8.1 DISCUSSION

Many optical techniques have been developed in the last 16 decades to assist physicians in an accurate diagnosis of eye diseases. To determine the added value of these techniques they need to be tested, preferably on samples that can accurately assess the precision and accuracy of the tested device (1). For example, the development of OCT, especially Fourier domain OCT, has dramatically changed everyday clinical ophthalmic practice. This imaging modality has evolved into a diagnostic imaging tool allowing in vivo optical biopsies of retinal tissue. This results in histology-like, optical cross-sections of the retina providing high resolution morphological information on its layered structure. The thickness of these retinal layers, in particular the RNFL and the thickness of the entire retina, is an important parameter in the diagnosis of diseases like glaucoma and diabetes (2). Many studies have been done to determine the reproducibility, accuracy and precision of these thickness measurements, but they were performed on human subjects from whom the real RNFL or retinal thickness was not known (see Chapter 2) (3-9). Therefore we developed a phantom eye model that mimics the various layers of the retina in structure and in optical properties (10). The analysis that is described in Chapter 2 is an important step towards interpretation of patient measurements, e.g. in Chapter 3 we used a commercially available OCT system to measure the retinal thickness in an amblyopia study in children. Studies providing information on interbrand and intrabrand variability (chapter 2) delineate the reliability of studies measuring retinal thickness (chapter 3).

THICKNESS MEASUREMENTS AND OPTICAL RESOLUTION

As mentioned in chapter 2, many factors can contribute to erroneous retinal thickness measurements. Besides the used segmentation algorithm, an inadequate dispersion compensation or faulty k-calibration of the spectrometer (directly translating into the scaling of the depth axis) can lead to an inaccurate thickness measurement but also specular reflection at the vitreo-retinal interface can hamper a reliable measurement.

Another feature of OCT systems that contributes to an erroneous thickness is the axial resolution. In chapter 2 we investigated the influence of the axial resolution of OCT systems on the determination of the transition of one layer to the next layer, and especially the coherent nature of this point spread function. From our simulation we conclude that an incoherent point spread function recovers the correct layer thickness. However, it is the coherent PSF that is involved in OCT signal formation.
DISCUSSION AND CONCLUDING REMARKS

To explain and investigate in more detail the difference we found between the real thickness and the measured thickness we would have needed full access to the calibration data of the systems. This involves knowledge about the depth dependent sensitivity of the systems, the measured axial resolution (i.e. not the theoretical axial resolution determined by the light source’s bandwidth) but most importantly the used segmentation algorithm. Without this information one can only speculate about the cause of the erroneous thickness measurement, but given the linear relation between recovered thickness and optical resolution found in our simulation, we propose that advanced segmentation algorithms may take the effect of the coherent point spread function into account.

Besides the determination of RNFL thickness, SD-OCT systems can also determine the thickness of the entire retina. In chapter 3 we used the retinal segmentation module of a 3DOCT-1000 of Topcon to determine the thickness of the retina in amblyopic and healthy children. We assumed that the error in thickness determination is mainly caused by the finite axial resolution of the OCT system, and therefore doesn’t scale with the layer thickness. This is however an interesting topic for further research. From literature we know that healthy subjects and healthy children have a linear relation between the axial length of the eye and the retinal thickness (11-14). Amblyopic children tend to have a shorter eye and therefore an increased retinal thickness can be expected. Our measurements confirmed that the axial length of amblyopic and fellow eyes is significantly shorter but a thicker retina is not found. From these results we conclude that there is an anomalous relation between axial length and retinal thickness is amblyopic children which can be caused by an anomalous retinal development (15) and that the development of the retina is also (partly) affected in amblyopia.

NORMATIVE DATABASES

In day-to-day clinical practice, a measured RNFL thickness and retinal thickness are compared to a normative database that is provided by the OCT manufacturer to aid physicians in their diagnosis. A normative database is therefore an important feature to take into account when RNFL thickness data are interpreted.

In addition to our phantom eye thickness measurements, we compared the RNFL normative databases of the used SD-OCT systems. We used GrapData software to extract the age-matched normative databases from the data sheets that are provided by the SD-OCT systems after each thickness measurement. Because all brands measured an equal overall average phantom RNFL thickness of 67 µm in the phantom eye, one would expect these normative values to be similar.
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Normative classification consists of 4 categories: the 95th to 100th percentiles are hyper normal (white color on thickness map displayed in Figure 8-1(A); 5th to 95th percentiles are normal (green); 1st to 5th percentiles are borderline (yellow); and 1st percentile is abnormal (red).

In Figure 8-1(A) the age-matching normative database of RTVue is displayed in color. On top of the RTVue database we plotted the Spectralis normative data base as lines. The dashed line, the dotted-dashed line and the solid line represent the upper boundary of the normal (green) area, the boundary of the yellow-green area, and the border of the red-yellow area of the Spectralis normative data base, respectively.

![Figure 8-1(A): The normative database of RTVue (in color) and Spectralis (lines) are displayed.](image)

In Figure 8-1(B) the normative database of Topcon and Cirrus are displayed in color and in lines, respectively. Comparing the four normative databases, large differences in classification by the different instruments are observed, e.g. in Figure 8-1(A) it is clear that for a thickness of 60 to 75 µm in the inferior area (240 degrees) a patient can be classified as healthy with Spectralis, but is classified as abnormal with RTVue: the boundary of the Spectralis abnormal area is 25.5 µm lower on average compared to the RTVue abnormal area which exceeds the depth resolution of these systems. Moreover, these differences are much larger than the mean differences in thickness measured using our phantom. This suggests that possible differences in diagnosis on the same patient using different machines may be due to differences in the databases, rather than performance of the OCT systems. Differences are, however, much less pronounced comparing Topcon with Cirrus (Figure 8-1B).
In Chapter 2 we indicated that patients can be reliably followed on different SD-OCT systems when the RNFL thickness is measured and used as an indicator for the progression of an eye disease. However, diagnosis based on normative databases of different manufacturers can be unreliable, especially when a subject’s RNFL thickness is close to the borderline of the abnormally thin nerve fiber layers.

**STRUCTURAL INFORMATION AND MOTION ARTIFACTS**

Structural information of the retina is important and factors that hamper the quality of the image (e.g. motions artifacts) should be minimized or corrected (16). OCT is a scanning imaging technique; therefore it takes time to acquire the data. Lateral and axial motions by the patient during acquisition can degrade the image or cause error in Doppler OCT measurements (mainly axial motions). Higher imaging speeds reduce the influence of the eye motions on the image quality, however, the amount of light that returns in the optical system is also reduced. This results in a lower signal to noise ratio and lower image contrast. Therefore these lateral and rotational eye motions need to be corrected as accurate as possible. To quantitatively measure flow with Doppler OCT, heart beat induced bulk motions by the patient need to be recorded and corrected (17, 18). For instance, imaging of the retina could take place in between a heart beat cycle of the patient (cardiac gating). This way the position of the retina, and the flow in the retina are maintained (19).

In chapter 4 we analyzed the cause of axial motions during the acquisition of consecutive OCT B-scans at the same location in the retina (18). We found that during a heart beat the position of the retina changes due to a head movement and that the position of the cornea relative to the retina does not change. Although it seems obvious that a heartbeat, as well as breathing by the patient, can cause motions during imaging finding the cause of these motions has not been easy. A frequency analysis of the motions initially showed strong (multiple) harmonics (around 2 and 3 Hz) of the heart beat. At first we investigated muscular motions around the eye by performing electroencephalography (EEG) measurements during OCT scanning that could cause this 2-3Hz movement. We found no relation between the muscular activities around the eye and the 3 Hz signal. In addition, micro-saccades were investigated by fixation by the patient to a moving object during OCT scanning, but no relation between the frequency of the lateral movement and the axial movement could be found. After analysis of the frequency content of the heart beat signal we found multiple harmonics at the same location as the frequency of the axial eye motions.

The results in chapter 4 can help in a better quantification of (Doppler) OCT data and improve medical diagnosis with OCT. Understanding the cause of image
artifacts improves diagnosis, for instance, knowledge about the heart beat cycle could be used to image at the same location on the retina and at exactly the same moment in between cycles. The response of cells to light stimuli could then be measured at the same moment during a heart beat cycle and at the same location. Furthermore, using a cylindrical lens two focal points can be created (chapter 4) that could enable measurements of intra ocular pressure. When a puff of air is presented at the cornea the anterior part of the eye moves relative to the posterior part. The response time (i.e., time is takes to recover it initial position) and the magnitude of the movement of the anterior segment could be related to intra ocular pressure or the elasticity of the entire eye.

The proposed retinal motion tracker described in chapter 5 can be a beneficial add-on for scanning retinal imaging techniques. The few components that are needed for the tracking system allow for a cheap and easy implementation of the tracker in other existing systems. More importantly, active motion correction allows for high resolution imaging of retinal structures and enables averaging of data to increase signal to noise ratio. Furthermore, motion free imaging improves the detection of small structures because the resolution in the image improved. In combination with adaptive optics, imaging of single cones and rods, the photosensitive parts of the retina, would in principle be possible. This would allow for functional imaging of single cells in the retina and may be a powerful tool in combination with devices like the Macubit (chapter 7). Furthermore, an improved resolution enables better segmentation of retinal layers that aids the physician in the diagnosis of e.g. glaucoma.

The evaluation of segmentation algorithms of OCT systems in chapter 2 together with the analysis and correction of motion artifacts in chapter 4 and chapter 5 are important to gain more precise structural information of the retina, which eventually will lead to a better diagnosis of eye diseases, like amblyopia (chapter 3). Adaptive optics in combination with an active retinal tracker (chapter 5) could in principle be a powerful diagnostic imaging modality that can evaluate the response of single cells in the retina to (light) stimuli. This way, the functional state of the retina can be fully mapped and followed over long periods of time.

OCT has been used in the ophthalmic clinic mainly to acquire high resolution 3D images of the retina. Although OCT has dramatically improved diagnosis of retinal diseases, the technique has only been used for morphological information. The current clinical systems lack the resolution both axially as well as laterally to provide the physician with functional information. Outside the clinic OCT has proven to be capable of imaging single cones and rods using adaptive optics. However, AO-OCT systems are large, and a have a limited field of view. This should
be improved before functional testing at the level of a single cell using OCT is possible for diseases like AMD and glaucoma.

**FUNCTIONAL TESTING OF THE RETINA**

In this thesis two devices were evaluated that measure the functional state or a quantity that is directly related to the functional state of the retina. In chapter 6 the Macuscope that is designed to assess the amount of macular pigment in the retina is tested on a group of healthy subjects and compared with a clinically accepted device (QuantifEye) and a device that uses spectroscopy, the macular pigment reflectometer (MPR)(20). This device where information from both the physician and the patient is needed, seemed incapable in determining the amount of macular pigment accurately. In chapter 7 the Macubit is tested on patients that were diagnosed with AMD in both eyes, and loss of vision in only one eye and on a healthy control group (21). Most of these devices use psychophysical testing. Here, the relationship between physical optical stimuli and their subjective response is an important parameter in testing the functional state of the retina. Psychophysical methods can be applied to various aspects of sensory and perceptual problems, from basic and comparative research to clinical diagnostics. The limitations in the use of psychophysical methods exist in that they require a conscious and cooperating subject, an understanding of the principle of the test and a way to reliably report the sensed events (22). However, potentially the tested subjects may influence the test results in order to simulate or exaggerate a performance loss. To some extent, methods such as those of the Macubit allow the experimenter or physician to check on the subject’s response bias and sensitivity, but it cannot be used to correct for purposely influencing the test results. Here we reach a principal limitation of subjective testing: cooperation on the part of the subject is required. This limitation of psychophysical methods can be taken into account and perhaps corrected for by combining subjective with objective methods in the examination of sensory function before they are used in the clinic as pointed out in chapter 6 where the Macuscope and the Quantifeye are both evaluated in comparison to the MPR.

Although the Macubit system uses psychophysical testing, it determines essentially the percentage of damaged neurons compared to the healthy by calculating the observed/shown dots ratio that are randomly shown without fixation mark. This functional information could be an interesting addition to the structural information that is provided by OCT scanning of the retina. Here, the subjective method of HFP can be combined by an objective measurement like OCT that lacks functional information of the retina.
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With (AO)-OCT functional measurements like temporal changes of cones is possible (23), however, AO the systems are bulky and the field of view is limited. Furthermore, motions of the retina hamper the image quality, but this problem can be solved with an AO-OCT imaging device and an active retinal tracker that will compensate for retinal motion. Retinal position tracking enables extended scan periods and averaging of multiple scans. The latter will diminish speckle in OCT images with minimal effect on imaging resolution. Furthermore, the retinal tracker that compensates for lateral motions uses physical landmarks and therefore precisely aligned scans can be compared months apart, which is beneficial in follow up studies involving patients with poor vision. Retinal morphology can thus be accurately recorded and monitored over time to detect subtle changes. Such benefits provide enhanced diagnostic sensitivity and specificity for several eye diseases and enable high resolution functional imaging of the photoreceptors in the retina.

Summarizing, when using psychophysical tests one should look for the easiest and most trustworthy method to answer the research question. More sophisticated measurement protocols are not by definition better than simpler methods, particularly if they introduce difficult technical problems. A combination of a psychophysical test with an objective test that can stage the health state of tissue (like OCT) can be a powerful diagnostic tool for ophthalmology. In combination with a retinal motion tracker and adaptive optics, spectroscopic OCT can potentially be of additional value to measurements where the amount of macular pigment is assessed using HFP. Therefore I recommend starting up studies where OCT images are analyzed on their spectroscopic content, in combination with the information that is provided by psychophysical tests like MacuBit and MPOD measurements.

CONCLUSION AND OUTLOOK

In this thesis several optical diagnostic techniques that are used in ophthalmic clinic are evaluated and a retinal motion tracker is presented to improve the quality of OCT images. We showed that a calibration of OCT systems on calibrated samples is essential for an accurate interpretation of the data and a retinal motion tracker for high resolution imaging is proposed. Furthermore, we evaluated devices that use psychophysical testing in order to map the functional state of the retina and to assess the amount of macular pigment. When OCT systems can be equipped with a retinal motion tracking system (chapter 5), low-cost adaptive optics(24) and the capability to perform spectroscopic measurements(25) to image the retina at a cellular level this could be a powerful imaging modality that can map the morphology and functional health state of the retina. However, the realization of a high quality combination of AO, and tracker with commercial OCT system is not depending on whether these techniques can be developed, but rather on the costs
of the entire system. Unfortunately, with increasing costs in the health care system, it will be inevitable that the focus of the research in optical diagnostic techniques in ophthalmology will be more in the development of low cost techniques (26) in the coming years.

8.2 REFERENCES


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19. T. Schmoll and R. A. Leitgeb, "Heartbeat-phase-coherent Doppler optical coherence tomography for measuring pulsatile ocular blood flow," J Biophoton. n/a-n/a


