and diagnosis of retinal diseases preferentially affecting the periphery.

Last, the combination of adaptive optics, confocal scanning laser ophthalmoscopy, and fluorescence detectors (FAOSLO) would enable the *in vivo* visualization of specific, fluorescently marked, retinal cells. This approximation has been yet successfully used to identify apoptotic retinal ganglion cells (Yap et al., 2018) or microglial cells (Zawadzki et al., 2015) in rodent models. Using the adequate fluorescent marker, this combination will allow to identify specific retinal cells of interest and to locate them in a retinal area and layer. Also, combining these techniques, the use of the retina as an early biomarker of neurodegenerative diseases could be proven and further validated, as for the identification of curcumin and β -amyloid plaques in Alzheimer's disease (Koronyo-

Hamaoui et al., 2011), or phosphorylated- α -synuclein accumulation in Parkinson's disease (Ortuno-Lizaran et al., 2018).

In conclusion, a deep knowledge of the retinal cell structure and remodeling during degeneration and therapy, combined with the advances of the OCT and OCTA in a near future, will allow the improvement of the ophthalmological practice.

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7. Technical Equipment

In this work, OCT and OCTA images were obtained using the OCT Spectralis imaging platform (Heidelberg Engineering, Germany). Confocal images were obtained in a Leica TCS SP8 confocal microscopy (Leica Microsystems, Wetzlar, Germany).

8. References

- Aboelela, S.W., Robinson, D.W., 2004. Physiological response properties of displaced amacrine cells of the adult ferret retina. *Vis Neurosci* 21, 135-144.
- Adachi, K., Takahashi, S., Yamauchi, K., Mounai, N., Tanabu, R., Nakazawa, M., 2016. Optical Coherence Tomography of Retinal Degeneration in Royal College of Surgeons Rats and Its Correlation with Morphology and Electroretinography. *PLoS One* 11, e0162835.
- Adhi, M., Duker, J.S., 2013. Optical coherence tomography--current and future applications. *Curr Opin Ophthalmol* 24, 213-221.
- Ahn, S.M., Ahn, J., Cha, S., Yun, C., Park, T.K., Goo, Y.S., Kim, S.W., 2019. Development of a Post-vitrectomy Injection of N-methyl-N-nitrosourea as a Localized Retinal Degeneration Rabbit Model. *Exp Neurobiol* 28, 62-73.
- Aizawa, S., Mitamura, Y., Baba, T., Hagiwara, A., Ogata, K., Yamamoto, S., 2009. Correlation between visual function and photoreceptor inner/outer segment junction in patients with retinitis pigmentosa. *Eye (Lond)* 23, 304-308.
- Alex, A.F.A., M.; Heiduschka, P.; Eter, N. , 2019. Retinal Fundus Imaging in Mouse Models of Retinal Diseases., in: Weber B., L.T. (Ed.), *Retinal Degeneration. Methods in Molecular Biology*. Humana Press, New York, NY.
- Alnawaiseh, M., Brand, C., Bormann, E., Wistuba, J., Eter, N., Heiduschka, P., 2017. Quantitative analysis of retinal perfusion in mice using optical coherence tomography angiography. *Exp Eye Res* 164, 151-156.

- Anger, E.M., Unterhuber, A., Hermann, B., Sattmann, H., Schubert, C., Morgan, J.E., Cowey, A., Ahnelt, P.K., Drexler, W., 2004. Ultrahigh resolution optical coherence tomography of the monkey fovea. Identification of retinal sublayers by correlation with semithin histology sections. *Exp Eye Res* 78, 1117-1125.
- Aplin, F.P., Luu, C.D., Vessey, K.A., Guymer, R.H., Shepherd, R.K., Fletcher, E.L., 2014. ATP-induced photoreceptor death in a feline model of retinal degeneration. *Invest Ophthalmol Vis Sci* 55, 8319-8329.
- Balaratnasingam, C., An, D., Sakurada, Y., Lee, C.S., Lee, A.Y., McAllister, I.L., Freund, K.B., Sarunic, M., Yu, D.Y., 2018. Comparisons Between Histology and Optical Coherence Tomography Angiography of the Periarterial Capillary-Free Zone. *Am J Ophthalmol* 189, 55-64.
- Barhoum, R., Martinez-Navarrete, G., Corrochano, S., Germain, F., Fernandez-Sanchez, L., de la Rosa, E.J., de la Villa, P., Cuenca, N., 2008. Functional and structural modifications during retinal degeneration in the rd10 mouse. *Neuroscience* 155, 698-713.
- Bartuma, H., Petrus-Reurer, S., Aronsson, M., Westman, S., Andre, H., Kvanta, A., 2015. In Vivo Imaging of Subretinal Bleb-Induced Outer Retinal Degeneration in the Rabbit. *Invest Ophthalmol Vis Sci* 56, 2423-2430.
- Berger, A., Cavallero, S., Dominguez, E., Barbe, P., Simonutti, M., Sahel, J.A., Sennlaub, F., Raoul, W., Paques, M., Bemelmans, A.P., 2014. Spectral-domain optical coherence tomography of the rodent eye: highlighting layers of the outer retina using signal averaging and comparison with histology. *PLoS One* 9, e96494.
- Bill, A., 1984. Circulation in the eye., in: Renkin EM, M.C. (Ed.), *Handbook in physiology. Microcirculation, Part 2*. The American Physiological Society pp. 1001-1034.

- Bringmann, A., Pannicke, T., Grosche, J., Francke, M., Wiedemann, P., Skatchkov, S.N., Osborne, N.N., Reichenbach, A., 2006. Muller cells in the healthy and diseased retina. *Prog Retin Eye Res* 25, 397-424.
- Bubis, E., Sher, I., Skaat, A., Sharvit-Ginon, I., Szalapak, A.M., Moroz, I., Kalter-Leibovici, O., Rotenstreich, Y., 2019. Blue Autofluorescence Fundus Imaging for Monitoring Retinal Degeneration in Royal College of Surgeons Rats. *Transl Vis Sci Technol* 8, 26.
- Camp, A.S., Ruggeri, M., Munguba, G.C., Tapia, M.L., John, S.W., Bhattacharya, S.K., Lee, R.K., 2011. Structural correlation between the nerve fiber layer and retinal ganglion cell loss in mice with targeted disruption of the *Brn3b* gene. *Invest Ophthalmol Vis Sci* 52, 5226-5232.
- Campbell, J.P., Zhang, M., Hwang, T.S., Bailey, S.T., Wilson, D.J., Jia, Y., Huang, D., 2017. Detailed Vascular Anatomy of the Human Retina by Projection-Resolved Optical Coherence Tomography Angiography. *Sci Rep* 7, 42201.
- Catier, A., Tadayoni, R., Paques, M., Erginay, A., Haouchine, B., Gaudric, A., Massin, P., 2005. Characterization of macular edema from various etiologies by optical coherence tomography. *Am J Ophthalmol* 140, 200-206.
- Chan, G., Balaratnasingam, C., Yu, P.K., Morgan, W.H., McAllister, I.L., Cringle, S.J., Yu, D.Y., 2012. Quantitative morphometry of perifoveal capillary networks in the human retina. *Invest Ophthalmol Vis Sci* 53, 5502-5514.
- Chang, B., Hawes, N.L., Hurd, R.E., Davisson, M.T., Nusinowitz, S., Heckenlively, J.R., 2002. Retinal degeneration mutants in the mouse. *Vision Res* 42, 517-525.

- Chang-Ling, T., 2010. Development of retinal vasculature., in: Dartt D, D.R., D'Amore P, Niederkorn J (Ed.), Immunology, inflammation and diseases of the eye. Elsevier, pp. 193-204.
- Cheng, J., Sohn, E.H., Jiao, C., Adler, K.L., Kaalberg, E.E., Russell, S.R., Mullins, R.F., Stone, E.M., Tucker, B.A., Han, I.C., 2018. Correlation of Optical Coherence Tomography and Retinal Histology in Normal and Pro23His Retinal Degeneration Pig. *Transl Vis Sci Technol* 7, 18.
- Cuenca, N., Fernandez-Sanchez, L., Campello, L., Maneu, V., De la Villa, P., Lax, P., Pinilla, I., 2014a. Cellular responses following retinal injuries and therapeutic approaches for neurodegenerative diseases. *Prog Retin Eye Res* 43, 17-75.
- Cuenca, N., Fernandez-Sanchez, L., Sauve, Y., Segura, F.J., Martinez-Navarrete, G., Tamarit, J.M., Fuentes-Broto, L., Sanchez-Cano, A., Pinilla, I., 2014b. Correlation between SD-OCT, immunocytochemistry and functional findings in an animal model of retinal degeneration. *Front Neuroanat* 8, 151.
- Cuenca, N., Ortuno-Lizaran, I., Pinilla, I., 2018. Cellular Characterization of OCT and Outer Retinal Bands Using Specific Immunohistochemistry Markers and Clinical Implications. *Ophthalmology* 125, 407-422.
- Daruich, A., Matet, A., Moulin, A., Kowalczyk, L., Nicolas, M., Sellam, A., Rothschild, P.R., Omri, S., Gelize, E., Jonet, L., Delaunay, K., De Kozak, Y., Berdugo, M., Zhao, M., Crisanti, P., Behar-Cohen, F., 2018. Mechanisms of macular edema: Beyond the surface. *Prog Retin Eye Res* 63, 20-68.

- dell'Omo, R., Tan, H.S., Schlingemann, R.O., Bijl, H.M., Lesnik Oberstein, S.Y., Barca, F., Mura, M., 2012. Evolution of outer retinal folds occurring after vitrectomy for retinal detachment repair. *Invest Ophthalmol Vis Sci* 53, 7928-7935.
- dell'Omo, R., Viggiano, D., Giorgio, D., Filippelli, M., Di Iorio, R., Calo', R., Cardone, M., Rinaldi, M., dell'Omo, E., Costagliola, C., 2015. Restoration of foveal thickness and architecture after macula-off retinal detachment repair. *Invest Ophthalmol Vis Sci* 56, 1040-1050.
- Drack, A.V., Dumitrescu, A.V., Bhattarai, S., Gratie, D., Stone, E.M., Mullins, R., Sheffield, V.C., 2012. TUDCA slows retinal degeneration in two different mouse models of retinitis pigmentosa and prevents obesity in Bardet-Biedl syndrome type 1 mice. *Invest Ophthalmol Vis Sci* 53, 100-106.
- Drexler, W., Fujimoto, J.G., 2008. State-of-the-art retinal optical coherence tomography. *Prog Retin Eye Res* 27, 45-88.
- Drexler, W., Morgner, U., Ghanta, R.K., Kartner, F.X., Schuman, J.S., Fujimoto, J.G., 2001. Ultrahigh-resolution ophthalmic optical coherence tomography. *Nat Med* 7, 502-507.
- Dubra, A., Sulai, Y., Norris, J.L., Cooper, R.F., Dubis, A.M., Williams, D.R., Carroll, J., 2011. Noninvasive imaging of the human rod photoreceptor mosaic using a confocal adaptive optics scanning ophthalmoscope. *Biomed Opt Express* 2, 1864-1876.
- Dysli, C., Wolf, S., Berezin, M.Y., Sauer, L., Hammer, M., Zinkernagel, M.S., 2017. Fluorescence lifetime imaging ophthalmoscopy. *Prog Retin Eye Res* 60, 120-143.
- Fan, S., Ding, X., Rao, P., Zheng, Y., Mao, F., Hu, Y., Liu, X., Fan, G., 2019. Multimodal imaging of the retina and choroid in healthy *Macaca fascicularis* at different ages. *Graefes Arch Clin Exp Ophthalmol* 257, 455-463.

- Fernandez-Sanchez, L., Bravo-Osuna, I., Lax, P., Arranz-Romera, A., Maneu, V., Esteban-Perez, S., Pinilla, I., Puebla-Gonzalez, M.D.M., Herrero-Vanrell, R., Cuenca, N., 2017. Controlled delivery of tauroursodeoxycholic acid from biodegradable microspheres slows retinal degeneration and vision loss in P23H rats. *PLoS One* 12, e0177998.
- Fernandez-Sanchez, L., Esquiva, G., Pinilla, I., Lax, P., Cuenca, N., 2018. Retinal Vascular Degeneration in the Transgenic P23H Rat Model of Retinitis Pigmentosa. *Front Neuroanat* 12, 55.
- Fernandez-Sanchez, L., Lax, P., Campello, L., Pinilla, I., Cuenca, N., 2015. Astrocytes and Muller Cell Alterations During Retinal Degeneration in a Transgenic Rat Model of Retinitis Pigmentosa. *Front Cell Neurosci* 9, 484.
- Fernandez-Sanchez, L., Lax, P., Esquiva, G., Martin-Nieto, J., Pinilla, I., Cuenca, N., 2012a. Safranal, a saffron constituent, attenuates retinal degeneration in P23H rats. *PLoS One* 7, e43074.
- Fernandez-Sanchez, L., Lax, P., Isiegas, C., Ayuso, E., Ruiz, J.M., de la Villa, P., Bosch, F., de la Rosa, E.J., Cuenca, N., 2012b. Proinsulin slows retinal degeneration and vision loss in the P23H rat model of retinitis pigmentosa. *Hum Gene Ther* 23, 1290-1300.
- Fernandez-Sanchez, L., Lax, P., Pinilla, I., Martin-Nieto, J., Cuenca, N., 2011. Tauroursodeoxycholic acid prevents retinal degeneration in transgenic P23H rats. *Invest Ophthalmol Vis Sci* 52, 4998-5008.
- Fischer, M.D., Huber, G., Beck, S.C., Tanimoto, N., Muehlfriedel, R., Fahl, E., Grimm, C., Wenzel, A., Reme, C.E., van de Pavert, S.A., Wijnholds, J., Pacal, M., Bremner, R., Seeliger, M.W., 2009. Noninvasive, in vivo assessment of mouse retinal structure using optical coherence tomography. *PLoS One* 4, e7507.

- Fleckenstein, M., Charbel Issa, P., Helb, H.M., Schmitz-Valckenberg, S., Finger, R.P., Scholl, H.P., Loeffler, K.U., Holz, F.G., 2008. High-resolution spectral domain-OCT imaging in geographic atrophy associated with age-related macular degeneration. *Invest Ophthalmol Vis Sci* 49, 4137-4144.
- Gabriele, M.L., Wollstein, G., Ishikawa, H., Kagemann, L., Xu, J., Folio, L.S., Schuman, J.S., 2011. Optical coherence tomography: history, current status, and laboratory work. *Invest Ophthalmol Vis Sci* 52, 2425-2436.
- Gadjanski, I., Williams, S.K., Hein, K., Sattler, M.B., Bahr, M., Diem, R., 2011. Correlation of optical coherence tomography with clinical and histopathological findings in experimental autoimmune uveoretinitis. *Exp Eye Res* 93, 82-90.
- Garcia Garrido, M., Beck, S.C., Muhlriedel, R., Julien, S., Schraermeyer, U., Seeliger, M.W., 2014. Towards a quantitative OCT image analysis. *PLoS One* 9, e100080.
- Gariano, R.F., Iruela-Arispe, M.L., Hendrickson, A.E., 1994. Vascular development in primate retina: comparison of lamellar plexus formation in monkey and human. *Invest Ophthalmol Vis Sci* 35, 3442-3455.
- Gekeler, F., Gmeiner, H., Volker, M., Sachs, H., Messias, A., Eule, C., Bartz-Schmidt, K.U., Zrenner, E., Shinoda, K., 2007. Assessment of the posterior segment of the cat eye by optical coherence tomography (OCT). *Vet Ophthalmol* 10, 173-178.
- Gloesmann, M., Hermann, B., Schubert, C., Sattmann, H., Ahnelt, P.K., Drexler, W., 2003. Histologic correlation of pig retina radial stratification with ultrahigh-resolution optical coherence tomography. *Invest Ophthalmol Vis Sci* 44, 1696-1703.
- Gomez-Vicente, V., Lax, P., Fernandez-Sanchez, L., Rondon, N., Esquivia, G., Germain, F., de la Villa, P., Cuenca, N., 2015. Neuroprotective Effect of Tauroursodeoxycholic Acid

on N-Methyl-D-Aspartate-Induced Retinal Ganglion Cell Degeneration. PLoS One 10, e0137826.

Grozdanic, S.D., Lazic, T., Kecova, H., Mohan, K., Kuehn, M.H., 2018. Optical coherence tomography and molecular analysis of sudden acquired retinal degeneration syndrome (SARDS) eyes suggests the immune-mediated nature of retinal damage. *Vet Ophthalmol*.

Guziewicz, K.E., Cideciyan, A.V., Beltran, W.A., Komaromy, A.M., Dufour, V.L., Swider, M., Iwabe, S., Sumaroka, A., Kendrick, B.T., Ruthel, G., Chiodo, V.A., Heon, E., Hauswirth, W.W., Jacobson, S.G., Aguirre, G.D., 2018. BEST1 gene therapy corrects a diffuse retina-wide microdetachment modulated by light exposure. *Proc Natl Acad Sci U S A* 115, E2839-E2848.

Hanna, J., Yucel, Y.H., Zhou, X., Mathieu, E., Paczka-Giorgi, L.A., Gupta, N., 2018. Progressive loss of retinal blood vessels in a live model of retinitis pigmentosa. *Can J Ophthalmol* 53, 391-401.

Hasegawa, E., Sweigard, H., Husain, D., Olivares, A.M., Chang, B., Smith, K.E., Birsner, A.E., D'Amato, R.J., Michaud, N.A., Han, Y., Vavvas, D.G., Miller, J.W., Haider, N.B., Connor, K.M., 2014. Characterization of a spontaneous retinal neovascular mouse model. *PLoS One* 9, e106507.

Hasegawa, T., Ikeda, H.O., Nakano, N., Muraoka, Y., Tsuruyama, T., Okamoto-Furuta, K., Kohda, H., Yoshimura, N., 2016. Changes in morphology and visual function over time in mouse models of retinal degeneration: an SD-OCT, histology, and electroretinography study. *Jpn J Ophthalmol* 60, 111-125.

Hayreh, S., 2010. Physiological anatomy of the retinal vasculature, in: Dartt D, D.R., D'Amore P, Niederkorn J (Ed.), Immunology, inflammation and diseases of the eye. Elsevier, pp. 207-212.

Hernandez-Merino, E., Kecova, H., Jacobson, S.J., Hamouche, K.N., Nzokwe, R.N., Grozdanic, S.D., 2011. Spectral domain optical coherence tomography (SD-OCT) assessment of the healthy female canine retina and optic nerve. *Vet Ophthalmol* 14, 400-405.

Hitchcock, P.F., Macdonald, R.E., VanDeRyt, J.T., Wilson, S.W., 1996. Antibodies against Pax6 immunostain amacrine and ganglion cells and neuronal progenitors, but not rod precursors, in the normal and regenerating retina of the goldfish. *J Neurobiol* 29, 399-413.

Ho, J.K., Stanford, M.P., Shariati, M.A., Dalal, R., Liao, Y.J., 2013. Optical coherence tomography study of experimental anterior ischemic optic neuropathy and histologic confirmation. *Invest Ophthalmol Vis Sci* 54, 5981-5988.

Hogan, M.J., Alvarado, J.A., Wedell, J.E., 1971. Retina, Histology of the human eye: an atlas and textbook. Saunders, pp. 508-519.

Huang, D., Swanson, E.A., Lin, C.P., Schuman, J.S., Stinson, W.G., Chang, W., Hee, M.R., Flotte, T., Gregory, K., Puliafito, C.A., et al., 1991. Optical coherence tomography. *Science* 254, 1178-1181.

Huber, G., Beck, S.C., Grimm, C., Sahaboglu-Tekgoz, A., Paquet-Durand, F., Wenzel, A., Humphries, P., Redmond, T.M., Seeliger, M.W., Fischer, M.D., 2009. Spectral domain optical coherence tomography in mouse models of retinal degeneration. *Invest Ophthalmol Vis Sci* 50, 5888-5895.

- Jeon, C.J., Strettoi, E., Masland, R.H., 1998. The major cell populations of the mouse retina. *J Neurosci* 18, 8936-8946.
- Jones, B.W., Marc, R.E., 2005. Retinal remodeling during retinal degeneration. *Exp Eye Res* 81, 123-137.
- Jonnal, R.S., Gorczynska, I., Migacz, J.V., Azimipour, M., Zawadzki, R.J., Werner, J.S., 2017. The Properties of Outer Retinal Band Three Investigated With Adaptive-Optics Optical Coherence Tomography. *Invest Ophthalmol Vis Sci* 58, 4559-4568.
- Jonnal, R.S., Kocaoglu, O.P., Zawadzki, R.J., Lee, S.H., Werner, J.S., Miller, D.T., 2014. The cellular origins of the outer retinal bands in optical coherence tomography images. *Invest Ophthalmol Vis Sci* 55, 7904-7918.
- Kashani, A.H., Chen, C.L., Gahm, J.K., Zheng, F., Richter, G.M., Rosenfeld, P.J., Shi, Y., Wang, R.K., 2017. Optical coherence tomography angiography: A comprehensive review of current methods and clinical applications. *Prog Retin Eye Res* 60, 66-100.
- Kiernan, D.F., Zelkha, R., Hariprasad, S.M., Lim, J.I., Blair, M.P., Mieler, W.F., 2012. En face spectral-domain optical coherence tomography outer retinal analysis and relation to visual acuity. *Retina* 32, 1077-1086.
- Kim, K.H., Puoris'haag, M., Maguluri, G.N., Umino, Y., Cusato, K., Barlow, R.B., de Boer, J.F., 2008. Monitoring mouse retinal degeneration with high-resolution spectral-domain optical coherence tomography. *J Vis* 8, 17 11-11.
- Kim, T.H., Son, T., Lu, Y., Alam, M., Yao, X., 2018. Comparative Optical Coherence Tomography Angiography of Wild-Type and rd10 Mouse Retinas. *Transl Vis Sci Technol* 7, 42.

- Kocaoglu, O.P., Cense, B., Jonnal, R.S., Wang, Q., Lee, S., Gao, W., Miller, D.T., 2011. Imaging retinal nerve fiber bundles using optical coherence tomography with adaptive optics. *Vision Res* 51, 1835-1844.
- Kocaoglu, O.P., Liu, Z., Zhang, F., Kurokawa, K., Jonnal, R.S., Miller, D.T., 2016. Photoreceptor disc shedding in the living human eye. *Biomed Opt Express* 7, 4554-4568.
- Koinzer, S., Hesse, C., Caliebe, A., Saeger, M., Baade, A., Schlott, K., Brinkmann, R., Roider, J., 2013a. Photocoagulation in rabbits: optical coherence tomographic lesion classification, wound healing reaction, and retinal temperatures. *Lasers Surg Med* 45, 427-436.
- Koinzer, S., Saeger, M., Hesse, C., Portz, L., Kleemann, S., Schlott, K., Brinkmann, R., Roider, J., 2013b. Correlation with OCT and histology of photocoagulation lesions in patients and rabbits. *Acta Ophthalmol* 91, e603-611.
- Koronyo-Hamaoui, M., Koronyo, Y., Ljubimov, A.V., Miller, C.A., Ko, M.K., Black, K.L., Schwartz, M., Farkas, D.L., 2011. Identification of amyloid plaques in retinas from Alzheimer's patients and noninvasive in vivo optical imaging of retinal plaques in a mouse model. *Neuroimage* 54 Suppl 1, S204-217.
- Ku, C.A., Ryals, R.C., Jiang, D., Coyner, A.S., Weller, K.K., Sinha, W., Robb, B.M., Yang, P., Pennesi, M.E., 2018. The Role of ERK1/2 Activation in Sarpogrelate-Mediated Neuroprotection. *Invest Ophthalmol Vis Sci* 59, 462-471.
- Lax, P., Esquivia, G., Altavilla, C., Cuenca, N., 2014. Neuroprotective effects of the cannabinoid agonist HU210 on retinal degeneration. *Exp Eye Res* 120, 175-185.

- Lax, P., Kutsyr, O., Esquiva, G., Altavilla, C., Maneu, V., Cuenca, N., 2019. Cannabinoid-mediated retinal rescue correlates with improved circadian parameters in retinal dystrophic rats. *Exp Eye Res* 180, 192-199.
- Lee, W., Noupou, K., Oll, M., Duncker, T., Burke, T., Zernant, J., Bearely, S., Tsang, S.H., Sparrow, J.R., Allikmets, R., 2014. The external limiting membrane in early-onset Stargardt disease. *Invest Ophthalmol Vis Sci* 55, 6139-6149.
- Lheriteau, E., Libeau, L., Stieger, K., Deschamps, J.Y., Mendes-Madeira, A., Provost, N., Lemoine, F., Mellersh, C., Ellinwood, N.M., Cherel, Y., Moullier, P., Rolling, F., 2009. The RPGRIP1-deficient dog, a promising canine model for gene therapy. *Mol Vis* 15, 349-361.
- Linberg, K., Cuenca, N., Ahnelt, P., Fisher, S., Kolb, H., 2001. Comparative anatomy of major retinal pathways in the eyes of nocturnal and diurnal mammals. *Prog Brain Res* 131, 27-52.
- Litts, K.M., Messinger, J.D., Freund, K.B., Zhang, Y., Curcio, C.A., 2015. Inner Segment Remodeling and Mitochondrial Translocation in Cone Photoreceptors in Age-Related Macular Degeneration With Outer Retinal Tubulation. *Invest Ophthalmol Vis Sci* 56, 2243-2253.
- Litts, K.M., Wang, X., Clark, M.E., Owsley, C., Freund, K.B., Curcio, C.A., Zhang, Y., 2017. Exploring Photoreceptor Reflectivity through Multimodal Imaging of Outer Retinal Tubulation in Advanced Age-Related Macular Degeneration. *Retina* 37, 978-988.
- Litts, K.M., Zhang, Y., Freund, K.B., Curcio, C.A., 2018. Optical Coherence Tomography and Histology of Age-Related Macular Degeneration Support Mitochondria as Reflectivity Sources. *Retina* 38, 445-461.

- Liu, Z., Kocaoglu, O.P., Miller, D.T., 2016. 3D Imaging of Retinal Pigment Epithelial Cells in the Living Human Retina. *Invest Ophthalmol Vis Sci* 57, OCT533-543.
- Liu, Z., Kurokawa, K., Zhang, F., Lee, J.J., Miller, D.T., 2017. Imaging and quantifying ganglion cells and other transparent neurons in the living human retina. *Proc Natl Acad Sci U S A* 114, 12803-12808.
- Makita, S., Hong, Y., Yamanari, M., Yatagai, T., Yasuno, Y., 2006. Optical coherence angiography. *Opt Express* 14, 7821-7840.
- Mammo, Z., Balaratnasingam, C., Yu, P., Xu, J., Heisler, M., Mackenzie, P., Merkur, A., Kirker, A., Albiani, D., Freund, K.B., Sarunic, M.V., Yu, D.Y., 2015. Quantitative Noninvasive Angiography of the Fovea Centralis Using Speckle Variance Optical Coherence Tomography. *Invest Ophthalmol Vis Sci* 56, 5074-5086.
- Marc, R.E., Jones, B.W., 2003. Retinal remodeling in inherited photoreceptor degenerations. *Mol Neurobiol* 28, 139-147.
- Marc, R.E., Jones, B.W., Watt, C.B., Strettoi, E., 2003. Neural remodeling in retinal degeneration. *Prog Retin Eye Res* 22, 607-655.
- Matsunaga, D., Yi, J., Puliafito, C.A., Kashani, A.H., 2014. OCT angiography in healthy human subjects. *Ophthalmic Surg Lasers Imaging Retina* 45, 510-515.
- McLellan, G.J., Rasmussen, C.A., 2012. Optical coherence tomography for the evaluation of retinal and optic nerve morphology in animal subjects: practical considerations. *Vet Ophthalmol* 15 Suppl 2, 13-28.
- Merino, D., Duncan, J.L., Tiruveedhula, P., Roorda, A., 2011. Observation of cone and rod photoreceptors in normal subjects and patients using a new generation adaptive optics scanning laser ophthalmoscope. *Biomed Opt Express* 2, 2189-2201.

- Meyer, C.H., Hotta, K., Peterson, W.M., Toth, C.A., Jaffe, G.J., 2002. Effect of INS37217, a P2Y(2) receptor agonist, on experimental retinal detachment and electroretinogram in adult rabbits. *Invest Ophthalmol Vis Sci* 43, 3567-3574.
- Milam, A.H., Li, Z.Y., Fariss, R.N., 1998. Histopathology of the human retina in retinitis pigmentosa. *Prog Retin Eye Res* 17, 175-205.
- Miller, A.R., Roisman, L., Zhang, Q., Zheng, F., Rafael de Oliveira Dias, J., Yehoshua, Z., Schaal, K.B., Feuer, W., Gregori, G., Chu, Z., Chen, C.L., Kubach, S., An, L., Stetson, P.F., Durbin, M.K., Wang, R.K., Rosenfeld, P.J., 2017. Comparison Between Spectral-Domain and Swept-Source Optical Coherence Tomography Angiographic Imaging of Choroidal Neovascularization. *Invest Ophthalmol Vis Sci* 58, 1499-1505.
- Minella, A.L., Occelli, L.M., Narfstrom, K., Petersen-Jones, S.M., 2018. Central retinal preservation in rdAc cats. *Vet Ophthalmol* 21, 224-232.
- Mitamura, Y., Mitamura-Aizawa, S., Katome, T., Naito, T., Hagiwara, A., Kumagai, K., Yamamoto, S., 2013. Photoreceptor impairment and restoration on optical coherence tomographic image. *J Ophthalmol* 2013, 518170.
- Mo, S., Krawitz, B., Efsthadiadis, E., Geyman, L., Weitz, R., Chui, T.Y., Carroll, J., Dubra, A., Rosen, R.B., 2016. Imaging Foveal Microvasculature: Optical Coherence Tomography Angiography Versus Adaptive Optics Scanning Light Ophthalmoscope Fluorescein Angiography. *Invest Ophthalmol Vis Sci* 57, OCT130-140.
- Moisseiev, E., Smit-McBride, Z., Oltjen, S., Zhang, P., Zawadzki, R.J., Motta, M., Murphy, C.J., Cary, W., Annett, G., Nolte, J.A., Park, S.S., 2016. Intravitreal Administration of Human Bone Marrow CD34+ Stem Cells in a Murine Model of Retinal Degeneration. *Invest Ophthalmol Vis Sci* 57, 4125-4135.

- Monai, N., Yamauchi, K., Tanabu, R., Gonome, T., Ishiguro, S.I., Nakazawa, M., 2018. Characterization of photoreceptor degeneration in the rhodopsin P23H transgenic rat line 2 using optical coherence tomography. *PLoS One* 13, e0193778.
- Muftuoglu, I.K., Mendoza, N., Gaber, R., Alam, M., You, Q., Freeman, W.R., 2017. Integrity of outer retinal layers after resolution of central involved diabetic macular edema. *Retina* 37, 2015-2024.
- Muniz, J.A., de Athaide, L.M., Gomes, B.D., Finlay, B.L., Silveira, L.C., 2014. Ganglion cell and displaced amacrine cell density distribution in the retina of the howler monkey (*Alouatta caraya*). *PLoS One* 9, e115291.
- Muraoka, Y., Ikeda, H.O., Nakano, N., Hangai, M., Toda, Y., Okamoto-Furuta, K., Kohda, H., Kondo, M., Terasaki, H., Kakizuka, A., Yoshimura, N., 2012. Real-time imaging of rabbit retina with retinal degeneration by using spectral-domain optical coherence tomography. *PLoS One* 7, e36135.
- Nakamura, T., Ueda-Consolvo, T., Oiwake, T., Hayashi, A., 2016. Correlation between outer retinal layer thickness and cone density in patients with resolved central serous chorioretinopathy. *Graefes Arch Clin Exp Ophthalmol* 254, 2347-2354.
- Nakano, N., Ikeda, H.O., Hangai, M., Muraoka, Y., Toda, Y., Kakizuka, A., Yoshimura, N., 2011. Longitudinal and simultaneous imaging of retinal ganglion cells and inner retinal layers in a mouse model of glaucoma induced by N-methyl-D-aspartate. *Invest Ophthalmol Vis Sci* 52, 8754-8762.
- Nishioka, C., Liang, H.F., Barsamian, B., Sun, S.W., 2019. Sequential phases of RGC axonal and somatic injury in EAE mice examined using DTI and OCT. *Mult Scler Relat Disord* 27, 315-323.

- Noailles, A., Maneu, V., Campello, L., Gomez-Vicente, V., Lax, P., Cuenca, N., 2016. Persistent inflammatory state after photoreceptor loss in an animal model of retinal degeneration. *Sci Rep* 6, 33356.
- Occelli, L.M., Tran, N.M., Narfstrom, K., Chen, S., Petersen-Jones, S.M., 2016. CrxRdy Cat: A Large Animal Model for CRX-Associated Leber Congenital Amaurosis. *Invest Ophthalmol Vis Sci* 57, 3780-3792.
- Ohno, Y., Makita, S., Shimazawa, M., Tsuruma, K., Yasuno, Y., Hara, H., 2013. Thickness mapping of the inner retina by spectral-domain optical coherence tomography in an N-methyl-D-aspartate-induced retinal damage model. *Exp Eye Res* 113, 19-25.
- Ooka, E., Mitamura, Y., Baba, T., Kitahashi, M., Oshitari, T., Yamamoto, S., 2011. Foveal microstructure on spectral-domain optical coherence tomographic images and visual function after macular hole surgery. *Am J Ophthalmol* 152, 283-290 e281.
- Orhan, E., Dalkara, D., Neuille, M., Lechauve, C., Michiels, C., Picaud, S., Leveillard, T., Sahel, J.A., Naash, M.I., Lavail, M.M., Zeitz, C., Audo, I., 2015. Genotypic and phenotypic characterization of P23H line 1 rat model. *PLoS One* 10, e0127319.
- Ortuno-Lizaran, I., Beach, T.G., Serrano, G.E., Walker, D.G., Adler, C.H., Cuenca, N., 2018. Phosphorylated alpha-synuclein in the retina is a biomarker of Parkinson's disease pathology severity. *Mov Disord* 33, 1315-1324.
- Osinchuk, S.C., Leis, M.L., Salpeter, E.M., Sandmeyer, L.S., Grahn, B.H., 2018. Evaluation of retinal morphology of canine sudden acquired retinal degeneration syndrome using optical coherence tomography and fluorescein angiography. *Vet Ophthalmol*.

- Padron-Perez, N., Catala-Mora, J., Diaz, J., Arias, L., Prat, J., Caminal, J.M., 2018. Swept-source and optical coherence tomography angiography in patients with X-linked retinoschisis. *Eye (Lond)* 32, 707-715.
- Pang, J.J., Dai, X., Boye, S.E., Barone, I., Boye, S.L., Mao, S., Everhart, D., Dinculescu, A., Liu, L., Umino, Y., Lei, B., Chang, B., Barlow, R., Strettoi, E., Hauswirth, W.W., 2011. Long-term retinal function and structure rescue using capsid mutant AAV8 vector in the rd10 mouse, a model of recessive retinitis pigmentosa. *Mol Ther* 19, 234-242.
- Patel, N.B., Hung, L.F., Harwerth, R.S., 2017. Postnatal maturation of the fovea in *Macaca mulatta* using optical coherence tomography. *Exp Eye Res* 164, 8-21.
- Pennesi, M.E., Michaels, K.V., Magee, S.S., Maricle, A., Davin, S.P., Garg, A.K., Gale, M.J., Tu, D.C., Wen, Y., Erker, L.R., Francis, P.J., 2012. Long-term characterization of retinal degeneration in rd1 and rd10 mice using spectral domain optical coherence tomography. *Invest Ophthalmol Vis Sci* 53, 4644-4656.
- Perez De Sevilla Muller, L., Shelley, J., Weiler, R., 2007. Displaced amacrine cells of the mouse retina. *J Comp Neurol* 505, 177-189.
- Pinilla, I., Fernandez-Sanchez, L., Segura, F.J., Sanchez-Cano, A.I., Tamarit, J.M., Fuentes-Broto, L., Eells, J.T., Lax, P., Cuenca, N., 2016. Long time remodeling during retinal degeneration evaluated by optical coherence tomography, immunocytochemistry and fundus autofluorescence. *Exp Eye Res* 150, 122-134.
- Pircher, M., Kroisamer, J.S., Felberer, F., Sattmann, H., Gotzinger, E., Hitzenberger, C.K., 2010. Temporal changes of human cone photoreceptors observed in vivo with SLO/OCT. *Biomed Opt Express* 2, 100-112.

- Popescu, D.P., Choo-Smith, L.P., Flueraru, C., Mao, Y., Chang, S., Disano, J., Sherif, S., Sowa, M.G., 2011. Optical coherence tomography: fundamental principles, instrumental designs and biomedical applications. *Biophys Rev* 3, 155.
- Provis, J.M., 2001. Development of the primate retinal vasculature. *Prog Retin Eye Res* 20, 799-821.
- Ramón y Cajal, S., 1893. La rétine des vertébrés. *La cellule* 9, 119-257.
- Roche, S.L., Wyse-Jackson, A.C., Gomez-Vicente, V., Lax, P., Ruiz-Lopez, A.M., Byrne, A.M., Cuenca, N., Cotter, T.G., 2016. Progesterone Attenuates Microglial-Driven Retinal Degeneration and Stimulates Protective Fractalkine-CX3CR1 Signaling. *PLoS One* 11, e0165197.
- Rösch, S., Aretzweiler, C., Muller, F., Walter, P., 2017. Evaluation of Retinal Function and Morphology of the Pink-Eyed Royal College of Surgeons (RCS) Rat: A Comparative Study of in Vivo and in Vitro Methods. *Curr Eye Res* 42, 273-281.
- Rösch, S., Johnen, S., Mataruga, A., Muller, F., Pfarrer, C., Walter, P., 2014a. Selective photoreceptor degeneration by intravitreal injection of N-methyl-N-nitrosourea. *Invest Ophthalmol Vis Sci* 55, 1711-1723.
- Rösch, S., Johnen, S., Muller, F., Pfarrer, C., Walter, P., 2014b. Correlations between ERG, OCT, and Anatomical Findings in the rd10 Mouse. *J Ophthalmol* 2014, 874751.
- Rovere, G., Nadal-Nicolas, F.M., Agudo-Barriuso, M., Sobrado-Calvo, P., Nieto-Lopez, L., Nucci, C., Villegas-Perez, M.P., Vidal-Sanz, M., 2015. Comparison of Retinal Nerve Fiber Layer Thinning and Retinal Ganglion Cell Loss After Optic Nerve Transection in Adult Albino Rats. *Invest Ophthalmol Vis Sci* 56, 4487-4498.

- Rovere, G., Nadal-Nicolas, F.M., Sobrado-Calvo, P., Garcia-Bernal, D., Villegas-Perez, M.P., Vidal-Sanz, M., Agudo-Barriuso, M., 2016. Topical Treatment With Bromfenac Reduces Retinal Gliosis and Inflammation After Optic Nerve Crush. *Invest Ophthalmol Vis Sci* 57, 6098-6106.
- Ryals, R.C., Andrews, M.D., Datta, S., Coyner, A.S., Fischer, C.M., Wen, Y., Pennesi, M.E., McGill, T.J., 2017. Long-term Characterization of Retinal Degeneration in Royal College of Surgeons Rats Using Spectral-Domain Optical Coherence Tomography. *Invest Ophthalmol Vis Sci* 58, 1378-1386.
- Sakata, L.M., Deleon-Ortega, J., Sakata, V., Girkin, C.A., 2009. Optical coherence tomography of the retina and optic nerve - a review. *Clin Exp Ophthalmol* 37, 90-99.
- Sandberg, M.A., Brockhurst, R.J., Gaudio, A.R., Berson, E.L., 2005. The association between visual acuity and central retinal thickness in retinitis pigmentosa. *Invest Ophthalmol Vis Sci* 46, 3349-3354.
- Santos-Ferreira, T., Volkner, M., Borsch, O., Haas, J., Cimalla, P., Vasudevan, P., Carmeliet, P., Corbeil, D., Michalakis, S., Koch, E., Karl, M.O., Ader, M., 2016. Stem Cell-Derived Photoreceptor Transplants Differentially Integrate Into Mouse Models of Cone-Rod Dystrophy. *Invest Ophthalmol Vis Sci* 57, 3509-3520.
- Shariati, M.A., Park, J.H., Liao, Y.J., 2015. Optical coherence tomography study of retinal changes in normal aging and after ischemia. *Invest Ophthalmol Vis Sci* 56, 2790-2797.
- Sho, K., Takahashi, K., Fukuchi, T., Matsumura, M., 2005. Quantitative evaluation of ischemia-reperfusion injury by optical coherence tomography in the rat retina. *Jpn J Ophthalmol* 49, 109-113.

- Snodderly, D.M., Weinhaus, R.S., Choi, J.C., 1992. Neural-vascular relationships in central retina of macaque monkeys (*Macaca fascicularis*). *J Neurosci* 12, 1169-1193.
- Somma, A.T., Moreno, J.C.D., Sato, M.T., Rodrigues, B.D., Bacellar-Galdino, M., Occelli, L.M., Petersen-Jones, S.M., Montiani-Ferreira, F., 2017. Characterization of a novel form of progressive retinal atrophy in Whippet dogs: a clinical, electroretinographic, and breeding study. *Vet Ophthalmol* 20, 450-459.
- Spaide, R.F., Curcio, C.A., 2011. Anatomical correlates to the bands seen in the outer retina by optical coherence tomography: literature review and model. *Retina* 31, 1609-1619.
- Spaide, R.F., Curcio, C.A., 2017. Evaluation of Segmentation of the Superficial and Deep Vascular Layers of the Retina by Optical Coherence Tomography Angiography Instruments in Normal Eyes. *JAMA Ophthalmol* 135, 259-262.
- Spaide, R.F., Fujimoto, J.G., Waheed, N.K., Sadda, S.R., Staurengi, G., 2018. Optical coherence tomography angiography. *Prog Retin Eye Res* 64, 1-55.
- Spaide, R.F., Klancnik, J.M., Jr., Cooney, M.J., 2015. Retinal vascular layers imaged by fluorescein angiography and optical coherence tomography angiography. *JAMA Ophthalmol* 133, 45-50.
- Sparrow, J.R., Hicks, D., Hamel, C.P., 2010. The retinal pigment epithelium in health and disease. *Curr Mol Med* 10, 802-823.
- Srinivasan, V.J., Monson, B.K., Wojtkowski, M., Bilonick, R.A., Gorczynska, I., Chen, R., Duker, J.S., Schuman, J.S., Fujimoto, J.G., 2008. Characterization of outer retinal morphology with high-speed, ultrahigh-resolution optical coherence tomography. *Invest Ophthalmol Vis Sci* 49, 1571-1579.

- Staurenghi, G., Sadda, S., Chakravarthy, U., Spaide, R.F., International Nomenclature for Optical Coherence Tomography, P., 2014. Proposed lexicon for anatomic landmarks in normal posterior segment spectral-domain optical coherence tomography: the IN*OCT consensus. *Ophthalmology* 121, 1572-1578.
- Strauss, O., 2005. The retinal pigment epithelium in visual function. *Physiol Rev* 85, 845-881.
- Tan, P.E., Yu, P.K., Balaratnasingam, C., Cringle, S.J., Morgan, W.H., McAllister, I.L., Yu, D.Y., 2012. Quantitative confocal imaging of the retinal microvasculature in the human retina. *Invest Ophthalmol Vis Sci* 53, 5728-5736.
- Tao, Y., Ding, L., Yao, A., Yang, Z., Yang, Q., Qin, L., Yu, L., Gao, Y., Huang, Y.F., Li, Z., Teng, D., 2018. Intravitreal Delivery of Alphas-Crystallin Ameliorates N-Methyl-N-Nitrosourea Induced Photoreceptor Degeneration in Mice: An in vivo and ex vivo Study. *Cell Physiol Biochem* 48, 2147-2160.
- Tian, C., Zhang, W., Mordovanakis, A., Wang, X., Paulus, Y.M., 2017. Noninvasive chorioretinal imaging in living rabbits using integrated photoacoustic microscopy and optical coherence tomography. *Opt Express* 25, 15947-15955.
- Tullis, B.E., Ryals, R.C., Coyner, A.S., Gale, M.J., Nicholson, A., Ku, C., Regis, D., Sinha, W., Datta, S., Wen, Y., Yang, P., Pennesi, M.E., 2015. Sarpogrelate, a 5-HT_{2A} Receptor Antagonist, Protects the Retina From Light-Induced Retinopathy. *Invest Ophthalmol Vis Sci* 56, 4560-4569.
- van Velthoven, M.E., Faber, D.J., Verbraak, F.D., van Leeuwen, T.G., de Smet, M.D., 2007. Recent developments in optical coherence tomography for imaging the retina. *Prog Retin Eye Res* 26, 57-77.

- Walters, S., Schwarz, C., Sharma, R., Rossi, E.A., Fischer, W.S., DiLoreto, D.A., Jr., Strazzeri, J., Nelidova, D., Roska, B., Hunter, J.J., Williams, D.R., Merigan, W.H., 2019. Cellular-scale evaluation of induced photoreceptor degeneration in the living primate eye. *Biomed Opt Express* 10, 66-82.
- Weinhaus, R.S., Burke, J.M., Delori, F.C., Snodderly, D.M., 1995. Comparison of fluorescein angiography with microvascular anatomy of macaque retinas. *Exp Eye Res* 61, 1-16.
- Wells-Gray, E.M., Choi, S.S., Bries, A., Doble, N., 2016. Variation in rod and cone density from the fovea to the mid-periphery in healthy human retinas using adaptive optics scanning laser ophthalmoscopy. *Eye (Lond)* 30, 1135-1143.
- Wilk, M.A., Hickenpähler, A.L., Collery, R.F., Link, B.A., Carroll, J., 2017. The Effect of Retinal Melanin on Optical Coherence Tomography Images. *Transl Vis Sci Technol* 6, 8.
- Wilk, M.A., McAllister, J.T., Cooper, R.F., Dubis, A.M., Patitucci, T.N., Summerfelt, P., Anderson, J.L., Stepien, K.E., Costakos, D.M., Connor, T.B., Jr., Wirostko, W.J., Chiang, P.W., Dubra, A., Curcio, C.A., Brilliant, M.H., Summers, C.G., Carroll, J., 2014. Relationship between foveal cone specialization and pit morphology in albinism. *Invest Ophthalmol Vis Sci* 55, 4186-4198.
- Wilson, J.D., Cottrell, W.J., Foster, T.H., 2007. Index-of-refraction-dependent subcellular light scattering observed with organelle-specific dyes. *J Biomed Opt* 12, 014010.
- Wu, K.C., Chen, X.J., Jin, G.H., Wang, X.Y., Yang, D.D., Li, Y.P., Xiang, L., Zhang, B.W., Zhou, G.H., Zhang, C.J., Jin, Z.B., 2019. Deletion of miR-182 Leads to Retinal Dysfunction in Mice. *Invest Ophthalmol Vis Sci* 60, 1265-1274.

- Xie, W., Zhao, M., Tsai, S.H., Burkes, W.L., Potts, L.B., Xu, W., Payne, H.R., Hein, T.W., Kuo, L., Rosa, R.H., Jr., 2018. Correlation of spectral domain optical coherence tomography with histology and electron microscopy in the porcine retina. *Exp Eye Res* 177, 181-190.
- Xiong, Y., Ji, H.P., Song, W.T., Yin, Y.W., Xia, C.H., Xu, B., Xu, Y., Xia, X.B., 2016. N-methyl-N-nitrosourea induces retinal degeneration in the rat via the inhibition of NF-kappaB activation. *Cell Biochem Funct* 34, 588-596.
- Yamauchi, Y., Agawa, T., Tsukahara, R., Kimura, K., Yamakawa, N., Miura, M., Goto, H., 2011. Correlation between high-resolution optical coherence tomography (OCT) images and histopathology in an iodoacetic acid-induced model of retinal degeneration in rabbits. *Br J Ophthalmol* 95, 1157-1160.
- Yap, T.E., Donna, P., Almonte, M.T., Cordeiro, M.F., 2018. Real-Time Imaging of Retinal Ganglion Cell Apoptosis. *Cells* 7.
- Yi, J., Puyang, Z., Feng, L., Duan, L., Liang, P., Backman, V., Liu, X., Zhang, H.F., 2016. Optical Detection of Early Damage in Retinal Ganglion Cells in a Mouse Model of Partial Optic Nerve Crush Injury. *Invest Ophthalmol Vis Sci* 57, 5665-5671.
- Yiu, G., Wang, Z., Munevar, C., Tieu, E., Shibata, B., Wong, B., Cunefare, D., Farsiu, S., Roberts, J., Thomasy, S.M., 2018. Comparison of chorioretinal layers in rhesus macaques using spectral-domain optical coherence tomography and high-resolution histological sections. *Exp Eye Res* 168, 69-76.
- Yu, P.K., Balaratnasingam, C., Cringle, S.J., McAllister, I.L., Provis, J., Yu, D.Y., 2010. Microstructure and network organization of the microvasculature in the human macula. *Invest Ophthalmol Vis Sci* 51, 6735-6743.

- Zanzottera, E.C., Ach, T., Huisinigh, C., Messinger, J.D., Freund, K.B., Curcio, C.A., 2016. Visualizing retinal pigment epithelium phenotypes in the transition to atrophy in neovascular age-related macular degeneration. *Retina* 36 Suppl 1, S26-S39.
- Zawadzki, R.J., Zhang, P., Zam, A., Miller, E.B., Goswami, M., Wang, X., Jonnal, R.S., Lee, S.H., Kim, D.Y., Flannery, J.G., Werner, J.S., Burns, M.E., Pugh, E.N., Jr., 2015. Adaptive-optics SLO imaging combined with widefield OCT and SLO enables precise 3D localization of fluorescent cells in the mouse retina. *Biomed Opt Express* 6, 2191-2210.
- Zeng, Y., Petralia, R.S., Vijayasarathy, C., Wu, Z., Hiriyanna, S., Song, H., Wang, Y.X., Sieving, P.A., Bush, R.A., 2016. Retinal Structure and Gene Therapy Outcome in Retinoschisin-Deficient Mice Assessed by Spectral-Domain Optical Coherence Tomography. *Invest Ophthalmol Vis Sci* 57, OCT277-287.
- Zhang, Y., Cense, B., Rha, J., Jonnal, R.S., Gao, W., Zawadzki, R.J., Werner, J.S., Jones, S., Olivier, S., Miller, D.T., 2006. High-speed volumetric imaging of cone photoreceptors with adaptive optics spectral-domain optical coherence tomography. *Opt Express* 14, 4380-4394.

Figure legends

Figure 1. Human macular anatomy. Dissected human eye in which the macula, its different regions, and the optic nerve head can be seen. This eye was treated with RNA-later[®] for its preservation, which changes the typical translucent appearance of the retina to a white coloration, allowing for a clearer view of the yellow pigment (A).

Magnification image of macular zone (B, C) indicating each of the macular region dimensions (C). Dimensions of macular regions at a retinal section (stained with GNB3, CRALBP and cytochrome-C antibodies) (D) and its correlation with an OCT image at the same area (E). Scale bars: (A) 5 mm; (D) 200 μm ; (5) (E) 200 μm x 200 μm .

Figure 2. Morphologic features of cones, rods and Müller cells along the human retina. Vertical section of a human retina in the fovea stained with GNB3 (green, cones), rhodopsin (blue, rods) and CRALBP (red, Müller cells and RPE) (A). Schematic drawing of the fovea showing morphology and location of Müller cells (red), cones (black), and rods (blue) (B). Schematic drawing and confocal images of the cone photoreceptor morphology and its cellular organization (C). Differences in cone morphology at the umbo (D), parafovea (1.5 mm from central fovea) (E) and in the peripheral retina (7mm from central fovea) (F). Schematic drawing of cone (black) and rod (blue) morphological variations at different eccentricities (G). Scale bars: (A) 200 μm ; (D-F) 20 μm .

Figure 3. Correlation of OCT images and histological retinal layers in the human retina. Vertical section of a human fovea, showing cones and cone bipolar cells in green, mitochondria in blue, and Müller and RPE cells in red (A), and its correlation with OCT bands (B). High-magnification of image (A) at the foveal slope showing the retinal layers (C). High-magnification OCT profile in the same area (D). Scale bars: (A, B) 200 μm ; (C, D) 50 μm .

Figure 4. Changes of the GCL and RNFL along the retina. Panoramic view of a human central retina section stained with Tuj1 (green) and RBPMS (red) antibodies, differentiating the nasal (N1, N2) and temporal zones (T1, T2) (A). Ganglion cells in the fovea (B), and the corresponding OCT profile (C). High magnification images of ganglion cells (red) and nerve fibers (green) in the temporal zone (D, F) and in the nasal zone (E, G). Scale bars: (A) 500 μm ; (B, C) 200 μm ; (D-G) 20 μm .

Figure 5. Histological interpretation of the human OCT outer hyperreflective retinal bands. Human vertical section immunostained to identify cone myoids (green), ellipsoids (blue), and outer segments (bright green) (A). Müller cell processes (red) and

microvilli (arrowheads) forming the ELM (B). Immunostained section showing Müller cells (red) forming the ELM, mitochondria (blue) at the ellipsoids, RPE interdigitations (red) and RPE cells (red) with their basal mitochondria (C), compared to the OCT profile (D). OCT profile (E) and its correspondent histological section showing cones (green), Müller and RPE cells (red), and phagosomes as green structures inside red RPE cells (F). Detail of RPE interdigitations ensheathing the entire length of the cone outer segment (arrowheads) (G, H). Histological images (A-C, G, H) were taken at approximately 800 μm from the foveal center and (F) at approximately 100 μm . Scale bars: (A, B, E) 25 μm ; (C, F) 20 μm ; (D) 50 μm ; (G, H) 10 μm . Modified from Cuenca *et al.* 2018.

Figure 6. Third and fourth outer hyperreflective retinal OCT bands interpretation by histology. Immunohistochemical sections of the human retina showing cones (green) rods (blue), RPE (red) and phagosomes (green or blue structures inside RPE at the foveola (A), at the parafovea (1.75mm from central fovea), and at the periphery (7 mm from the central retina (C). High-magnification image of the RPE, showing the layer where the phagosomes are located; the middle zone of the RPE occupied by the

melanosomes (arrowheads); and the mitochondria zone (blue) located at the basal portion of the RPE (arrows) (D). Scale bars: (A-D) 20 μm .

Figure 7. Summary of the proposed interpretation of OCT bands based on histology. Graphical summary showing the different hyperreflective and hyporefective bands at the foveal outer retina and their cellular origin (A). High magnification of a foveal cross-section showing retinal layers (B). Nomenclature of OCT bands (C) and their correspondent layers in a histological section stained with immunohistochemistry (D). Scale bars: (B) 50 μm ; (C, D) 50 μm x 50 μm . Modified from Cuenca *et al.* 2018.

Figure 8. Vascular plexuses variations at different retinal eccentricities. OCT image at a central region (A) and the equivalent location in a retinal histological section showing cones (green), amacrine and bipolar cells (red), and blood vessels (blue) (B). Magnifications at different retinal eccentricities allow the visualization of the vascular plexuses in each region (C-J). This information is summarized in a schematic representation of the different vascular plexuses, RPCN (green), SVP (blue), ICP (red) and DCP (yellow), depending on the retinal position (K-N). The positions analyzed

were: near the optic nerve (C, D, K), at the parafovea (E, F, L), at the fovea (G, H, N), and at the peripheral retina (I, J, M). Scale bars: (A) 200 μm x 200 μm ; (B) 250 μm x 250 μm ; (C-J) 50 μm .

Figure 9. Segmentation of vascular plexuses in *en face* OCTA and confocal images.

En face OCTA images (A-D, I-L, Q, S) and retinal wholemount confocal images (E-H, M-P, R, T, U) show retinal vasculature at equivalent locations: near the optic nerve (A-H), at the fovea (I-P), at 11 mm eccentricity (Q-T) and at 15 mm eccentricity (U). Arrow in [U] points the avascular zone at the most peripheral retina. Note the vascular loops magnification in the ICP (inserts in [C] and [G]) and the spider-like structures in the DCP (inserts in [D] and [H]; arrowheads in [P]). A diagram represents the retinal location of each image (V). Scale bar (A-U) 500 μm .

Figure 10. Histological correlation of mouse retinal layers with OCT bands.

OCT images shows hyperreflective and hyporefective bands corresponding to different retinal layers (A, D, H). Confocal image sections showing the three retinal nuclear layers (B, E-G). Schematic drawing of these retinal layers and their correspondent cell types

(C). RGCs (red) and their axons, corresponding to RNFL (green) (E). Outer segments of photoreceptors (green); RPE and Müller cells (red), (F). Double immunostaining showing bipolar cells (red); amacrine and ganglion cells (green), (G). Immunolabeling sections showing plexiform layers corresponding to photoreceptor synaptic ribbons (red) and their synaptic contacts (yellow) with rod bipolar cells (green) (I-K). Scale bar: 40 μ m

Figure 11. Histological interpretation of mouse outer hyperreflective retinal bands

in OCT. OCT images highlighting with red box the four outer hyperreflective bands of the retina (A). High magnification of the indicate zone enumerating hyperreflective bands (B). Confocal images at the end of ONL (blue) shows the ELM (green, arrowheads) (C). Same immunolabeling (CRALBP) detects RPE cells and their membrane interdigitations (C, F, G, I, J). Mitochondria staining (blue) shows the ellipsoid zone (square bracket) and mitochondria in basal portion of RPE (arrows) (D, F). Diagram showing the different hyperreflective and hyporefective bands at the outer retina (E). Immunolabeling showing outer (red) and inner (blue) segments of photoreceptors (F). Arrowheads pointing interdigitations of RPE (green), along the entire outer segments (red); outer segments phagocytosis (arrows) establish a continuous line along apical

portion of RPE (G-J). High magnification of phagosome zone (H-J) permits to detect the empty space when the green channel is removed (compare [I] vs. [J]). Scale bars: (A) 200 μm ; (B-D, F-J) 20 μm .

Figure 12. Retinal structural, morphological and functional changes during retinal degeneration in rd10 mice. Comparison along age (from P20 to P60) of retinal degeneration with OCT images (A-D) and confocal images (E-L). Immunostaining of cones (green), nuclei (blue) and rod-bipolar cells (red). ERGs corroborate retinal degeneration in retinitis pigmentosa mice (M-P). Scale bar 50 μm .

Figure 13. Advantages and limitations of using OCT scanning in mouse models. Analysis of degenerated retina in rd10 mice by OCT could generate different images depending on scanning zone (A-C). Note that retinal detachment in the closest optic nerve area (line 2) does not appear in the furthest one (line 1), (compare [B] vs. [C]). OCT scanning only evaluates center-middle retina, around the optic nerve (E). Conversely, confocal scanning of immunostained sections, allows to detect structural changes throughout whole retina (D, F-H). The square bracket identifies the OCT

scanning area (D). In degenerate retina of rd10 mice, center (F) or middle retina (G) has thicker IPL and ONL compared to peripheral area (H). Scale bars: (A, D) 500 μm ; (B, C) 100 μm ; (E-H) 50 μm .

Figure 14. Retinal remodeling at late stages of degeneration in P23H rat. OCT images along age in wild type (A) and P23H rat model of retinitis pigmentosa (D, G, J, M) and its correlation with immunostained retinal sections in wild type (B, C) and P23H rat (E, F, H, I, K, L, N, O). It is possible to visualize with both techniques a progressive retina invasion by blood vessels accompanied by RPE (pointed with arrows). Scale bar 100 μm .

Figure 15. Vascular plexuses in the retina of murine models. Vascular plexuses of C57Bl/6 wild type mice retina by confocal scanning of wholemount retina stained with collagen-IV for blood vessels (A, C, E) correlates with *en face* OCTA obtained images at equivalent locations (B, D, F). Note capillary loops detection using immunohistochemistry and OCTA (arrowheads). Low magnification of the wholemount retina plexuses in C57Bl/6 mouse (G-I). Scale bars: (A-F) 500 μm ; (G-I) 1 mm.

Table 1

Identification	Correspondence	Reflectivity	Species	Authors
Ganglion cell complex (GCC)	RNFL	High	Pig, cat, primates	Aplin et al. 2014; Cheng et al., 2018; Yiu et al., 2018
	GCL		Rodents, rabbit	Koinzer, 2013b; Pinilla et al, 2016
	NFL+/-GCL		Monkey	García Garrido et al. 2014
RGC	RGC	Low	Only close to ON head, in pig, dog, monkey	Xie et al 2018 (pig)
Inner plexiform layer (IPL)	IPL	High	all	Bartuma et al. 2015; Cheng, 2018; Pinilla et al, 2016; Yiu et al., 2018
Inner nuclear layer (INL)	INL	Low	all	Bartuma et al. 2015; Pinilla et al, 2016; Cheng, 2018; Yiu et al., 2018
Outer plexiform layer (OPL)	OPL	High	all	Bartuma et al. 2015; Cheng, 2018; Pinilla et al, 2016; Yiu et al., 2018
Outer nuclear layer (ONL)	ONL	Low	all	Bartuma et al. 2015; Cheng, 2018; Pinilla et al, 2016; Yiu et al., 2018
External hyperreflective 1	ELM	High	Pig, rabbit, cat, monkey, rodents	Adachi et al. 2016, Anger et al. 2004, Bartuma 2015, Berger et al. 2014, Cuenca et al. 2014b, Fan et al 2019, García Garrido 2014, Gloessmann et al, 2003, Huber et al., 2009, Koinzer et al. 2013, Ocelli et al. 2016, Pinilla et al., 2016, Ryals et al. 2017, Tian 2017, Xie et al., 2018, Yiu et al 2018, Zeng, et al., 2016. See Fig. 15
External hyporeflective 1	Myoid region	Low	Monkey, pig, rabbit, rodents	Anger et al. 2004, Bartuma et al., 2015, Berger et al. 2014, Gloesmann et al., 2003, Pinilla et al., 2016, Xie et al., 2018, Tian 2017, Zeng et al., 2016. See Fig. 15
External hyperreflective 2	EZ	High	Cat, monkey, pig, rabbit, rodents	Adachi et al., 2016, Anger et al. 2004, Bartuma et al. 2015, Berger et al., 2014, Fan et al 2019, Gloesmann et. al, 2003, Minella et al., 2018, Monai et al., 2018, Ocelli et al., 2016, Pinilla et al., 2016, Ryals et al., 2017, Tian et al. 2017, Xie et al., 2018, Zeng et al., 2016. See Fig. 15
	IS/OS junction		Rabbit, cat, monkey, rodents	Aplin 2014, García Garrido et al. 2014, Huber et al., 2009, Koinzer et al. 2013, Yiu et al 2018
External hyporeflective 2	OS/interdigitations	Low	Mouse	See Fig.15
External hyperreflective 3	RPE	High	Cat, monkey, rodents, pig	Anger et al. 2004, Aplin et al. 2014, Berger et al., 2014, García Garrido et al. 2014, Gloesmann et. al, 2003, Huber et al., 2009, Yiu et al 2018
	Cone OS tips		Monkey	García Garrido et al., 2014
	OS		Rodents	Pinilla et al, 2015, Zeng et al., 2016
	OS/RPE/Bruch membrane		Rabbit	Koinzer et al. 2013
	IZ		Cat, monkey, rodents, pig, rabbits	Adachi et al., 2016, Bartuma 2015, Berger et al., 2014, Fan et al 2019; Minella et al., 2018, Monai et al., 2018, Ocelli et al., 2016, Ryals et al., 2017, Tian 2017, Xie et al., 2018
	RPE phagosome zone		Mouse	See Fig. 15
External hyporeflective 3	Melanosomes/nuclei	Low	Mouse	See Fig. 15
External hyperreflective 4	RPE	High	Monkey, rabbit, rodents	Adachi et al., 2016, Bartuma et al. 2015, Berger et al., 2014, Cuenca et al., 2014b, Fan et al 2019, García Garrido et al. 2014, Monai et al., 2018, Pinilla et al, 2015, Ryals et al., 2017, Tian et al. 2017, Zeng et al., 2016
	RPE basal mitochondria		Mouse	See Fig. 15

OCT profile bands as assigned by different authors in several animal models. Hyperreflective bands are shown in white, and hyporeflective bands in grey shade. Our interpretation of the outer retinal OCT bands is shown in red. 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 limitant membrane; EZ: Ellipsoid zone; IS Photoreceptor inner segments; OS: Photoreceptor outer segments; IZ: Interdigitation zone; RPE: retinal pigment epithelium.

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Percentage of work contributed by each author in the production of the manuscript is as follows: Nicolás Cuenca 10%, Isabel Ortuño-Lizarán 12%, Xavier Sánchez-Sáez 12%, Oksana Kutsyr 12%, Henar Albertos-Arranz 8%, Laura Fernández-Sánchez 8%, Natalia Martínez-Gil 8%, Agustina Noailles 4%, José Antonio López-Garrido 2%, Maribel López-Gálvez 2%, Pedro Lax 6%, Victoria Maneu 8%, Isabel Pinilla 8%.