Aspects of Optical Coherence Tomography (OCT) in Healthy Eyes and Eyes with Retinal Diseases

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Abstract

Optical coherence tomography (OCT) is a technique in which cross-sectional images from intraocular tissue can be obtained. The quantitative and qualitative examinations are used for evaluating retinal diseases. Conventional OCT (Stratus) is mainly used, but the new Spectral domain (Cirrus) OCT, which has improved technology, may provide more reliable measurements.

The aim of the study was to collect normal values of macular thickness in children and adults and to evaluate the effect of age and/or gender, to compare measurement variability in healthy eyes and eyes with age-related macular degeneration (AMD), to compare Stratus and Cirrus OCT and to study the effect of cataract surgery on macula.

Sixty-seven healthy adults and 56 children, 30 patients with AMD, 34 patients with diabetes and cataract and 35 healthy controls were included. The quantitative maps in Stratus and Cirrus were used and manual correction of foveal location was evaluated. Qualitative OCT was compared to fluorescein angiography (FA) after cataract surgery.

The mean values of macular thickness in Stratus OCT were 207 μm in adults and 204 μm in children. The measurement variability was low. Macular thickness decreased with age in adults, but not in children. No correlation with gender was found. In eyes with wet AMD, there were small differences in measurement variability comparing Stratus and Cirrus OCT. After manual correction in Cirrus OCT, the coefficients of repeatability were improved to values close to the repeatability in normal eyes. Two thirds of the diabetic and half of the control eyes showed leakage on FA after cataract surgery. Qualitative OCT corresponded poorly to FA in diabetic eyes. A thicker macula, assessed with OCT, was often observed without any obvious effect on visual acuity. OCT was as good as FA in revealing clinically relevant changes in macula after surgery, and was the technique recommended for follow-up.

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To my family
List of Papers

This thesis is based on the following papers, which are referred to in the text by their Roman numerals:


IV Eriksson U, Alm A, Larsson E. Is quantitative spectral-domain superior to time-domain optical coherence tomography (OCT) in eyes with age-related macular degeneration? A study comparing repeatability and reproducibility in time domain (Stratus) and spectral domain (Cirrus) OCT. Submitted for publication.


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### Abbreviations

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<tr>
<td>AMD</td>
<td>Age-related macular degeneration</td>
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<tr>
<td>CCD-Cam</td>
<td>Charge-coupled device camera</td>
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<td>CME</td>
<td>Cystic macular edema</td>
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<td>D</td>
<td>Diopter</td>
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<td>DME</td>
<td>Diabetic macula edema</td>
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<td>DR</td>
<td>Diabetic retinopathy</td>
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<td>DRC-net</td>
<td>Diabetic Retinopathy Clinical Research network</td>
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<td>EDTRS</td>
<td>Early Treatment Diabetic Retinopathy Study Research group</td>
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<td>FA</td>
<td>Fluorescein angiography</td>
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<td>HD-OCT</td>
<td>High definition OCT</td>
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<td>ME</td>
<td>Macula edema</td>
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<td>MPS</td>
<td>Macular Photocoagulation Study Group</td>
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<td>nAMD</td>
<td>Neovascular age-related macular degeneration</td>
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<td>OCT</td>
<td>Optical coherence tomography</td>
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<td>RNFL</td>
<td>Retinal nerve fibre layer</td>
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<td>RPE</td>
<td>Retinal pigment epithelium</td>
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<td>SD-OCT</td>
<td>Spectral domain optical coherence tomography</td>
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<td>SLD</td>
<td>Superluminescent diode</td>
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<td>TD-OCT</td>
<td>Time-domain optical coherence tomography</td>
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<td>UHR-OCT</td>
<td>Ultrahigh resolution optical coherence tomography</td>
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<td>VA</td>
<td>Visual acuity</td>
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Introduction

Macula lutea is the central part of the retina. It allows high-resolution central visual acuity. Different conditions, like diabetic retinopathy, age-related macular degeneration (AMD) or retinal vein occlusion can affect the macular structure, which may result in severe loss of visual acuity. Traditionally, examinations of the macula are performed by ophthalmoscopy or slit-lamp examination with a contact-lens. From the 1970s fluorescein angiography (FA) on silver film, has been a valuable aid for clinical evaluation of the retina. The rapidly evolving technology of digital imaging from the mid 1980s, has added several new diagnostic modalities for retinal disease. It has permitted computer-assisted analysis of both FA and indocyanin green angiographies, as well scanning laser ophthalmoscope imaging of the ocular fundus. One of the most useful techniques developed is optical coherence tomography (OCT),\(^{47}\) which was introduced to the market in 1996. OCT provides in vivo cross-sectional information of macular structure with micrometer resolution, without requiring physical contact with the patient. The ‘non-contact’ feature makes the technique very useful when examining children. In 1995, the first in vivo images from the human eye was reported\(^ {36}\) and thereafter, OCT has been used to demonstrate morphological changes in different macular diseases\(^ {83,37-39,76}\) and has contributed to the understanding of the pathogenesis of many retinal diseases i.e. the formation of macular holes.\(^ {38,30,101}\)

OCT also provides a possibility for quantitative measurements of retinal and nerve-fibre layer thickness\(^ {40,41,92}\) with high repeatability and reproducibility in normal eyes.\(^ {59,68,93}\) Therefore, OCT has rapidly become established as a useful tool for treatment decisions and follow-up. However, image artefacts, particularly in eyes with structural changes in the macula, as seen in AMD, have been reported by several investigators\(^ {85,42,89,31,79}\) and may prevent accurate measurements in the images.

The first commercial instruments for OCT of the retina were based on time domain detection of coherence, Recently, a new generation of OCT instruments, spectral domain OCT, with even higher image resolution, has recently been introduced.\(^ {107-109,74}\) These instruments achieve faster scanning speed along the retinal surface thereby allowing more measurement points per time thus reducing artefacts due to eye movements. Together with an improved algorithm for determining the boundaries of the retina, this could be expected to provide more reliable measurements, especially in eyes affected by retinal disease.
This thesis focuses mainly on the quantitative aspects of OCT measurements. Normal values in healthy eyes have been obtained and the instrument variability has been evaluated both in healthy eyes and in eyes affected by macular disease. The conventional Time domain OCT technique (Stratus) has been compared to the new Spectral domain OCT (Cirrus) in eyes with morphological changes in the macula.

Macula

The retina develops from the walls of the embryologic optic cup which is an outgrowth from the foetal forebrain. The outer, thinner layer of the cup becomes the pigment epithelium and the inner, thicker, layer of the cup differentiates into the neural layers of the retina. The macula is the part of the retina that is responsible for high-resolution visual acuity. Anatomically it is defined as the central area of the retina that contains xanthophyll and features more than two layers of ganglion cells. The central retina develops over a prolonged period. In the early stage of development, the macular region is a central elevation due to accumulation of ganglion cells. By the sixth month of gestation, this region starts thinning as the cells of the inner retina are displaced centrifugally and develop elongated processes. This displacement results in the fovea, the central depression of the macula. The fovea thins progressively towards its centre, the foveola, which is the thinnest part of the macula and consists of cone cells only. Before birth, the fovea has a diameter that is considerably larger than the adult fovea, and at birth the cone density is only 20 percent of that of the adult fovea. Cones then elongate, migrate centrally, and increase their packing density. This process is completed five to eight years after birth.

One third to one half of the neurons in the central nerve system are estimated to be lost during a lifetime. It therefore appears reasonable that the number of retinal neurons also decreases with age. Histological studies of the human retina and optic nerve have shown a decreased density with age of photoreceptors, ganglion cells, pigment epithelial cells and optic nerve fibres. However, if this results in a thinning of the retina with age is not known.

Age related macular degeneration (AMD)

Age-related macular degeneration (AMD) is a progressive disease of the retina and is the leading cause of legal blindness in people over 50 years of age in the Western world. The disease is divided into two main forms: dry (geographic) and neovascular (also termed exudative or wet) The dry form, in which there is a progressive atrophy of the retinal pigment epithelium (RPE) and photorecep-
tors, is the most common, accounting for 85 percent of cases. The visual impairment in the dry form progresses slowly in the majority of cases.\textsuperscript{53} The less frequent, neovascular form (15 percent of cases) usually results in more severe visual disturbance and develops rapidly, over months rather than years.\textsuperscript{111} Newly formed vessels originating from the choriocapillaris grow under the retinal pigment epithelium (RPE), penetrate through Bruch’s membrane and invade the subretinal space.\textsuperscript{34,103,4} These newly formed vessels are fragile and lack the tight junctions that connect endothelial cells in normal retinal vessels. This results in serous leakage, macular edema, haemorrhages, photoreceptor damage and finally development of a fibrotic scar. Although only 15 percent of patients with AMD have the wet form, it is responsible for almost 90 percent of those with severe visual loss (20/200 or worse).\textsuperscript{24}

Treatment based on inhibition of vascular endothelial growth factor (anti-VEGF-injections), given as intravitreal injections, has recently been introduced.\textsuperscript{33,88,6} The treatment has a marked effect on the progression of the disease, but repeated injections are usually necessary.\textsuperscript{27,63}

Diabetic retinopathy and macular edema

Diabetes mellitus is a metabolic disorder with, if left untreated, chronic hyperglycaemia. The worldwide prevalence of diabetes is estimated to be 2.8 percent\textsuperscript{105,113} and in Sweden three to four percent.\textsuperscript{104}

There are two dominant forms of the disease: classified as diabetes type 1 and type 2. Type 1 occurs because of insufficient insulin production due to early autoimmune destruction of the beta cells of the pancreatic islets of Langerhans. Type 2 is characterized by an insulin-secretor defect in combination with factors that oppose peripheral action of insulin (insulin resistance). About 85-90 percent of all diabetes is type 2.

Diabetic retinopathy (DR) is the main ocular complication of diabetes, and is the leading cause of blindness in working-age people in the western world.\textsuperscript{72,112,106,87} The incidence of retinopathy increases with duration of the disease and the majority of patients will have developed some retinopathy after 20 years.\textsuperscript{54,55} Microvascular occlusions, resulting in ischemia, is a major component of the retinopathy. The ischemia may stimulate formation of new vessels, proliferative retinopathy. Another feature of diabetic retinopathy is diabetic macula edema (DME) characterized by retinal thickening in the macular area due to leakage from the perifoveal capillaries.\textsuperscript{11} In order to define a level of edema in which it is appropriate to initiate laser treatment, the term “clinically significant macular edema” was established by the Early Treatment Diabetic Retinopathy Study (ETDRS) research group.\textsuperscript{21,22}

As with DR, the prevalence of DME increases with the duration of the disease, and with the severity of retinopathy.\textsuperscript{57,72} The prevalence of macular edema (ME) in the large Wisconsin Epidemiologic Study of Diabetic Reti-
nopathy (WESDR 1984) was reported to be 11.1 percent. It was slightly higher in type 1 (young onset) diabetes, where the 10 year rate to develop ME was 20.1 percent, compared to 13.9 percent in type 2 (old onset) not taking insulin. In type 2 patients on treatment with insulin the risk of developing ME was the highest, 25.4 percent. This high incidence could be suspected to be an effect of poor glycaemia control.

Patients with diabetes mellitus develop cataract earlier than healthy individuals, and cataract is a common cause of visual impairment in diabetic eyes. Cataract surgery normally restores good vision but it can also provoke ME in subjects with DR. Eyes with pre-existing DME are at highest risk for persisting ME and poor final visual outcome after surgery.
Optical coherence tomography (OCT)

Basic principles

Optical coherence range measurement is the optical analogue of ultrasound range measurement, but instead of back scattering of sound waves, optical-coherence range measurement relies on a backscattering of short-wavelength infrared radiation. For both measurement strategies, the ranges can be systematically sampled at multiple close angles, thus providing adjacent range measurements that can be presented as a depth profile, perpendicular to the beam and several depth profiles can be added together to provide a topographic image.

In ultrasound range measurement, the time of flight of a sound pulse reflected against a target is measured. The axial resolution therefore is limited by the pulse frequency of the ultrasound used. However, at the same time the penetration depth in acoustically complex media like biological tissue decreases with increasing pulse frequency setting the resolution limit to on the order of $100 \mu m$, and in order to obtain images within the eye the transducer has to be applied in contact with the eye.\textsuperscript{13}

Short coherence measurement relies on the fact that if an electromagnetic wave is split into two paths, the two ‘beams’, if being brought together, will only produce optical interfere if the time of flight difference is within the coherence length.\textsuperscript{100,115} Therefore, measurement of the time of flight for the path length of the reference beam, distance divided by speed, when optical interference occurs, provides information on the time of flight of the measurement beam. The time of flight of the measurement beam divided by the speed of the measurement beam provides the measured distance. The axial resolution therefore is limited by the coherence length of the electromagnetic wave which is on the order of $1 \mu m$. Near-infrared radiation penetrates the air cornea interface with limited losses and therefore allows no contact measurement.
Time domain OCT

The conventional OCT technique used in clinical practice is referred to as time-domain OCT (TD-OCT). The first commercial OCT instrument (OCT 1) was introduced to the market in 1996, followed shortly after by the more clinically applicable OCT 2 (Zeiss Humphrey, Inc, Dublin, CA, USA). The next generation OCT instrument, Stratus OCT (OCT 3) (Carl Zeiss, Meditec, Dublin, CA, USA), was introduced 2002.

OCT is based on the classical optical technique known as low-coherence interferometry. Figure 1 is an example of an optic interferometer (The Michelson interferometer). From a superluminescent diode (SLD) source, near infrared radiation, with low-coherent length and broad bandwidth, is directed onto a semi-transparent mirror (beam splitter).

Conventional TD-OCT instruments are built as a fibre optic system (Swanson) and a fibre coupler with a function analogue to the mirror “beam splitter” (Figure 2).

Figure 1. Schematic presentation of an Optic interferometer (Michelsons interferometer). SLD= Super luminescent diode source. a=beam reflected towards the reference mirror. b= beam passing through the beamsplitter to the tissue sample. c=beam reflected back from the reference mirror. d=beam reflected back from the sample. signal is recorded in the photodetector, and transferred to a computer for signal analysis.
Figure 2. A schematic fibre optic OCT setting. SLD=Super luminescent light source, Optic “beamsplitter” (coupler). Moving reference mirror for axial scanning (A-scan). Mirror for transverse scanning of the beam over the retina to construct a B-scan.

After the beam splitter, the beam is divided into a measurement arm directed into the eye and a reference arm, directed on a reference mirror with known relative position. By moving the reference mirror, the length of the reference arm will vary so that at some moment the absolute difference of time of flight from the beam splitter to the reference mirror and back (distance divided by speed), and the time of flight from the beam splitter to the back scattering target in the retina and back (distance divided by speed) will be less than the coherence time of the source. At that moment, the two beams when coinciding on the beam splitter, will produce constructive and destructive interference, sensed by the detector as increased intensity. By back calculation from the speed of light in air and the the speed of light in the eye media the depth of the back scattering target can be estimated. Since, targets at varying axial depth in the retina, corresponding to the various layers in the retina, will similarly be detected at various scan positions of the reference mirror, information about the reflectivity as a function of axial depth in the retina, A-scan, is obtained and stored in a computer (Figure 2). The fact that the measurements are based on measurement of time of flight is the bases of the name ‘Time domain OCT’ (TD-OCT). By transversally moving the beam after each axial scan of the reference mirror for reflectivity at various depths while recording the scanning angle, a two dimensional image, B-scan can later be reconstituted with the computer, In Stratus OCT ~ 400 A-scans can be obtained in one second. In the cross-sectional ‘pseudo-histological’ B-scan image, the strength of reflectivity from different structures can be presented in greyscale or as a false-colour scale (Figure 3).
Scan quality and image resolution

The quality of an OCT scan depends mainly on the image resolution and image acquisition time. Increased acquisition speed reduces image distortion caused by eye movements. The image resolution in axial and transversal directions, respectively, is determined by different physical mechanisms. The axial (depth) resolution is determined by the coherence-length of the source, which is inversely proportional to the bandwidth of the light source used. In TD-OCT, an SLD source with a bandwidth centred at ~800 nm (near-infrared) with a bandwidth of 20 nm is used which supports an axial image resolution of ~10µm. The lateral (transverse) resolution is determined by the spot-size and the focusing optics in the instrument which is similar to a conventional microscope. The image resolution is also dependent on the B-scan length of the tomogram, where higher lateral resolution is obtained if the A-scans are more densely packed (higher scan-density).

Since axial image resolution depends mainly on the bandwidth of the examination light source, one obvious way to improve axial resolution would be to use a broader bandwidth, and in 2003 the first use of ultrahigh resolution OCT (UHR-OCT) was reported by Drexler et al. The basic setup of UHR-OCT is similar to standard TD-OCT, but a Titanium:Sapphire femtosecond laser with a broader bandwidth is used. This results in an axial resolution of ~3 µm, which improves image quality, resulting in a considerably better differentiation between the different retinal layers. However, a notable limitation of the HD-OCT technology is that higher axial resolution slows down acquisition-speed for each A-scan thereby increasing intensity in the same spot close to threshold limit for retinal damage. Further, the longer sampling time at each A-scan, reduces the number of A-scans recordable before the eye moves. In B-scan. These technical difficulties, and the costly laser, have been the major drawbacks in the commercialization of UHR-OCT.

A possibility to improve image quality is by post-processing images to reduce noise although this will not change the resolution. One classical way
to suppress noise is by averaging multiple scans taken from the same position. Sander et al. developed a correlation algorithm for aligning individual A-scans from corresponding retinal locations by performing multiple B-scans\textsuperscript{91}. This resulted in improved image quality, comparable to that obtained with UHR-OCT (see example Figure 5D). One limitation of this strategy is that eye-movements may misplace B-scans during scanning. To ensure that the same location is examined repeatedly, and thereby enable good alignment of A-scans, an eye-tracking system has been developed. In this system, typical fundus features are used to detect transverse eye-movements and reposition the beam during scanning.\textsuperscript{35}

**Spectral domain OCT**

Spectral (or Fourier) domain OCT (SD-OCT), is a new generation of OCT instruments recently introduced to the market.\textsuperscript{107-109,74} The principle set up is similar to TD-OCT with an SLD source (~840 nm), a beam splitter providing, one measurement beam into the eye and a reference beam reflected on a reference mirror. In contrast to TD-OCT, the reference mirror in SD-OCT is stationary. The spectral distribution of intensity detected as the interference between the measurement beam and the reference beam contains information about back scattering delay caused by echoes from different depths in the retina.\textsuperscript{107-109} In SD-OCT, the beam resulting from interference of the measurement beam and the reference beam is therefore dispersed in space with e.g. with a grating and and captured by a high speed charge-coupled device (CCD) camera, resulting in a measurement of intensity as a function of wavelength (Figure 4). The spectral component of the spectral intensity distribution is then analyzed by a Fourier transform that provides information about the backscattered intensity as a function of time of flight difference between the measurement arm and the reference arm. The time of flight difference is finally converted to distance based on the speed of light in the tissue. In SD-OCT all echoes along the axis of the measurement beam are thus recorded simultaneously rather than as in TD-OCT consecutively by moving the mechanical reference mirror. Therefore, a considerably larger number of A-scans can be acquired per time unit with FD-OCT than with TD-OCT. TD-OCT allowing a wider field without eye movement artefacts if sampling density matches lateral resolution defined by the optics. Further, the sampling of all depths in one instant along one axial scan allows higher intensity, implying better sampling from larger depth, without exceeding the exposure limit. Figure 5 is an example of different image qualities that can be obtained with the Stratus and Cirrus OCT.
Figure 4. Simplification of a spectral domain OCT setting. SLD=Super luminescent light source. The reference mirror is fixed. DG=diffraction grating. A full field detector (CAM) act as spectrometer. DSP= digital signal processing.

Figure 5. Image A is a single B-scan from the Stratus fast program (128 A-scans) and B from the standard (512 A-scans) of a normal macula. Image C is a B-scan from Cirrus, (512 A-scans), of the same macula. Note the better quality compared to Stratus standard (B) composed from equal number of A-scans. This improvement is primarily due to the faster acquisition speed in Cirrus. Image D is a Cirrus image further improved by “averaging” of multiple scans.
Quantitative measurements

OCT provides a unique possibility to measure the retinal thickness in vivo.\textsuperscript{47,36} A quantitative tool for topographic measurements, the mapping technique, was first developed and introduced in 1998.\textsuperscript{41} The boundaries that delineate the retinal thickness in the Stratus OCT are defined as the distance between the vitreoretinal interface and the highly reflective band close to the retinal pigment epithelium (Figure 6a), thought to represent the junction between the inner and outer photoreceptor segments.\textsuperscript{18,12} The two-dimensional ‘thickness-map’ is then built from six single B-scans, equally spaced $30^\circ$ apart and scanned over a common axis through the area of fixation. The retinal thicknesses between the scans are extrapolated by the instrument. The map is constructed from three circular fields with diameters of one, three and six mm. The three and six mm circles are further divided into eight thickness areas in an ETDRS grid-pattern fashion\textsuperscript{21} where the average thickness in each area is presented (Figure 6A).

The central field (A1) represents the average thickness in the one mm area, whereas the foveal minimum is the thickness of the central point where the six scans intersect. With this scanning technique, the A-scan density is highest in the central field (512 A-scans), and decrease towards the periphery.

In Cirrus OCT, the area under examination is scanned in a 6x6 mm raster built from >20 times the numbers of measurement points compared to the Stratus maps (Figure 6B). With this scanning technique the A-scans are evenly distributed over the posterior pole which allows a more complete coverage of the macula. As in Stratus, the inner retinal border is the vitreoretinal interface, but in Cirrus the boundary for the outer retina is in the centre of the highly reflective RPE (Figure 6b). A traditional ETDRS-grid as described for Stratus OCT, is automatically placed over the posterior pole. Unlike in Stratus OCT, where the central fovea is determined by the patient’s fixation, the Cirrus instrument identifies the foveal location by the reduced reflectivity below the internal limiting membrane in the central macula.\textsuperscript{9}
a. B-scan in Stratus OCT. The inner retinal border (white arrow) and the outer retinal border (yellow arrow) at the thin highly reflective band adjacent to the pigment epithelium.

B. In Cirrus OCT the macula is scanned in a 6x6 mm square over the posterior pole. A single B-scan is composed of 512 or 200 A-scans. The thin dotted line symbolizes the raster of horizontal B-scans (200 or 128)

b. B-scan in Cirrus OCT. The inner retinal border (white arrow) and the outer retinal border (yellow arrow) centrally located in the pigment epithelium.
Aims

- To collect normal values of macular thickness assessed with OCT in children and adults and to evaluate the repeatability (reliability of the instrument) (Study I and II).

- To compare the “high-resolution” (standard) protocol with the “low-resolution” fast protocol in Stratus OCT (Study I).

- To evaluate whether age and/or gender affect retinal thickness (study II and III).

- To evaluate repeatability and reproducibility of quantitative optical coherence tomography measurements in eyes with neovascular macular degeneration and low levels of visual acuity comparing the conventional TD-OCT with new SD-OCT technique (study IV).

- To study the effect of cataract surgery on the macular thickness and structure in subjects with diabetes retinopathy, assessed with OCT and fluorescein angiograms and compared to a control group (study V).
Material

Healthy volunteers

Sixty-seven healthy individuals – 43 women (64 percent) and 24 men (36 percent) – were included in the ‘adult’ studies (studies I and II). They were recruited from the staff of the eye clinic at Uppsala University Hospital. The median age was 34.4 years (range 12-74). Only those subjects without a history of any ophthalmologic disease, and only eyes with a normal visual acuity (20/20), and a refractive error of less than 6 diopters (D) spherical, and/or 3 D cylindrical were included.

For study III, 56 full-term children (≥ 37 weeks gestational age or normal birth weight [> 2500g]) were randomly selected from the birth register of the Swedish National Board of Health and Welfare. Parents were asked by letter whether they wanted their child to participate in the study. The mean age was 10.1 years (range 5-16) with an equal number of boys and girls. Eyes with a VA < 0.65 and/or spherical equivalent > +3 or < -3 D and/or > 2 D of astigmatism were excluded.

Patients

All patients were recruited from the outpatient clinic of the ophthalmology department of Uppsala University Hospital. For study IV, 30 patients, (10 men and 20 women) with a mean age of 80 years (range 64-90) with neovascular (wet) AMD were recruited. The majority had been recently diagnosed with the condition and were about to receive their first anti-VEGF injection. In study V, 34 patients, (27 men and seven women), with a mean age of 71 years (range 54-82). With diabetes type 2, mild-to-moderate retinopathy, and who had been scheduled for cataract surgery were included. Thirty-five otherwise healthy control subjects (16 men and 19 women, median age 70 years range [58-81]) planned for cataract extraction were recruited for comparison.

The study protocols for both healthy controls and patients were approved by the ethics committee of Uppsala University. The study participants were given full written information and signed informed consent documents.
Methods

OCT

The TD-OCT Stratus OCT (Carl Zeiss, Meditec, Dublin, CA, USA) with software 4.0.1 was used in all studies and measurements were obtained through dilated pupils. The examiner controlled the fixation by observing the macula and fixation object on the live fundus image. No external fixation was used. Images with low signal strength (less than five) and obvious artefacts were repeated in order to obtain the best quality possible. The quantitative measurements were performed with the ‘macular thickness map protocol’ (standard) in adults and the fast macular protocol (fast) in both children and adults. The B-scans in the standard protocol are manually obtained one-by-one with an acquisition time of around one second/B-scan. Each B-scan consists of 512 A-scans, thus making the entire map made up from 3072 A-scans (512 x 6). The fast macular thickness map protocol compresses the six macular thickness B-scans into one scan, composed of 768 A-scans (128 x 6) with an acquisition time of 1.92 seconds for the entire map. To allow calculation of repeatability for studies I and II, there were three maps made in both eyes, and the patients leaned back for a short rest between each map scanning. Both eyes were examined and one eye was randomly chosen for establishing normal retinal thickness values for the in all nine ETDRS-areas, for the foveal minimum and the macular volume.

In order to separate the retinal nerve fibre layer (RNFL) thickness from the total retinal thickness (study III), one out of the six 6-mm scans that formed part of a map was selected. This scan extends horizontally from the nasal to the temporal side of the macula. In this scan, one specific A-scan, (number 50 of 512), counting from the nasal side was used. This A-scan examines the retina in the papillomacular nerve fibre bundle, within the ETDRS area nine. The instrument algorithm can calculate both the total retinal thickness and the RNFL-thickness in each A-scan.

In study V, OCT was evaluated both qualitatively and quantitatively. Subjects were examined with the ‘macular thickness map’ (standard) protocol, pre-operatively, at six weeks and at six months after cataract surgery. The nine ETDRS-areas of the macular map were merged into three circular fields: central subfield (A1), inner (A2-A5) and outer circle (A6-A8) in which the retinal thickness was calculated. The total macular volume was also recorded.
The six B-scans that constituted the macular map were also evaluated qualitatively by an independent observer and classified into three types: Type 1, homogenous thickening of the fovea, Type 2, sub-foveal macular detachment and Type 3, cystoid changes in the outer retinal layers. If no changes were found they were classified as normal.

In study IV, the SD Cirrus HD-OCT model 4000 (Carl Zeiss Meditec Inc. Dublin CA.USA) software version 4.0.1 was used together with the Stratus OCT. The standard and fast map protocols in Stratus (see above) were compared to the quantitative 512x128 and 200x200 cube protocols in Cirrus. The 512x128 cube is generated from 512 horizontal A-scans and 128 vertical B-scan lines with an acquisition time of 2.4 seconds, and the 200x200 cube with 200 horizontal A-scans and 200 vertical B-scans with an acquisition time of 1.5 seconds (see further quantitative measurements page 21).

**Fluorescein angiography**

Fluorescein angiography (FA) which is the gold standard to demonstrate diseases affecting the macula was used in study V. Sodium fluorescein is a small molecule of which 75-85 percent binds to serum proteins the rest circulating freely. The tight junctions between the endothelial cells in the retinal vessels form the inner blood-retinal barrier. This barrier limits the passage of fluorescein. A small leakage from healthy vessels that can be detected on fluorescein angiography is considered pathological.

The Topcon digital system model TRC-50 IX was used. Half a ml of a 10 percent solution of sodium fluorescein followed by a flush of 0.9 percent saline was given in the anticubal vein. Images were taken up to five minutes after the injection (late phase). Fluorescein angiograms were performed pre-operatively, and at six weeks after cataract surgery. In cases where cataract caused poor angiographic quality and made analysis impossible, the angiography was performed seven days ± one day after the operation and classified as preoperative. The angiograms were qualitatively classified into three leakage patterns: Pattern 1, cystoid leakage with pooling of dye in cystic spaces in the macula in the late phase. Pattern 2, focal leakage with well-defined areas of leakages from micro aneurysms and dilated capillaries. Pattern 3, diffuse, ill-defined, widespread late leakage.

**Visual acuity testing**

In studies I, II and III, subjects with normal visual acuity (VA) were included. In studies I and III, VA was tested with a Snellen chart at five metre. In Study II, the best corrected VA was assessed monocularly with linear log
MAR charts (Anders Hedin chart) at five meter and with a HVOT-chart at three metre.

In studies IV and V, which included subjects with macular diseases, the VA was determined with an ETDRS -chart. This self-illuminated chart is mainly used to test VA in eyes with macular disease and low levels of VA. Testing was performed at two metres. The chart consists of five optotypes on each line. The lines are separated by 0.1 log unit and the logarithmic scale enables statistical analysis. A visual acuity score of 85 letters is equivalent to Snellen VA 20/20.

In study V, a loss of > five letters between day seven and week six, in combination with macular changes on FA and/or OCT was the definition of clinical cystic macular edema (CME).
Repeatability is mainly a measure of reliability. One examiner performs repeated measurement on the same object, with the same instrument within a short time. Reproducibility is the variation in repeated measurements on the same object, when the instrument, the examiner and/or the time of examination is not the same. For comparison with other authors, repeatability was estimated using three different methods: As intraclass correlation coefficient (ICC) (Study I and II), coefficient of variance (CV) (Study I, II and IV), and as the coefficient of repeatability (CR) (Study I) as described by Bland and Altman. Thus the CR was calculated as two standard deviations (SD) of the difference between two of the three measurements made with the same protocol for each area and volume. As three measurements had been made there is three combinations of two measurements resulting in three different CR:s. The mean of these three CR:s was used as the CR of the technique.

In study IV, coefficients of repeatability (intravisit – two scans at first visit) and reproducibility (intervisit – one scan at each visit) were calculated with the definition $1.96 \times SD$ for the intravisit difference between the measurements (repeatability) or intervisit difference (reproducibility).

Limits of agreement, which illustrate the agreement between two methods, protocols or examinations, were calculated between Standard and Fast protocols for all areas and for the volume, as described by Bland and Altman. (Study I).

The independent t-test, the Mann-Whitney test or ANOVA were used for comparison between groups. Intra-individual comparison was performed using the paired sample t-test.

For analysis of correlations, Pearson’s test was used. Linear regression of retinal thickness and volume with age was based on the mean values of three measurements in order to reduce the effect of measurement error (Study III).
Results

Study I:
All 67 subjects underwent three measurements with the standard protocol and 45 of them also 3 measurements taken with the fast protocol. Normal values for both protocols were within one µm in the one and three mm circles (A1-A5) and a slightly larger difference (0.3-2.5 µm) in the outer six mm circle (A6-A9), see figure 7. The inner superior, inferior and nasal quadrants (A5, A2 and A4) were thickest for both protocols and the temporal quadrants (A3 and A7) were thinnest. Mean values were higher in men for all nine areas and volume, but were only statistically significant in A2 and A5. The variability was low, and of the same order for both protocols with intraclass correlation coefficient (ICC) values ranging from 0.92-0.98, coefficient of repeatability (CR) 6-8 µm and coefficients of variance (CV) below one percent. The exception was area A1 for which the variability was twice as large with the fast protocol as with the standard protocol. Limits of agreement between the two protocols were, as a rule, less than 10 µm.
Figure 7. Box-plots for thickness values and volume obtained with the standard and fast protocols. Medians are drawn as thick dark lines. Boxes include interquartile range (25-75%). Whiskers extend to the most extreme data point which is not more than 1.5 times the length of the box. Extremes are drawn as circles.

Study II:

Fifty-six children aged five to 16 years were examined. One child was excluded according to the refractive exclusion criteria. Thus 55 randomised eyes were included in a database for normal values in children. All children co-operated well. Mean ± SD central macular thickness was 204 ± 19 µm. No correlations were found between age, gender and macular thickness. Coefficients of variance (CV) were < two percent and intraclass correlation (ICC) > 0.9 in all areas except in the foveal minimum (0.69).

Study III:

The relationship between thickness in each ETDRS area including the foveal minimum and macular volume with age was analyzed. Using linear regression analysis, we found a statistically significant negative relationship between retinal thickness and age for all nine ETDRS areas in the macular thickness map (p=0.042 for A1, p<0.01 for all other areas).
Figure 8 presents the change of retinal thickness with age for all nine ETDRS areas. Retinal thickness decreased by 0.26-0.46 µm, and the total retinal volume by about 0.01 mm³ per year. The proportion of this tissue loss with age that was due to reduction of RNFL was also analyzed in a specific point in the papillomacular nerve fibre bundle (see methods). As for the total retinal thickness, there was a statistically significant thinning of the RNFL with age, indicating a nerve fibre layer thinning of 0.09-0.12 µm per year in this area. Twenty percent of the thinning was due to RNFL thinning and eighty percent due to thinning in other retinal layers.

Figure 8. Dependency between thickness and age for Areas A1 to A9 in one randomly chosen eye in 67 individuals between 12 and 74 years of age. The regression line and its 95% CI is plotted for each area.
Study IV:

The purpose of this study was to determine repeatability and reproducibility of macular measurements in eyes with neovascular (wet) age-related macular degeneration (nAMD) and compare time-domain Stratus OCT 3 to spectral domain Cirrus HD OCT 4000.

Twenty-nine eyes with neovascular AMD were examined. The macular thickness was assessed with the high-resolution macular thickness map (standard) and the fast protocol in Stratus OCT, and with the 512x128 and 200x200 cube protocols in Cirrus OCT. Two measurements, with a short rest in between, were performed at the first visit to analyse repeatability. One up to two weeks later, a third measurement was performed to analyse reproducibility. In Cirrus OCT, a manual correction of foveal location was also performed.

The repeatability for central macular thickness, (expressed as CV) was about three percent for all protocols, and the coefficient of repeatability between 34-54 μm. Reproducibility expressed as CV was between four to seven percent and the coefficient of repeatability between 64-89μm. After manual adjustment of foveal location in Cirrus OCT the coefficient of repeatability improved to 12-18 μm and coefficient of reproducibility to 44-47 μm.

Study V:

Thirty-four patients with type 2 diabetes and 35 controls were enrolled in this study.

Fluorescein angiography (FA)
The fluorescein leakage was classified into three patterns (see methods). At 6 weeks, cystic fluorescein leakage (Pattern 1) was observed in 23 percent of the control eyes. Apart from that no focal or diffuse leakage was seen. Fluorescein leakage was seen in 76 percent of the diabetic eyes. Ten percent showed angiographic CME only (Pattern 1), 12 percent well-defined focal areas of leakage outside the fovea (Pattern 2), 18 percent diffuse leakage (Pattern 3), and 34 percent a combination of more than one pattern.

Qualitative OCT
At six weeks 20 percent in the control group and 44 percent of diabetics showed visible changes on OCT. The changes were classified into three types (see method). The main difference between the groups was that Type 1, a homogenous thickening of the fovea, was seen only in diabetic eyes (26 percent). The other two Types were equally frequent in eyes with and without diabetes.

Quantitative OCT
For quantitative measurements the macular map was divided into three areas (see methods). There were no statistically significant differences in thickness between the groups preoperatively. In both groups, a statistically significant increase in thickness was observed for all the macular areas as well as in the macular volume, at six weeks. The thickness and the volume remained significantly increased at six months compared to preoperative values. When comparing the diabetic and control eyes, there were no differences for the central area or inner circle at any visit. At six weeks however, the outer circle and the total macula volume was significantly higher in the diabetic eyes (p= 0.005, p=0.020 respectively) remaining statistically significant at six months (p=0.013 and p= 0.026).

OCT and FA qualitative changes were compared. For diabetic eyes the correspondence between the different FA patterns and OCT types was poor. For the control eyes, the correlation was better with seven of nine FA patterns that corresponded to OCT Types 2 and/or 3.

Visual acuity (VA)
There was no statistically significant difference in median VA preoperatively (p=0.06), or at day seven (p=0.12) but at week six after surgery a significant difference was observed with lower median VA in eyes with diabetic retinopathy (p=0.004). At six months, however, the VA was nearly equivalent, with no statistically significant difference between the groups (p=0.97). Two control eyes (six percent) and four diabetic eyes (12 percent) experienced a clinical CME defined loss of > five letters between day seven and week six.

The correlation between VA at week six and the percentage change in central subfield (A1) thickness between the preoperative/baseline and week six was calculated and found to be weak.
Discussion

Normal values, interchangeability of protocols, and effect of age and gender (studies I, II and III).

In this thesis different aspects of OCT have been studied and evaluated. In Stratus OCT there are two types of quantitative macular thickness protocols, the higher resolution (standard) and the fast protocol (see Methods). In software version 4.0.1, normal values for retinal thickness are provided, but only for the fast map protocol and for ages above 18. In the clinical situation it may sometimes be necessary to compare examinations performed with different protocols at different visits, and we therefore wanted to find out if the two protocols were interchangeable and also to determine normal values for children younger than 18 years (study II). In study I, normal values from 67 healthy subjects with an age range of 12-77 years was determined. Normative data obtained from the protocols were very close with a mean central macular thickness of 207±19 µm for both protocols. There was a wide range of normal macular thicknesses but values below 170µm or above 250µm were unusual in normal eyes. The limits of agreement between the two protocols were as a rule less than 10µm, showing that they were interchangeable in the clinical situation.

Normal values for spectral domain (SD)-OCT Cirrus were not evaluated in this thesis. However, in study IV where OCT Stratus and Cirrus were compared in eyes with neovascular (wet) AMD, the macula was approximately 30 percent thicker with the Cirrus compared to the Stratus instrument. This is consistent with other reports on normal eyes with Cirrus OCT or other SD instruments and is probably an effect of a different (deeper) boundary used in Cirrus to outline the outer retinal border (see Figure 6).

Testing VA and examining the retina with ophthalmoscopy or the slit-lamp in children requires good co-operation. OCT takes only a few seconds and can be a helpful and objective tool to examine and document retinal findings. Study II was the first population-based study to cover an age range of about 10 years in full-term children selected by randomized sampling based on the birth registers. Study I concluded that normal values were almost identical with the fast the standard protocols and since use of the fast-protocol shortened the examination procedure, the fast protocol was used to collect normative data in study II. In children the mean values were slightly
lower in the central subfield, 204 ± 19 µm, compared to 207 ± 19 µm in the study on adults. The OCT technique is fast and non-invasive, and in our experience a suitable examination technique for most children. The use of the fast-protocol shortened the examination, which facilitated co-operation and no child was excluded due to lack of concentration.

Study III concluded that age had an influence on retinal thickness, and that thickness decreased with 2.6-4.6 µm each decade, depending on which macular area was examined. Retinal thinning with age has been reported by others⁵,⁵² but to our knowledge this was the first study to document a thinning with age in the map protocols. Similar finding have subsequently been reported by Kyung et al. (2009) and Neuville et al. (2009).⁷⁵ Like previous authors⁶⁹,¹⁰ we did not find a significant difference in retinal thickness between women and men, but there was a tendency towards thicker maculae in men in all ETDRS areas and larger macular volume. Also, in study II, boys generally had thicker retinas than girls but no to a statistically significant degree. It seems reasonable that there is a small difference in retinal thickness between males and females, but to confirm this difference a larger study with better power is needed. In fact, a recent report from the Handan Eye Study which studied 2,230 eyes of healthy Chinese subjects found significantly greater macular thickness in men compared to women.²⁰ For large differences in macular thickness, as in diabetic macula edema (DME), vein occlusions, or wet AMD the thinning with age may be of limited importance. However, over a lifetime, there is a retinal thinning of approximately 25-30 µm, which one should be aware of when performing studies on diseases where the changes in retinal thickness can be expected to be small. The age-related thinning of the macula was not found in children (study II), though the age range was quite narrow (5-16 years). In fact, there was, if anything, a trend towards a retinal thickening with age in this group. Huynh et al. examined 1,543 six-year-old children.⁵⁰ The central macular thickness reported in their study was thinner (194µm) than in the present study, and a statistically significant positive correlation with age was found. One could speculate that this apparent ‘thickening’ in early age could be an effect of the developing macula in childhood⁴⁴,¹¹⁴ resulting in a slight thickening of the central retina before it is fully developed, but the finding could also be due to algorithm problems in a growing eye.

Repeatability and reproducibility (Studies I, II and IV).

Repeatability is mainly a measure of instrument variability where repeated measurements are performed with the same instrument by the same operator, while reproducibility can include variations due to change of operator, instrument or examination on a different occasion. Good repeatability and reproducibility have been reported in normal eyes for the high resolution
map protocols\textsuperscript{68} and the fast protocols.\textsuperscript{81} Studies I and II found excellent repeatability for the fast protocol as well as the standard protocol (Study I), on normal eyes both in children and adults. The two protocols have different potential advantages. The fast map is automated, quicker to perform, requires less skill from the examiner. It is therefore the protocol most frequently used in the clinic as well as in clinical trials. The standard protocol, on the other hand, has a higher image resolution and since the scans are obtained one-by-one, a well-trained examiner has a better control of the mapping procedure. The repeatability was excellent and very similar for the standard and fast maps in all areas examined, with the exception of central subfield (A1) where the repeatability was twice as good with the standard map. The central subfield is the area of greatest importance regarding visual function and it is the area preferred when evaluating macular thickness in clinical trials. Information on the better repeatability with the standard protocol might be of interest when planning future studies on the macula. However, one should be aware that the better repeatability found with the standard protocol in study I might not apply to eyes with low levels of VA and pathological changes in the macula.

Previous studies with time domain OCT have not shown any marked difference between repeatability and reproducibility in normal eyes and eyes with DME.\textsuperscript{68,81,14} The situation may well be different in eyes affected by wet AMD, where the reliability of thickness measurement with macular maps is known to be low. This is primarily a problem with poor fixation and measurement artefacts due to morphological damage to the retinal structures, resulting in algorithm errors and incorrect identification of the retinal borders.\textsuperscript{85,89,31,79,60} Initially, the macular thickness map algorithm, on which Stratus OCT maps are based, was developed to measure retinal thickness in eyes with DME.\textsuperscript{42} In DME, there is a thickening of the neurosensory retina, but the RPE, which is used as the outer retinal boundary by the OCT algorithm, is not affected. In AMD on the other hand, the echo from the RPE is often disturbed by neovascular lesions, often resulting in erroneous measurements.

Effective treatment of wet AMD by intravitreal injections of inhibitors of vascular endothelial growth factor has recently been introduced.\textsuperscript{33,88,6} This treatment has a marked effect on the disease progression but repeated injections are usually necessary.\textsuperscript{27,63} Macular thickness seems to reflect disease activity and changes in macular thickness, if reliable, could be a valuable tool in re-treatment decisions. The latest guidelines regarding treatment with ranibizumab\textsuperscript{71} strongly recommend OCT in the management of neovascular AMD. However, as discussed above, there are considerable measurement problems with the quantitative OCT maps in eyes with AMD. It is important to be able to differentiate a true change from variations in the measurement technique. So far, there are only a few published studies reporting measurement variability in eyes with AMD. Patel et al. reported repeatability for Stratus fast maps\textsuperscript{80} and Krebs et al. compared repeatability and reproducibility in high-resolution and fast maps with the Stratus OCT.\textsuperscript{61} Menke et al.
published the first results from mapping with the SD-OCT technique and recently Parravano et al. reported reproducibility in Cirrus OCT.

In study IV, we wanted to compare the conventional Stratus and the new spectral domain Cirrus OCT in eyes with wet AMD, and find out if there were any major differences between the quantitative protocols within and between each of the instruments. Both repeatability and reproducibility were evaluated since it was not self-evident which of these two that provide the best information. Repeatability is mainly a measure of instrument variability, whereas reproducibility also can include short-term variations in macular thickness. We found that, in contrast to normal eyes, repeatability was considerably better than reproducibility with all map protocols. Visit two (reproducibility) was performed after one-to-two weeks, and in that time a true change could have occurred due to disease progression. In fact there was a trend towards thicker maculae at the follow-up visit, however, not statistically significant. Although it cannot be excluded that the difference between reproducibility and repeatability in the present study was due to progression of the disease, the possibility that eyes with wet AMD show marked ‘short-time’ variation should be kept in mind if changes in retinal thickness determined with OCT should be used to determine re-treatment. We found that an increase or a decrease in macular thicknesses at the second examination were equally common. This does suggest that there is some variation in macular thickness not related to disease progression. Diurnal variations of macular thickness in eyes with macula edema (ME) due to diabetes retinopathy have been reported. This ‘short-time’ variation can also be expected to occur in eyes with ME due to AMD.

In study I, the repeatability for the central subfield (A1) with the standard map was twice as good as with the fast map. However, in eyes with wet AMD neither repeatability nor reproducibility could be improved by using the manual standard protocol with the Stratus OCT, although the scanning was performed by the same examiner in both studies. This indicates that a quick acquisition time is probably more important in reducing measurement variability, than a higher scan resolution or manual correction of scan location during the examination procedure.

In study IV, the new SD-OCT Cirrus was also evaluated. The SD technology provided images with higher resolution, quicker acquisition time, and around 70 times more measuring points compared to TD-OCT (see methods). Two different quantitative protocols (200x200, 512x128 cubes) were compared to the standard and fast maps in Stratus OCT. Less artifacts have been reported with the new algorithm in Cirrus compared to Stratus OCT in eyes with AMD. At first, this seems self-evident, because one could expect the algorithm to more easily identify the retinal borders in an image with higher resolution, but paradoxically, enhanced resolution with more reflective layers in the retina, requires a more refined boundary-detection algorithm (for examples of image resolutions see Figure 5). Unexpect-
edly, despite this improved algorithm, study IV found only small differences in repeatability and reproducibility when comparing the two OCT instruments. One option to further improve the algorithm measurements is manual correction of algorithm errors. Sadda and his co-workers have developed software for Stratus OCT maps, where misplaced retinal boundaries can be manually re-drawn, and these corrections have resulted in a very good reproducibility in eyes with AMD.\textsuperscript{90,51} A major improvement with the SD-technology is the possibility to evaluate the quality of the map and adjust for algorithm errors after the examination. A similar ‘drawing tool’ as used by Sadda et al. is included in the Cirrus software\textsuperscript{9} and could probably have improved the measurements, but when there are 128-200 A-scans that might need to be re-drawn, this is not an option in a clinical setting. Another possible explanation for the small improvement in repeat- and reproducibility in Cirrus compared to Stratus could be the differences in how the instruments locate the central macula (fovea). In Stratus, the fovea is determined by patient fixation while in Cirrus the software places the ETDRS-grid automatically over the central macula. The Cirrus software identifies the fovea by the reduced reflectivity in the central macula in a healthy retina. This reflectivity pattern can be disturbed in an eye with AMD, resulting in incorrect positioning of the grid. In the Cirrus software there is an option to correct the location of a ‘misplaced’ fovea. If the evaluating physician disagrees with the algorithm about the true location of the fovea, manual adjustments can be performed. This subjective correction was performed and evaluated as a sub-analysis in Study IV. The corrections resulted in a considerably lower variability between measurements and the coefficient of repeatability improved so as to be close to the repeatability found in Stratus fast maps in healthy eyes (study I).

OCT and correlation with visual acuity and fluorescein angiography (Study V)

One major advantage of quantitative OCT is the possibility to follow macular changes objectively over time. This option was used in study IV to study and follow macula changes induced by phacoemulsification up to six months after surgery performed in patients with diabetic retinopathy (DR) and healthy controls.

One aspect of OCT often discussed is the correlation with visual function, and whether OCT measurements could replace VA testing. Correlations between OCT and VA were evaluated in study V. Already in 1995, Hee et al. reported good correlation between VA and retinal thickness in eyes with diabetic retinopathy,\textsuperscript{40} and this finding has since been confirmed by others. However, the reported correlations vary widely.\textsuperscript{76,32,49,15} As found in study I, and as previously reported,\textsuperscript{10} normal values for macular thickness vary over
a wide range. Therefore, it seems reasonable that a change in thickness better could correlate to visual function, and we decided to evaluate the correlation between VA and retinal ‘thickening’ six weeks after cataract surgery. The correlation found was weak. In the diabetic group, there were some maculae with profound thickening but very good VA. Thickened maculae and good VA were also observed in eyes with DME after grid laser treatment in a report from the DRC network study group. One explanation for the low correspondence with VA, and thickening in study V, could be that we only included eyes with no ME and presumably well preserved photoreceptor function. An edema may then have less effect on VA than in eyes with long-standing ME.

Fluorescein angiography (FA) is the gold standard to evaluate macula leakage after cataract surgery. Fluorescein angiograms and qualitative OCT scans were performed, classified and compared after surgery in study V (see Methods). A majority of diabetic eyes (76 percent) showed leakage on FA, and about half, (44 percent) revealed change on OCT. Otanio at al. reported a good correlation between FA and specific OCT findings in eyes with diabetic ME. Soliman et al. further refined the OCT qualitative patterns and also reported a good correlation with FA. In study V, we did not find a strong connection between FA and qualitative OCT in eyes with DR. Classification of FA-patterns was found to be difficult when many eyes revealed a mixture of leakage patterns. However, in control eyes, where only one leakage pattern on FA was seen, the correspondence with qualitative OCT was good.

The findings in study V revealed that OCT was as good as FA to detect and follow clinically important CME after cataract surgery, and thus we concluded that OCT was the superior technique. It should however be kept in mind that quantitative or qualitative changes on OCT are often seen after surgery without any obvious effect on visual function, and such sub-clinical changes do not require immediate treatment.
Concluding remarks

- Normal values of macular thickness were equal between the Stratus standard and fast protocols. Repeatability was excellent with both protocols and they were found to be fully interchangeable.

- Macular thickness throughout the macular map decreased significantly with age in normal eyes with exception of the central point (foveola).

- Normal thickness values for the fast macular map in Stratus OCT were determined for full-term children aged five to 16 years. There was no statistically significant correlation between age/gender and macular thickness. Repeatability in children was good, and close to that observed in adults. OCT seemed to be suitable for examination of the macula also in children.

- In eyes affected by neovascular (wet) AMD, there were only small differences in repeatability and reproducibility, comparing the quantitative mapping programs in conventional Stratus and the corresponding maps in the new Cirrus OCT. The repeatability was higher than in normal eyes. However, a sub-analysis evaluating the Cirrus software for manual correction of foveal position, showed a significant decrease in variability.

- Quantitative OCT corresponded poorly to FA patterns in eyes with diabetic retinopathy after cataract surgery. There was a weak correlation with macular thickening and VA after cataract surgery. Changes in OCT were often observed without any obvious effect on VA.

- OCT was as good as the ‘gold standard’ fluorescein angiography in revealing clinical CME after cataract surgery, and is the technique recommended for follow-up after surgery.
Sammanfattning på svenska

Näthinnans centrala del (makula eller gula fläcken) är av största betydelse för skarpt seende. Flera sjukdomar kan drabba makula, t.ex. diabetes, blodproppar och åldersdegeneration. Bedömning av makula, både gällande funktion (synskärpa) och struktur är en väsentlig del vid undersökning av dessa patienter. Den kliniska bedömningen av makula har traditionellt varit genom undersökning i ögonmikroskop. Optisk koherens tomografi (OCT) är en relativt ny teknik för undersökning av ögat med hög förstoring och precision, vilket också möjliggör direkta mätningar av ögats vävnader, t.ex makulas tjocklek. OCT liknar ultraljud, men använder sig av reflekterat ljus istället för ljud. Ljusets egenskaper gör att undersökningen ger en mycket fin bildupplösning med stor detaljriksdom.

OCT har funnits i praktisk sjukvård sedan slutet av 1990-talet och man har de senaste åren sett en mycket snabbt ökande användning av tekniken. Utvärdering av den kliniska nytta av instrument är angelägen, inte minst mot bakgrunden av att det också sker en snabb utveckling av behandlingsmöjligheter vid olika makulasjukdomar. Man behöver helt enkelt veta om man kan lita på mätningarna. Detta kräver både en uppfattning om mätmetodens repeterbarhet (tillförlitlighet) och reproducerbarhet (förväntad variation mellan mätningar utförda vid olika tillfällen).

Tre av de i avhandlingen ingående arbetena har utvärderat OCT i friska ögon och två arbeten i sjuka ögon (diabetes- samt åldersförändringar i makula). Till arbete 1 och 3 rekryterades 67 ögonfriska individer, bestående av personal, anhöriga och vänner med normal syn och mellan åldrarna 12-74 år. Studiedeltagarna undersöktes med två olika mätprogram i OCT instrumentet (Stratus). Det ena programmet är ”högupplöst”, dvs. ger bättre detaljriksdom, och kräver lite större erfarenhet av undersökaren. Det andra programmet är ett automatiserat ”snabbprogram” som är enklare att utföra och används därför oftast i kliniska sammanhang. Vi ville samla in ett eget normalmaterial över makulas tjocklek och också jämföra om de två mätprogrammen överensstämde. Vi fann att normalvärden för programmen var närmast identiska. Repeterbarheten, dvs. instrumentets tillförlitlighet var också mycket god. I centrala makula som är viktigaste för synfunktionen var repeterbarheten för det högupplösta programmet bäst, men snabbprogrammet visade sig också vara ett bra alternativ.

Att näthinnans och synnervens nervfibrerlagret förtunnas med åren är visat i histologiska (mikroskopiska) undersökningar av vävnad från avlidnas ögon eller ögon som undersöks efter att de opererats bort pga. sjukdom. Man skulle därför förvänta sig, att makulas tjocklek, också skulle förtunnas med åren, men något sådant samband har ännu inte rapporterats för OCT mätprogrammen. OCT har den unika egenskapen att man kan göra mätningar på näthinnan hos en levande människa och slipper därför artefakter (fel) som kan uppstå när man bearbetar ett vävnadspreparat för mikroskopi. Vi mätte tjocklek av makula och också tjockleken på synnernens nervtrådslager och fann ett att makulas såväl som nervfiberlagrets tjocklek avtog signifikant med stigande ålder även mätt med OCT. Tjugo procent av förtunningen sågs i nervfiberlagret och åttio procent i näthinnans övriga lager.

Arbete 1 och 2 samt andra publikationer har visat att tillförlitligheten av OCT mätningar är mycket bra i friska ögon med god syn. Från den kliniska vardagen är dock viktigt att tillförlitligheten är mycket sämre i ögon med sjukdom i makula, framför allt i ögon med så kallade våta åldersförändringar (våt makuladegeneration). Detta är ett problem då resultat av moderna makulabehandlingar, vid vilka läkemedel injiceras i ögat, rutinmässigt följs upp med OCT. Dessa läkemedel är mycket effektiva i att bromsa upp sjukdomen, men tyvärr läcker den oftast inte ut och upprepad injektion behövs som regel. OCT undersökningens resultat är en viktig del i beslutet om ny behandling behöver ges, och vi måste därför veta hur mycket vi kan lita på undersökningen. En ny generations OCT, sk "Fourier-domain" OCT finns sedan en kortare tid tillgänglig på marknaden. Dessa OCT instrument har mycket högre detaljupplösning på bilden och ger en mer heltäckande och snabb undersökning av makula jämfört med vår traditionella OCT. Man kan förvänta sig att dessa förbättrade egenskaper också ska ge bättre och mer tillförlitlig mätinformation. Vi ville därför jämföra vår vanliga OCT (Stratus) mot det nya instrumentet (Cirrus). Vi studerade patienter med våt makulade-
generation och låg synskärpa och fann, något oväntat, att tillförlitligheten inte var mycket bättre med Cirrus OCT, när man jämförde med Stratus OCT. Men Cirrus erbjuder andra möjligheter. Datorprogrammets mätfunktion i Cirrus är vidareutvecklat och ger bland annat en möjlighet att manuellt justera uppenbart felaktiga mätningar i efterhand. I en delanalys utvärderade vi den möjligheten och fann att tillförlitligheten av mätningarna förbättrades markant och Cirrus blev då överlägsen Stratus. Man skall dock vara medveten om, att dessa justeringar är subjektiva och resultatet påverkas därför sannolikt mycket av vem som gör justeringarna.

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References

8. Bressler NM. Age-related macular degeneration is the leading cause of blindness. *JAMA* 2004;291:1900-1901.


54. Klein R, Klein BE, Moss SE, Davis MD, DeMets DL. The Wisconsin Epidemiologic Study of Diabetic Retinopathy. II. Prevalence and risk of diabetic reti-
nopathy when age at diagnose is 30 or less years. *Arch Ophthalmol* 1984 Apr; 102:520-526.


102. van Velthoven M EJ, Faber DJ, Verbraak FD, van Leeuwen TG, de Smet MD. Recent developments in optical coherence tomography for imaging the retina. *Progress in retinal and eye research* (26); 2007: 55-77.


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