Optical Coherence Tomography (OCT) Device Independent Intraretinal Layer Segmentation

Alexander Ehnes^{1,2}, Yaroslava Wenner^{1,3}, Christoph Friedburg¹, Markus N. Preising¹, Wadim Bowl¹, Walter Sekundo³, Erdmuthe Meyer zu Bexten², Knut Stieger¹✉, and Birgit Lorenz¹

¹ Department of Ophthalmology, Justus-Liebig-University, Giessen, Germany

² Department of Medical Informatics, University of Applied Sciences, Giessen, Germany

³ Department of Ophthalmology, Phillips University, Marburg, Germany

Correspondence: Knut Stieger, DVM, PhD, Department of Ophthalmology, Justus-Liebig-University, Friedrichstrae 18, 35392 Giessen, Germany. e-mail: knut.stieger@uniklinikum-giessen.de

Received: 9 June 2013

Accepted: 30 November 2013

Published: 11 February 2014

Keywords: optical coherence tomography; retinal layer segmentation; retina; graph theory optimization

Citation: Ehnes A, Wenner Y, Friedburg C, et al. Optical Coherence Tomography (OCT) device independent intraretinal layer segmentation. *Trans Vis Sci Tech.* 2014;3(1):1, <http://tvstjournal.org/doi/full/10.1167/tvst.3.1.1>, doi:10.1167/tvst.3.1.1

Purpose: To develop and test an algorithm to segment intraretinal layers irrespective of the actual Optical Coherence Tomography (OCT) device used.

Methods: The developed algorithm is based on the graph theory optimization. The algorithm's performance was evaluated against that of three expert graders for unsigned boundary position difference and thickness measurement of a retinal layer group in 50 and 41 B-scans, respectively. Reproducibility of the algorithm was tested in 30 C-scans of 10 healthy subjects each with the Spectralis and the Stratus OCT. Comparability between different devices was evaluated in 84 C-scans (volume or radial scans) obtained from 21 healthy subjects, two scans per subject with the Spectralis OCT, and one scan per subject each with the Stratus OCT and the RTVue-100 OCT. Each C-scan was segmented and the mean thickness for each retinal layer in sections of the early treatment of diabetic retinopathy study (ETDRS) grid was measured.

Results: The algorithm was able to segment up to 11 intraretinal layers. Measurements with the algorithm were within the 95% confidence interval of a single grader and the difference was smaller than the interindividual difference between the expert graders themselves. The cross-device examination of ETDRS-grid related layer thicknesses highly agreed between the three OCT devices. The algorithm correctly segmented a C-scan of a patient with X-linked retinitis pigmentosa.

Conclusions: The segmentation software provides device-independent, reliable, and reproducible analysis of intraretinal layers, similar to what is obtained from expert graders.

Translational Relevance: Potential application of the software includes routine clinical practice and multicenter clinical trials.

Introduction

Twenty-years ago, optical coherence tomography (OCT) technology, a noninvasive examination technique, revolutionized clinical ophthalmology. It provided new insight into the physiology and pathology of the retina through in vivo visualization of intraretinal microstructures in various pathologies.^{1–9} The time domain (TD)-OCT devices were later outperformed by spectral domain (SD)-OCT devices, which allowed significantly faster acquisition and

higher axial resolution of retinal images.^{10,11} The latest SD-OCT devices combine additional features such as scanning laser ophthalmoscopy, eye tracking, noise reduction, or B-scan averaging to further improve image quality.^{12–16}

Since the introduction of OCT, measurements of retinal thickness in healthy and affected eyes became possible with high accuracy and reproducibility.^{16–20} Nonetheless, the segmentation algorithms have several limitations. Firstly, until to date, algorithms have been demonstrated only on scans from a specific device. The variety of OCT-devices has led to

significant variability in retinal thickness measurements. Compounding this, confusion arose regarding the interpretation of the inner and outer retinal boundaries.^{21–34} Secondly, many device-dependent analysis algorithms are limited to the segmentation of the inner and outer retinal boundary. However, retinal disorders present with disease-related stratification and therefore, segmentation of retinal layers is an important task.^{35,36} Manual segmentation of retinal layers is not only challenging even for expert graders, but also extremely time consuming in clinical use and especially in large scale, multicenter trials.^{35,36}

To overcome these limitations, several groups developed layer segmentation algorithms, based on different approaches of pattern recognition. The number of layers segmented varied and most were specifically developed for individual OCT devices.^{37–50,58} The diversity of methods and normative values for each device makes comparisons difficult and impedes the design of multicenter clinical trials.^{21–34}

The software presented here, addresses this problem by providing intraretinal layer segmentation that is independent of the OCT devices used. Based on the graph theory optimization problem, we developed an algorithm, which employs a consistent layer boundary definition and segments up to 11 intraretinal layers in OC-tomograms of different devices (included in this study: Stratus OCT 3 [Carl Zeiss Meditec, Jena, Germany], Spectralis OCT [Heidelberg Engineering, Heidelberg, Germany], and RTVue100 [Optovue Inc., Fremont, CA]). The software comprises different subsequent analysis methods such as thickness profiling, thickness mapping, layer combination, and application of the early treatment of diabetic retinopathy study (ETDRS) grid, which allows equivalent analysis of data recorded with different OCT devices.

Material and Methods

Segmentation Procedure

The problem of contour detection in OCT B-scans was handled analogue to a cost optimization problem. Determination of a cost-effective link between two contour points, in our case the object contour, forms the basic operation of the employed method. To obtain a cost optimal path corresponding to the object contour, a cost function that results in low costs along object contours was defined. The cost function for two adjacent pixels p and q consists of two linearly combined components: the axial intensity gradient and a binary image,

$$c_{static}(p, q) = w_{gradient} * c_{gradient}(q) + w_{binary} * c_{binary}(q) \quad (1)$$

with the weighting of each cost term component $w_{gradient}$ = (gradient magnitude weight), w_{binary} = (binary cost weight) $\in [1]$ and $c_{gradient}(q)$ = (gradient magnitude at point q), $c_{binary}(q)$ = (binary cost at point q) the cost value to an adjacent pixel q . Empirical tests demonstrated that applying equivalent weights (0.5) provided the optimal solution.

The cost function was transformed into a seed point-specific graph using the Dijkstra-Algorithm, and the adjacency list representation of the seed point-specific graph was applied.^{51,52} We decided to implement the Dijkstra-Algorithm due to its robustness and rapid generation of data. Since we are not dealing with negative edge weights in our cost functions, implementing algorithms that can deal with negative weights such as the Bellman-Ford algorithm are not necessary for solving our problem.⁵² The algorithm addresses the adjacency list directly with edge weights limited to natural numbers. This leads to a significant improvement in execution time.⁵² The resulting seed point-specific graph describes all cost-effective links between one-seed point (pixel) and any other pixel. Further information on the segmentation process is provided in the Supplementary Material and Methods section.

In order to avoid wavy layer edges following segmentation, the edges were smoothed using the *cubic spline curve fitting* procedure.⁵³ The parameters (degrees of freedom M and p) for this fit varied depending on the boundary. For the contour nerve fibre layer (NFL)/ganglion cell layer (GCL) and GCL/inner plexiform layer (IPL), the degrees of freedom M equals 100 and p equals 3 were used. For the remaining layers, M equals 100 and p equals 1 were applied. Empirical tests demonstrated that applying the cubic spline fit leads to optimal smoothing results without distorting the original course.

Cost Function Definition

The algorithm was developed by transforming the segmentation problem into a graph theory optimization problem. In order to find a cost optimal path corresponding to object boundaries, we defined a cost function c , which has low costs along object boundaries. The definition of the cost function is an important component in determining the layers that differ in their signal intensity. In general, layers are distinguished in an OCT image by the assumption

that a dark layer is always adjacent to a bright layer. For this reason, the gradient cost function proposed by Mortensen and Barrett was modified.⁵⁴ We calculated the axial gradient and split the cost function into two components, the “dark to light transition” referring to the positive gradient and the “light to dark transition” referring to the negative gradient. In addition, we normalized the cost functions in the range of 0 to 255 and generated two different cost functions. The first cost function “dark to light transition” corresponds to the NFL/GCL, IPL/INL, outer plexiform layer (OPL)/outer nuclear layer (ONL), external limiting membrane (ELM)/inner segments (IS), inner segment ellipsoid (ISE)/outer segment (OS), and retinal pigment epithelial (RPE)/choroid boundaries. The second cost function “light to dark transition” corresponds to the vitreous/NFL, GCL/IPL, INL/OPL, ONL/ELM, IS/ISE, and OS/RPE boundaries.

The binarized axial gradient was used as additional cost term. As a first step the grey value image was smoothed with a large average filter of 30 pixels in horizontal (x) dimension and 3 pixels in vertical dimension (y). The averaged results were processed with a 5×5 median filter. The axial gradient was calculated to split the image into light to dark transition and dark to light transition and to binarize the result with the Hysteresis procedure (upper and lower threshold for the “light to dark transition” set to the grey values 28 and 10, and for the “dark to light transition” to 10 and 5, respectively, as determined empirically). These additional cost terms acted as a stabilizer and reduced segmentation errors in the presence of inhomogeneous layer progression. Specific cost functions were combined linearly for the segmentation of different intraretinal layer boundaries:

- Axial gradient edge term favouring light to dark transition. Used for the upper boundary of the layers NFL, IPL, OPL, ELM, ISE, and RPE.
- Axial gradient edge term favouring dark to light transition. Used for the upper boundary of the layers GCL, INL, ONL, IS, OS, and choroid.
- Smoothed binarized, axial gradient cost term favouring light to dark transition. Used for the upper boundary of the layers OPL, ISE, and RPE.
- Smoothed binarized, axial gradient cost term favouring dark to light transition. Used for the upper boundary of the layers GCL, INL, ONL, OS, and choroid.

OCT Devices

Optical coherence tomographs were obtained with the Stratus OCT 3 (Carl Zeiss Meditec), the RTVue-100 (Optovue Inc.), and the Spectralis OCT (Heidelberg Engineering). The technical specifications of the OCT devices are provided in Supplementary Material and Methods Table S1. All devices function in the 800-nm band region.

Subjects and Patient

Research presented in this paper followed the tenets of the Declaration of Helsinki. Informed consent was obtained from the subjects after explanation of the nature and possible consequences of the study. Only data from clinically healthy subjects with no history of glaucoma, intraocular inflammation, or neovascular disorder were included. The study was approved by the institutional review board of the University of Giessen. The 21 subjects (age 18–45 years, mean 25 years, 12 male and 9 female) enrolled in the cross-device examination study underwent clinical examination for visual acuity and visual field. The 10 subjects enrolled in the reproducibility study were a subset of this cohort.

One OCT C-scan from a patient with X-linked retinitis pigmentosa (XLRP) due to a mutation in the *RPGR* gene was analyzed. The scanned area was 6×6 mm in size and consisted of 19 B-scans. At the time of the examination the patient was 17 years old.

Algorithm Validation

The algorithm validation procedure was performed on 91 single B-scans captured with the Spectralis OCT (Heidelberg Engineering). The B-scans with a digital resolution of 3.9 μm were chosen randomly, and varied greatly in image quality, noise, and contrast. The whole set was split into two subsets for each validation procedure. Separate subsets of B-scans were used to test the accuracy of the determined layer boundary and layer thickness. The first subset included 50 B-scans, the second 41 B-scans.

Reproducibility

For analysing intraday variability, 60 C-scans were recorded in 10 healthy subjects, three per subject, each with the Spectralis OCT (Heidelberg Engineering) and the Stratus OCT (Carl Zeiss Meditec). The set obtained with the Stratus OCT (Carl Zeiss Meditec) had a digital resolution of 10 μm and comprised six radial B-scans. The set obtained with the Spectralis

Figure 1. Visualization of the iterative 11-layer segmentation process. For a detailed description of the process see the Supplementary Material and Methods section. (A) Original Spectralis OCT B-Scan. (B) First segmentation step, the extraction of Vitreous/ILM and IS/ISe boundaries. (C) Second segmentation step, the extraction of RPE/choroid boundary. (D) Third segmentation step, the extraction of the OS/RPE boundary. (E) Fourth segmentation step, the extraction of ISe/OS boundary. (F) Fifth segmentation step, the extraction of NFL/GCL boundary. (G) Sixth segmentation step, the extraction IPL/INL and OPL/ONL boundaries. (H) Seventh segmentation step, the extraction of GCL/IPL and INL/OPL boundaries. (I) Last segmentation step, the extraction of ONL/ELM and ELM/IS boundaries. (J) Entire segmentation of a B-scan.

OCT (Heidelberg Engineering) had a digital resolution of 3.9 μm and comprised 26 B-scans with an interscan distance of 0.26 mm. The follow-up tracking option was used to obtain data from the same retinal location.

Between each replication of recording the subjects rested 5 minutes. The calculation was performed on a pixel by pixel location basis.

Cross Device Examination

The scan settings for each device are provided in the Supplementary Material and Methods Table S2. One eye in each of the 21 healthy subjects (total number of scans = 84) was scanned with: one radial and one volume scan with the Spectralis OCT (Heidelberg Engineering), one radial scan with the Stratus OCT (Carl Zeiss Meditec), and one volume scan with the RTVue-100 OCT (Optovue Inc.; for scanning protocols, see also Fig. 4).

Statistical Analysis

The data was statistically evaluated with MedCalc (<http://www.medcalc.org/>) and Matlab statistic tool-

box (Matworks, Natick, MA). Normal distribution of thickness values for the ETDRS grid was verified with the Kolmogorov-Smirnov test and significance of mean value differences tested with the interpaired *t*-test with *P* values set to 0.05. A Bland-Altman analysis was used with the limit of agreement set to a 95% confidence interval (CI). The variability of the distribution was quantified by the coefficient of variation (CV), and agreement of intraday measurements in the reproducibility analysis was assessed by the intra class correlation (ICC).

Results

Algorithm Performance

We solved the optimization problem in linear calculation time employing the Dijkstra-Algorithm in adjacency list representation.^{51,52} Under optimal conditions, the algorithm distinguished up to 11 layers in a B-scan of healthy subjects (Fig. 1I-A, further information regarding the different steps during the segmentation process provided in the Supplementary Material and Methods section). The

Figure 2. Visualization of the position differences of the boundaries that have been segmented by the algorithm and those segmented by three experts. The differences are presented unsigned, which means that negative and positive differences are displayed both as positive. (A) Comparison of the differences between the algorithm and each of the three experts separately. (B) Comparison of the differences among the three experts. (C) Comparison of the average differences (from A and B) between any of the three experts against the algorithm and the average differences among the experts.

automatic segmentation process is very rapid compared with the manual segmentation by expert graders. Even though we did not measure the time exactly, on average, full segmentation of an OCT B-scan took about 2 to 3 seconds, while a clinical grader needed between 5 and 10 minutes for the same task. To facilitate clinical application, when certain layers are absent in the B-scan because of a specific disease, and hence might not be segmented correctly, we added an option to combine segmented layers into layer groups.

Validation of Segmentation Results

We validated the operation of the algorithm by comparing its segmentation with that of three expert graders using two validation approaches on OCT B-scans obtained with the Spectralis OCT (Heidelberg Engineering).

In the first approach, 12 contour boundary positions for unsigned deviations in micrometers on 50 scans (Fig. 2, and Supplementary Tables S1 and S2) were compared. The differences between the algorithm and each expert ranged from 1.9 to 15 μm (Fig. 2A). The largest deviations occurred for GCL/IPL and

ONL/ELM boundaries, and the lowest deviations for ELM/IS boundary. In comparison, deviations among the three experts (Fig. 2B) ranged from 1.9 to 14.2 μm . Experts mostly differed with regard to the contour GCL/IPL, the smallest deviation occurred for the contour of the ISe. The mean deviations for each layer boundary position between experts versus algorithm and among the experts ranged from 1.9 to 9.62 μm , and both the smallest and the largest deviation occurred in between the experts (Fig. 2C).

The second approach was to compare the accuracy of layer thickness measurements. The intraretinal layers ONL, IS, and ELM were determined as ONL⁺, and the layer thickness was measured in ETDRS grid areas #2, #4, #6, #8, and #9 on 41 scans (Fig. 3A). The individual measurements were averaged. The comparison demonstrated a good agreement between the results of the algorithm and experts #1 and #2 (Table 1). The smallest difference of 0.1 μm (0.12%) was observed between the algorithm and expert #1 in the ETDRS grid subfield 2. The largest difference of 6.1 μm (6.4%) was observed between the algorithm and expert #2 in subfield 9. The mean difference for both experts #1 and #2 versus the algorithm was $1.29 \pm$

Figure 3. Comparison of mean ONL+ thickness values obtained with the algorithm and those obtained from the three experts. (A) ETDRS grid and central OCT B-scan with corresponding regions showing the area of the sectors 4, 8, 9, 6, and 2. The layer group ONL+ is defined as ONL+ELM+IS, and was segmented in ETDRS grid sector 9. (B) Bland-Altman plots showing the differences of the segmentation results between the algorithm and the three experts. The solid line shows the average difference. The dotted line shows the 95% limit of agreement. (Left) Comparison between expert 1 and the algorithm. (Center) Comparison between expert 2 and the algorithm. (Right) Comparison between expert 3 and the algorithm.

1.33 μm . The measurements of expert #3 versus the algorithm varied significantly more. For example, a difference of 6.1 μm (6.4%) was observed for section #8, whereas the algorithm and the other two experts differed by 3.76 μm . In summary, the differences among the experts were larger than those between the experts and algorithm (Table 1), because expert three segmented the scans slightly differently than the other two experts.

The differences in segmentation between the algorithm and each of the three experts were within the 95% CI, as shown in three Bland-Altman plots in Figure 3B. Data points for all three comparisons were distributed evenly within a range of 110 to 140 μm .

There was no correlation between values outside the CI and thickness.

Reproducibility of the Algorithm Data

For analysing intraday variability, 30 volumetric scans of 10 healthy subjects were obtained either with the Stratus OCT (Carl Zeiss Meditec) or the Spectralis OCT (Heidelberg Engineering) ($n = 60$ scans in total). The ETDRS-grid related layer thicknesses of 11 layers with the Spectralis OCT (Heidelberg Engineering) and nine layers with the Stratus OCT (Carl Zeiss Meditec) were calculated. For statistical reasons, the center of the ETDRS grid was placed at the center of the fovea in each

Table 1. Subfield Related Comparison of ONL+ELM+IS Thicknesses (in μm) Between Algorithm and Three Experts

Subfield	Alg.		Expert 1		Expert 2		Expert 3	
	Avg.	SD	Avg.	SD	Avg.	SD	Avg.	SD
2	83.6	9.5	83.5	11.5	83.8	10.1	86.7	12.4
4	85.1	11.2	86.2	15.1	83.5	12.8	85.8	21.1
6	100.6	15.9	103.1	14.5	100.9	16.3	106	17.3
8	104.4	15.0	104.0	18.0	100.6	18.8	110.5	16.6
9	124.4	12.3	127.2	9.9	124.3	12.5	129.9	11.6

Table 2. Reproducibility Results by Analyzing the Segmentation Results of Three Volumetric Scans Replications of 10 Healthy Subject With the Devices Stratus OCT and Spectralis OCT

Upper	Lower	SD-OCT (Spectralis OCT)			TD-OCT (Stratus OCT)		
		ICC	CV, %	SD, m	ICC	CV, %	SD, m
Vitreous/ILM	NFL/GCL	0.997	3.1	1.1	0.776	15.6	4.9
Vitreous/ILM	GCL/IPL	0.989	3	2.3	0.812	8.3	6.2
Vitreous/ILM	IPL/INL	0.997	1.3	1.5	0.893	6.2	6.3
Vitreous/ILM	INL/OPL	0.997	1.3	1.9	0.869	5.5	8.0
Vitreous/ILM	OPL/ONL	0.987	2.5	4.4	0.902	4.6	7.5
Vitreous/ILM	ONL/ELM	0.997	1.3	3	-	-	-
Vitreous/ILM	ELM/IS	0.999	0.9	2.2	-	-	-
Vitreous/ILM	IS/ISe	0.999	0.8	2	0.922	5.0	12.1
Vitreous/ILM	ISe/OS	0.999	0.8	2	0.925	4.7	12.1
Vitreous/ILM	OS/RPE	0.994	1.5	4.4	0.919	4.3	12.1
Vitreous/ILM	RPE/Choroid	0.998	0.7	2.2	0.922	3.8	11.8

Figure 4. Set up for comparing the ETDRS grid related thickness values obtained with the different OCT devices. Scanning protocols are indicated adjacent to the thickness maps. (A) Segmentation results of a Stratus OCT tomogram. (B) Thickness map of the retina, obtained using the Stratus OCT radial scan protocol with the respective ETDRS grid position. (C) Segmentation results of a Spectralis OCT tomogram. (D) Thickness map of the retina, obtained using the Spectralis OCT volume scan protocol with the respective ETDRS grid position. (E) Thickness map of the retina, obtained using the Spectralis OCT radial scan protocol with the respective ETDRS grid position. (F) Segmentation results of an RTVue-100 tomogram. (G) Thickness map of the retina, obtained using the RTVue-100 EMM5 scan protocol with the respective ETDRS grid position. The *black arrows* illustrate, which thickness maps have been compared.

Figure 5. Bland-Altman Plot for agreement of the mean retinal thickness values obtained on B-scans from the three different devices. The solid line shows the average difference. The dotted line shows the 95% limit of agreement. (Left) Comparison between radial macular scans of the Spectralis OCT and the Stratus OCT. (Right) Comparison between volumetric macular scans of the Spectralis OCT and the RTVue-100.

volumetric scan. The layer thickness was calculated based on this design. The layer thickness from the vitreous/ILM boundary up to OPL/ONL boundary in the central ETDRS grid subfield #9 was omitted for the statistical reproducibility calculation, because values in this sector become zero due to the anatomy of the fovea.

The reproducibility was quantified on a pixel by pixel basis using the ICC, the mean CV and the mean standard deviation (SD) (Table 2). For Spectralis OCT (Heidelberg Engineering) scans, the mean ICC was 0.995. The mean CV was 1.56%, and 9 of 11 CV values were in the range between 0.7% and 1.5%. The two higher CV values of 3.1% and 3.0% were obtained for the GCL/IPL and the NFL/GCL border, respectively. The mean SD was 2.45 μm , which is less than the digital resolution of 3.9 μm (i.e., less than 1 pixel). Analysing Stratus OCT (Carl Zeiss Meditec) scans, the mean ICC was 0.882, the mean CV was 6.4%, and the SD was 9.0 μm . The highest CV values of 15.6% and 8.3% were also obtained for the border GCL/IPL and NFL/GCL.

Cross-Device Examination Setup

Four OCT scans protocols (one each with the Stratus [Carl Zeiss Meditec] and the RTVue-100 [Optovue Inc.] and two with the Spectralis OCT [Heidelberg Engineering]), of 21 healthy subjects were analyzed with the layer segmentation algorithm. Spectralis OCT (Heidelberg Engineering) versus

Stratus OCT (Carl Zeiss Meditec) were compared using the radial scan protocols (Figs. 4B, 4E), and Spectralis OCT (Heidelberg Engineering) versus RTVue-100 (Optovue Inc.) using the “volume scans” protocols (Figs. 4D, 4G). The volume scans obtained with the Spectralis OCT (Heidelberg Engineering) were segmented for 11 layers (ONL, ELM, and IS were combined to ONL⁺ afterwards), while eight layers and one layer group (ONL⁺) were segmented in scans of the Stratus OCT (Carl Zeiss Meditec) and RTVue-100 (Optovue Inc.) (Figs. 4A, 4C, 4F). The ETDRS grid was manually centered on the fovea, ensuring comparability of defined areas among the devices. Subsequently, using the segmentation results, the ETDRS grid related thickness for each layer was calculated.

Comparison of Thickness Values Measuring the Entire Retina

Segmentation of the “entire retina,” defined as the area between the vitreous/NFL boundary and the RPE/choroid boundary, was compared with a Bland-Altman plot in sector #9 of the ETDRS grid, with the results of the Spectralis OCT (Heidelberg Engineering) as the reference (Fig. 5). All three devices provided highly comparable measurements with retinal thickness between 250 and 380 μm , most of them within the 95% limit of agreement (Fig. 5). The mean difference between the Spectralis OCT (Heidelberg Engineering) and the Stratus OCT (Carl Zeiss Meditec) was 4.74

(I.) Spectralis OCT and Stratus OCT

(II.) Spectralis OCT and RTVue-100

- Layer thickness measured on Spectralis OCT is significantly thicker than layer thickness measured on (I.) Stratus OCT, (II.) RTVue-100
- Layer thickness measured on Spectralis OCT is significantly thinner than layer thickness measured on (I.) Stratus OCT, (II.) RTVue-100
- No significant difference in layer thickness
- Not applicable

- A: Nerve fiber layer
- B: Ganglion cell layer
- C: Inner plexiform layer
- D: Inner nuclear layer
- E: Outer plexiform layer
- F: Outer nuclear layer + External limiting membrane + Inner segment
- G: Inner Segment ellipsoid
- H: Outer segment
- I: Retinal pigment epithelium
- J: Retina

Figure 6. Comparative chart of the ETDRS grid related thickness values among the three OCT devices using the paired *t*-test. (I) Comparison between Spectralis OCT and Stratus OCT. (II) Comparison between Spectralis OCT and RTVue-100. The significance of the obtained differences of the thickness values among the intraretinal layers is color coded. Colors other than green illustrate a rejection of the null hypothesis with a 5% level of significance, with a 95% CI. As the null hypothesis assumes that the layer thickness measured on B-scans of different OCT devices did not differ. The numerical values inside the ETDRS grid sectors illustrate the mean difference in micrometers.

Figure 7. Segmentation results of a C-scan of a retina from a patient with XLRP compared with a healthy retina. (I) Results of the segmentation of seven boundaries and six layer groups. (II) ETDRS grid analysis of a healthy retina (*left*) and the diseased retina (*right*) based upon the segmentation result. (I, A) unsegmented and (I, B) segmented B-scan of a healthy subject. The layers GCL+IPL, OPL+ONL+IS+OLM, and ISe were modified by the combination feature to layer groups. (I, C) unsegmented and (I, D) segmented B-scan of

→

the diseased retina. (II, A) Thickness map of the RNFL. (II, B) Thickness map of the layer group GCL+IPL. (II, C) Thickness map of the INL. (II, D) Thickness map of the layer group OPL+ONL+IS+OLM. (II, E) Thickness map of the layer group ISe+OS. (II, F) Thickness map of the RPE. (II, G) Thickness map of the entire retina.

μm , and the mean difference between the Spectralis OCT (Heidelberg Engineering) and the RTVue-100 OCT (Optovue Inc.) was $6.39 \mu\text{m}$.

Interestingly, the entire retina thickness values obtained with the Stratus (Carl Zeiss Meditec) and the RTVue OCT (Optovue Inc.) devices tended to be slightly lower than the values obtained with the Spectralis OCT (Heidelberg Engineering). According to a paired *t*-test, this difference was not significant (Figs. 6I-J, 6II-J).

Comparing the Thickness Values When Measuring Different Intraretinal Layers

Mean absolute thickness values obtained for any layer, ETDRS grid sector, and device are provided in the Supplementary Tables S3 to S6. The three devices provided highly comparable values as tested with the *t*-test (corresponding *P* values provided in Supplementary Tables S7 and S8). Figure 6 shows the differences of the mean values between the three devices. The null hypothesis was that the mean layer thickness is equal measured with any of the devices. Colours other than green illustrate a rejection of the null hypothesis with a 5% level of significance.

Mean values of intraretinal layers obtained with the Spectralis OCT (Heidelberg Engineering) and the Stratus OCT (Carl Zeiss Meditec) did not differ for most ETDRS grid sectors regarding 6 of 10 analysed layers or layer groups, (Figs. 6I-B, 6I-D, 6I-F, 6I-H–J). The remaining four layers/layer groups (NFL, IPL, OPL, and IS/OS) were significantly thicker measured with the Spectralis OCT (Heidelberg Engineering) in most ETDRS grid sectors (Figs. 6I-A, 6I-C, 6I-E, 6I-G) by $1.1 \mu\text{m}$ to $13.5 \mu\text{m}$.

The Spectralis OCT (Heidelberg Engineering) and the RTVue-100 (Optovue Inc.) showed an overall higher agreement in retinal layer thickness measurements. In 7 of 10 layers, no differences in the mean values were observed (Figs. 6II-B–D, 6II-F, 6II-H–J). Only the values of the layers NFL, OPL, and ISe measured on the Spectralis OCT tomograms (Heidelberg Engineering) were significantly thicker compared with RTVue-100 (Carl Zeiss Meditec) tomograms in certain grid sectors (Figs. 6II-A, 6II-E, 6II-G). With significant differences between 2.1 and $5.6 \mu\text{m}$, these were smaller than the differences between the

Spectralis OCT (Heidelberg Engineering) and the Status OCT (Optovue, Inc.).

Depiction of Intraretinal Layer Changes in a Patient with RP

To demonstrate the potential practical use of the algorithm in the analysis of intraretinal changes in retinal degenerative disorders, an automated segmentation of retinal layers was performed in an OCT volume scan of a patient with advanced XLRP. The algorithm contains two features specifically designed for segmenting retinæ affected with centripetal degeneration like RP, in which intraretinal layers undergo thinning and will eventually disappear towards the periphery: (1) the possibility to combine layers when necessary, and (2) a feature that eliminates layer boundaries when layer thickness values become zero. Results of the segmentation of seven boundaries and six layer groups are shown in Figure 7I. Functionally important layers, such as the ISe+OS, which often disappear parafoveal in RP, can be exactly segmented until the end point (Fig. 7I, arrows), and the thickness can be displayed in a heat map that shows the residual island around the fovea (Fig. 7II). Furthermore, thickness values of the layer group OPL+ONL+OLM+IS, which corresponds to the functional unit of the photoreceptor cell except ISe+OS can similarly be precisely measured and displayed in a heat map (Fig. 7II). Inner retinal layers remain largely unchanged, except for some evidence of thickening of the retinal nerve fiber layer (RNFL), a phenomenon referred to as “remodeling” during RP. All data can also be exported and subsequently processed in other data analysis programs.

Discussion

This paper describes the first comparison of retinal and retinal layer thickness obtained with different OCT devices but using the same segmentation algorithm. The algorithm was specifically developed to allow device independent OCT analysis employing a consistent definition of layer boundaries. The software so far includes standard features such as thickness profiling, thickness mapping and ETDRS

Table 3. Mean Normal Retinal Thickness (in μm) of Healthy Human Subjects in the Central Subfield of the ETDRS Grid, Obtained in This and Other Studies

References	Stratus OCT (Radial lines)	3D OCT-1000 (3D Macular)	RTVue-100 (MM5)	Cirrus Cube 512×128	Spectralis OCT (Volume Scan)	SOCT Copernicus
23	201.6 $\pm$ 18.1				277.4 $\pm$ 16.8	
24	203.3 $\pm$ 17	231 $\pm$ 16	267 $\pm$ 15	262 $\pm$ 16		
25	195.6 $\pm$ 17.2	216.4 $\pm$ 18.0				
28	202.3				271.4	
29	198	224		256	274	
30	206 $\pm$ 25			259 $\pm$ 19	279 $\pm$ 21	
32	213 $\pm$ 19		245 $\pm$ 28	276 $\pm$ 17	288 $\pm$ 16	246 $\pm$ 23
33	201 $\pm$ 24	229 $\pm$ 24	259 $\pm$ 21	269 $\pm$ 27	283 $\pm$ 27	182 $\pm$ 42
This study	268.6 $\pm$ 11		270.1 $\pm$ 11.2		275.5 $\pm$ 15.1	

Values were obtained through manual or automatic segmentation. Empty fields illustrate devices that were not investigated in the present study.

grid analysis, which in recent years have been used as standard procedures in clinical practice.

Evaluating the accuracy of the algorithm compared with three experts (Fig. 2), we observed that the deviations between the algorithm and the experts (Fig. 2A) were comparable with that of the experts among themselves (Fig. 2B). The most obvious deviations were found for the layer boundaries GCL/IPL and ONL/ELM between one of the experts and the two other experts or equally the algorithm. We suspect that the deviation is due to the relatively low contrast of these contours.

Comparison of our results to five other studies on device dependent manual and automatic segmentation shows similar levels of accuracy of the segmentation results.^{42,44,47,48,58} Three-dimensional (3D) segmentation algorithms showed a mean difference in the position of a given layer boundary of $5.69 \pm 2.41 \mu\text{m}$,⁴² $6.1 \pm 2.9 \mu\text{m}$,⁴⁴ and $5.75 \pm 1.37 \mu\text{m}$ ⁵⁸ between the algorithm and the experts, those with two-dimensional (2D) segmentation $3.39 \pm 0.96 \mu\text{m}$ ⁴⁷ and $3.03 \pm 2.65 \mu\text{m}$.⁴⁸ Hence, the absolute difference of $5.5 \pm 1.9 \mu\text{m}$ found in our study compares well with those of the 3D segmentation algorithms,^{42,44,58} but is slightly higher than those with 2D segmentation algorithms.^{47,48} We suspect that this is mainly due to the challenging task of intraretinal segmentation, which had significantly larger deviations especially regarding the GCL/IPL boundary.

In addition to the boundary position differences, we evaluated layer thickness measurements. The mean difference between the first two experts and the algorithm was $1.29 \pm 1.33 \mu\text{m}$. Interestingly, the data of the algorithm and experts #1 and #2 showed

significant differences compared to the third expert. The discrepancy is most likely due to the fact that the layer to be segmented included ONL+ELM+IS and excluded the OPL. Thickness and boundaries of the OPL can vary enormously on OCT B-scans, depending on the reflection intensity of the Henle fiber layer (HFL) that may fuse with the OPL to one layer. OCT images, which have been captured with a pupil entry point of the subject different from the center of the pupil show an increased reflection of the HFL, and thus the OPL appears to be thicker.⁵⁵ In contrast, in cases where the entry point is central in the pupil, the reflectivity of the HFL layer is low and thus the overall OPL layer appears smaller. In such cases, the manual segmentation of the expert is highly depending on the individual experience of the grader. Conversely, the algorithm always segments along the strongest edge of the image and therefore the segmentation may be different from the grader. The experience of the expert graders #1 and #2 apparently differed in this evaluation method from the one of expert #3, emphasizing the advantages of an objective evaluation method as obtained with the algorithm. In order to avoid incorrect segmentation of the OPL, ONL and OPL layer could be combined to one layer group as proposed by Hood et al.³⁵

The main advantage of our approach is that raw OCT data from different devices can be segmented consistently with the same algorithm, independent from built-in software solutions of the OCT devices, which in recent years have frequently been compared.^{21–34} And, changes to the built-in software in the course of updates potentially affect the measurement results. Table 3 allows a comparison of results in

those studies from up to six different OCT-devices with that from our study. The variability of thickness values obtained with device-specific evaluation algorithms imply that a comparison of data between different OCT-devices is almost impossible: mean retinal thickness of the central subfield differed between 196 and 288 μm . The main reason for this discrepancy lies in the definition of the outer retinal boundary position definition, which varies among different OCT evaluation algorithms. Therefore, all device embedded algorithms contribute to systematic variability in retinal thickness measurements. When designing multicenter clinical trials in which OCT data are crucial, it is more and more difficult to handle different OCT devices. In order to solve this problem, we propose the use of an algorithm with a consistent definition of the layer boundaries among the compared raw data and the application of a single segmentation algorithm for all devices.

The results of our study suggest that the mean retinal thickness is highly comparable between the Spectralis OCT (Heidelberg Engineering), and either the Stratus OCT (Carl Zeiss Meditec), or the RTVue-100 OCT (Optovue, Inc.) devices, under circumstances when the inner and outer boundary definition of the retina is consistent. The mean retinal thickness between the devices did not differ significantly as supported by the paired *t*-test analysis (Figs. 6I-J, 6II-J). The Bland-Altman analysis in Figure 5 illustrates that the retinal thickness measurement in a single subject using different OCT devices can be different, but most of them are within the 95% limit of agreement.

Most individual layer thickness measurements did not deviate significantly among the three devices (Fig. 6). In cases where values differed, those obtained with the Spectralis OCT (Heidelberg Engineering) were typically higher than those obtained with the Stratus OCT (Carl Zeiss Meditec) or with the RTVue-100 OCT (Optovue, Inc.). We attribute the higher variability among Spectralis OCT (Heidelberg Engineering) and Stratus OCT (Carl Zeiss Meditec), even in the presence of a consistent layer boundary definition to the fact that it compared SD-OCT and TD-OCT technology. It is known that the properties of SD-OCTs, such as higher axial resolution, faster recording speeds, eye tracking, B-scans averaging, and noise reduction result in a more favourable view of retinal structures, and in a significant higher image quality, possibly enabling the algorithm to increase the sharpness of the boundary segmentation.¹⁰⁻¹⁶

In this regard, the significant difference in IPL

thickness values between the Spectralis OCT (Heidelberg Engineering) and the Stratus OCT (Carl Zeiss Meditec) may be attributed to the low contrast between the GCL and the IPL (Fig. 6I-C). The boundary between these two layers is not clearly visible in the Stratus OCT (Carl Zeiss Meditec) tomograms in most cases, while it is often fairly well detectable in the Spectralis OCT (Heidelberg Engineering) scans. This may explain why authors of layer segmentation algorithms based on the Stratus OCT (Carl Zeiss Meditec) tomograms never separated the GCL and IPL.^{37-39,42,56} It remains to be seen whether segmentation of GCL and IPL, even with the Spectralis OCT, is helpful in daily clinical practice.

Interestingly, significant differences in the retinal layer thickness values of the ISe were observed among all devices. This is a highly reflective layer having a very high contrast against the adjacent layers. Morphologically, this structure corresponds to the IS of photoreceptors containing a high number of mitochondria.⁵⁷ Due to the high reflectivity of this layer, the ISe is segmented very precisely by all three devices (Figs. 4A, 4C, 4F). However, due to the low total thickness of this layer, even a small difference in layer thickness that is less than the axial resolution, would lead to the definition of a significant difference, based on the null hypothesis of the paired *t*-test (see results part, and Figs. 6I-G, 6II-G). It remains to be seen, whether ISe layer thickness rather than the length of the ISe will be a meaningful read out parameter in clinical applications.

Comparison of the NFL layer thickness values also showed significant differences, with the Spectralis OCT (Heidelberg Engineering) data being higher than those for the other two devices (Figs. 6I-A, 6II-A). Differences in the NFL layer thickness measurements in healthy subjects have been published.^{23,27,34} Knight and colleagues²³ compared the NFL thickness values between the Stratus OCT and Cirrus OCT (Carl Zeiss Meditec, SD-OCT) using device embedded algorithms and also documented significant differences. The Cirrus OCT (Carl Zeiss Meditec) measured a thinner NFL in healthy eyes compared with the Stratus OCT (Carl Zeiss Meditec). Buchser and colleagues²⁷ compared the NFL thickness measured on three different OCT devices (Cirrus HD-OCT [Carl Zeiss Meditec], RTVue-100 [Optovue, Inc.], and 3D OCT-1000 [Topcon Medical Systems, Oakland, CA], all SD-OCT devices): measurements with the RTVue-100 were significantly higher compared with both the Cirrus and the 3D OCT-1000 values in healthy subjects.²⁷ Likewise,

Kim and colleagues³⁴ described comparable NFL data between the Stratus OCT and the Cirrus (SD-OCT) (both, Carl Zeiss Meditec) and also reported that Cirrus (SD-OCT) measured a thinner NFL in healthy eyes compared with the Stratus OCT. The reason for this variability among the different devices remains to be solved.

In order to demonstrate the usefulness of the algorithm for segmenting OCT data in diseased retinæ, we analysed one C-scan of a patient with XLRP. The segmentation results show correct layer segmentation in the presence of pathological retinal abnormalities. The number of segmented layers was reduced to avoid segmentation errors caused by degenerated photoreceptors in the peripheral retina, nicely demonstrating the usefulness of this feature. Whether the algorithm can be applied to any given disease entity, is subject of ongoing investigations.

We developed a software solution for device independent OCT data analysis, in which the two most likely sources of variability (i.e., the inconsistent definition of the retinal layer boundaries and the device dependency of the segmentation algorithm) were eliminated. We applied the algorithm to raw data obtained from three different devices and obtained a high agreement of thickness values among the devices. We therefore propose the use of our software solution in multicenter clinical trials where different OCT devices are employed.

Acknowledgments

The authors thank Matthew Ellinwood, Iowa State University, for critical reading and editing. Supported by Grant number 57-0016 from the von Behring Röntgen Foundation.

Disclosure: **A. Ehnes**, None; **Y. Wenner**, None; **C. Friedburg**, None; **M.N. Preising**, None; **W. Bowl**, None; **W. Sekundo**, None; **E. Meyer zu Bexten**, None; **K. Stieger**, None; **B. Lorenz**, None

References

1. Huang D, Swanson EA, Lin CP, et al. Optical coherence tomography. *Science*. 1991;254:1178–1181.
2. Hee MR, Izatt JA, Swanson EA, et al. Optical coherence tomography of the human retina. *Arch Ophthalmol*. 1995;113:325–332.
3. Puliafito CA, Hee MR, Lin CP, et al. Imaging of macular diseases with optical coherence tomography. *Ophthalmology*. 1995;102:217–229.
4. Coscas F, Vismara S, Zourdani A, Li Calzi CL. Optical coherence tomography in age-related macular degeneration. Heidelberg, Germany: Springer Medizin Verlag; 2009.
5. Cense B, Nassif N, Chen T, et al. Ultrahigh-resolution high-speed retinal imaging using spectral-domain optical coherence tomography. *Opt Express*. 2004;12:2435–2447.
6. Apushkin MA, Fishman GA, Janowicz MJ. Monitoring cystoids macular edema by optical coherence tomography in patients with retinitis pigmentosa. *Ophthalmology*. 2004;111:1899–1904.
7. Ergun E, Hermann B, Wirtitsch M, et al. Assessment of central visual function in Stargardt's disease/fundus flavimaculatus with ultrahigh-resolution optical coherence tomography. *Invest Ophthalmol Vis Sci*. 2005;46:310–316.
8. Schmidt-Erfurth U, Leitgeb RA, Michels S, et al. Three-dimensional ultrahigh-resolution optical coherence tomography of macular diseases. *Invest Ophthalmol Vis Sci*. 2005;46:3393–3402.
9. Peroni CG, Witkin AJ, Ko TH, et al. Ultrahigh resolution optical coherence tomography in non-exudative age related macular degeneration. *Br J Ophthalmol*. 2006;90:191–197.
10. De Boer JF, Cense B, Park BH, Pierce MC, Tearney GJ, Bouma BE. Improved signal to noise ratio in spectral-domain compared with time-domain optical coherence tomography. *Opt Letters*. 2003;28:2067–2069.
11. Coscas G. Principles of OCT—examination technique—main OCT systems. In: Coscas G, ed. *Optical Coherence Tomography in Age-Related Macular Degeneration*. Heidelberg, Germany: Springer Medizin Verlag; 2009:5–14.
12. Sander B, Larsen M, Thrane L, Hougaard JL, Jorgensen TM. Enhanced optical coherence tomography imaging by multiple scan averaging. *Br J Ophthalmol*. 2005;89:207–212.
13. Sakamoto A, Hangai M, Yoshimura N. Spectral-domain optical coherence tomography with multiple B-Scans averaging for enhanced imaging of retinal diseases. *Ophthalmology*. 2008;115:1071–1078.
14. De Smet MD, Van Velthoven MEJ. Combined optical coherence tomography and confocal ophthalmoscopy (OCT/SLO). In: Coscas G, ed. *Optical Coherence Tomography in Age-Related Macular Degeneration*. Heidelberg, Germany: Springer Medizin Verlag; 2009.

15. Pappuru RR, Briceno C, Ouyang Y, Walsh AC, Sadda SR. Clinical significance of B-scans averaging with SD-OCT. *Ophthalmic Surg Lasers Imaging*. 2012;43:63–68.
16. Chin EK, Sedeek RW, Li Y, et al. Reproducibility of macular thickness measurement among five OCT instruments: effects of image resolution, image registration, and eye tracking. *Ophthalmic Surg Lasers Imaging*. 2012;43:97–108.
17. Polito A, Del Borrello M, Isola M, Zemella N, Bandello F. Repeatability and reproducibility of fast macular thickness mapping with stratus optical coherence tomography. *Arch Ophthalmol*. 2005;123:1330–1337.
18. Stetson PF, Durbin MK, Callan TM. Repeatability and reproducibility. In: Stetson PF, ed. *Repeatability and Reproducibility of Macular Thickness Measurements from the Cirrus Spectral-Domain Optical Coherence Tomography System*. Dublin, Ireland: Carl Zeiss Meditec; 2007:1–6.
19. Patel PJ, Chen FK, Xing W, et al. Repeatability of stratus optical coherence tomography measures in neovascular age-related macular degeneration. *Invest Ophthalmol Vis Sci*. 2008;49:1084–1088.
20. Bruce A, Pacey IE, Dharni P, Scally AJ, Barrett BT. Repeatability and reproducibility of macular thickness measurements using Fourier domain optical coherence tomography. *Open Ophthalmol J*. 2009;3:10–14.
21. Wang XG, Penq Q, Wu Q. Comparison of central macular thickness between two spectral-domain optical coherence tomography in elderly non-mydiatic eyes. *Int J Ophthalmol*. 2012;5:354–363.
22. Ho J, Sull AC, Vuong LN, et al. Assessment of artifacts and reproducibility across spectral and time domain optical coherence tomography devices. *Ophthalmology*. 2009;116:1960–1970.
23. Chopovska Y, Jaeger M, Rambow R, Lorenz B. Comparison of central retinal thickness in healthy children and adults measured with the Heidelberg spectralis OCT and the Zeiss stratus OCT 3. *Ophthalmologica*. 2010;225:27–36.
24. Sull AC, Vuong LN, Price LL, et al. Comparison of spectral / Fourier domain optical coherence tomography instruments for assessment of normal macular thickness. *Retina*. 2010;30:235–245.
25. Leung CK, Cheung CY, Weinreb RN, et al. Comparison of macular thickness measurements between time domain and spectral domain optical coherence tomography. *Invest Ophthalmol Vis Sci*. 2008;49:4893–4897.
26. Knight OJ, Chang RT, Feuer WJ, Budenz DL. Comparison of retinal nerve fiber layer measurements using time domain and spectral domain coherence tomography. *Ophthalmology*. 2009;116:1271–1277.
27. Buchser NM, Wollstein G, Ishikawa H, et al. Comparison of retinal fiber layer thickness measurements bias and imprecision across three spectral-domain optical coherence tomography devices. *Invest Ophthalmol Vis Sci*. 2012;53:3742–3749.
28. Grover S, Murtby RK, Brar VS, Chalam KV. Comparison of retinal thickness in normal eyes using stratus and spectralis optical coherence tomography. *Invest Ophthalmol Vis Sci*. 2010;51:2644–2647.
29. Mylonas G, Ahlers C, Malamos P, et al. Comparison of retinal thickness measurements and segmentation performance of four different spectral and time domain OCT devices in neovascular age related macular degeneration. *Br J Ophthalmol*. 2009;93:1453–1460.
30. Han IC, Jaffe GJ. Comparison of spectral and time domain optical coherence tomography for retinal thickness measurements in healthy and diseased eyes. *Am J Ophthalmol*. 2009;147:847–858.
31. Foroogbian F, Cukras C, Meyerle CB, Chew EY, Wong WT. Evaluation of time domain and spectral domain optical coherence tomography in the measurement of diabetic macular edema. *Invest Ophthalmol Vis Sci*. 2008;49:4290–4296.
32. Wolf-Schnurrbusch UE, Ceklic L, Brinkmann CK, et al. Macular thickness measurements in healthy eyes using six different optical coherence tomography instruments. *Invest Ophthalmol Vis Sci*. 2009;50:3432–3437.
33. Giani A, Cigada M, Choudhy N, et al. Reproducibility of retinal thickness measurements on normal and pathologic eyes by different optical coherence tomography instruments. *Am J Ophthalmol*. 2012;150:815–824.
34. Kim SJ, Ishikawa H, Gabriele ML, et al. Retinal nerve fiber layer thickness measurement comparability between time domain optical coherence tomography (OCT) and spectral domain OCT. *Invest Ophthalmol Vis Sci*. 2010;51:896–902.
35. Hood DC, Lin CE, Lazow MA, et al. Thickness of receptor and post-receptor retinal layers in patients with retinitis pigmentosa measured with frequency-domain optical coherence tomography. *Invest Ophthalmol Vis Sci*. 2009;50:2328–2336.
36. Hood DC, Lazow MA, Locke KG, et al. The transition zone between healthy and diseased

- retina in patients with retinitis pigmentosa. *Invest Ophthalmol Vis Sci*. 2011;52:101–108.
37. Fernández DC, Salinas HM, et al. Automated detection of retinal layer structures on optical coherence tomography images. *Opt Express*. 2005; 13:10200–10216.
 38. Haeker M, Abramoff M, Kardon R, Sonka M. Segmentation of surfaces of the retinal layer from OCT images. *Med Image Comput Comput Assist Interv*. 2006;9:800–807.
 39. Haeker M, Sonka M, Kardon R, Sonka M. Automated segmentation of intraretinal layers from macular optical coherence tomography images. *Proc SPIE*. 2007;6512:651214.
 40. Niemeijer M, Garvin M, van Ginneken MK, et al. Vessel segmentation in 3D spectral OCT scans of the retina. *Proc SPIE*. 2008;6914:69141R1–69141R8.
 41. Ahlers C, Simader C, Geitzenauer W, et al. Automatic segmentation in three-dimensional analysis of fibrovascular pigment epithelial detachment using high-definition optical coherence tomography. *Br J Ophthalmol*. 2008;92:197–203.
 42. Garvin MK, Abramoff MD, Kardon R, Russell SR, Wu X, Sonka M. Intraretinal layer segmentation of macular optical coherence tomography images using optimal 3-D graph search. *IEEE Trans Med Imaging*. 2008;27:1495–1505.
 43. Mishra A, Wong A, Bizheva K, Clausi DA. Intraretinal layer segmentation in optical coherence tomography images. *Opt Express*. 2009;17:23719–23728.
 44. Garvin MK, Abramoff MD, Wu X, Russell SR, Burns TL, Sonka M. Automated 3-D intraretinal layer segmentation of macular spectral-domain optical coherence tomography images. *IEEE Trans Med Imaging*. 2009;28:1436–1447.
 45. Yazdanpanah A, Hamarneh G, Smith B, Sarunic M. Segmentation of intra-retinal layers from optical coherence tomography images using an active contour approach. *IEEE Trans Med Imaging*. 2010;32:484–496.
 46. Kajic V, Povazay B, Hermann B, et al. Robust segmentation of intraretinal layers in the normal human fovea using a novel statistical model based on texture and shape analysis. *Opt Express*. 2010; 18:14730–14744.
 47. Chui SJ, Li XT, Nicholas P, et al. Automatic segmentation of seven retinal layers in SD-OCT images congruent with expert manual segmentation. *Opt Express*. 2010;18:19413–19428.
 48. Yang Q, Reisman CA, Wang Z, et al. Automated layer segmentation of macular OCT images using dual-scale gradient information. *Opt Express*. 2010;18:21293–21307.
 49. Gloesmann M, Hermann B, Schubert C, Sattmann H, Ahnelt PK, Drexler W. Histologic correlation of pig retina radial stratification with ultrahigh-resolution optical coherence tomography. *Invest Ophthalmol Vis Sci*. 2003;44:1696–703.
 50. Huang D, Kaiser PK, Lowder CY, et al. Optical coherence tomography (OCT). In: Huang D, ed. *Retinal Imaging*. St. Louis, MO: Elsevier Mosby; 2006:47–65.
 51. Handles H, Pöpl S. Segmentierung medizinischer Bilddaten. In: Sandten U, ed. *Medizinische Bildverarbeitung*. Wiesbaden, Germany: Vieweg & Teubner; 2009:95–157.
 52. Krumke SO, Noltmeier H. Kürzeste Wege. In: Sandten U, ed. *Graphentheoretische Konzepte und Algorithmen*. Wiesbaden, Germany: Vieweg & Teubner; 2009:167–192.
 53. Loan V, Charles F. Cubic splines. In: Robbins T, ed. *Introduction to Scientific Computing*. Upper Saddle River, NJ: Prentice Hall; 1997:112–113.
 54. Barrett WA, Mortensen EN. An interactive live-wire boundary extraction. *Med Image Anal*. 1997; 1:331–341.
 55. Lujan B, Roodra A, Knighton RW, Carroll J. Revealing Henle's fiber layer using spectral domain optical coherence tomography. *Invest Ophthalmol Vis Sci*. 2011;52:1486–1492.
 56. Vámos R, Tatrai E, Nemeth J, Holder GE, DeBuc DC, Somfai GM. The structure and function of the macula in patients with advanced retinitis pigmentosa. *Invest Ophthalmol Vis Sci*. 2011;52:8425–8432.
 57. Hood DC, Zhang X, Ramachandran R, et al. The inner segment/outer segment border seen on optical coherence tomography is less intense in patients with diminished cone function. *Invest Ophthalmol Vis Sci*. 2011;52:9703–9709.
 58. Quellec G, Lee K, Dolejsi M, et al. Three-dimensional analysis of retinal layer texture: identification of fluid-filled regions in SD-OCT of the macula. *IEEE*. 2010;29:1321–1330.