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Assessing the validity of a cross-platform retinal image segmentation tool in normal and diseased retina

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Abstract

Purpose-

To compare automated retinal image segmentation using cross-platform and proprietary software on images captured using Heidelberg HRA + OCT in normal and diseased eyes.

Methods-

Study of retinal layer segmentations of normal, intermediate dry Age-related Macular Degeneration (iAMD) and Diabetic Macular Edema (DME) eyes performed using Heidelberg Spectralis HRA + OCT and automated OCT segmentation software Orion.

Results-

Orion was significantly better than Heidelberg in the segmentation of NFL and INL layers in normal eyes. Orion generated significantly better segmentation only for NFL in iAMD and for INL and OPL layers in DME eyes when compared to the 'gold standard' of manual segmentation. To understand where differences lay, we directly compared layer volumes between Orion and Heidelberg software. In normal eyes, all retinal layer volumes calculated by the two softwares were moderate-strongly correlated except OUTLY. In iAMD eyes, GCIPL, INL, ONL, INLY, TRV layer volumes were moderate-strongly correlated between softwares. In eyes with DME, all layer volume values were moderate-strongly correlated between softwares.

Conclusion

- Findings suggest that cross-platform Orion retinal layer segmentation software can be used reliably to study the retinal layers and compares well against manual segmentation and the commonly used proprietary software for normal eyes and in particular for diseased eyes.

Introduction

Since its invention in 1991, Optical Coherence Tomography (OCT) has greatly assisted both ophthalmic clinical and research imaging[1]. Spectral Domain Optical Coherence Tomography (SD-OCT) systems are now able to rapidly capture high-resolution, three-dimensional (3D) volume scans of the retina for identifying, monitoring, and quantitatively assessing various pathologic conditions of the macula[1]. Alongside the improvement in scanning speed, SD-OCT is capable of visualizing the retina and its sublayers at a greater resolution. The use of SD-OCT has increased rapidly during the last two decades.

Manual segmentation of retinal layers from SD-OCT images is time consuming[2]. Automated segmentation potentially allows for rapid, accurate and repeatable delineation of individual retinal layers assisting the investigation of retinal diseases[2]. While automated retinal segmentation algorithms have traditionally performed well in normal retina to segment major retinal landmarks, there is a relative lack of data for automated segmentation of inner retinal layers in pathology[3,4].

The Heidelberg HRA + OCT system is now used globally for retinal studies in both the clinic and research settings. The proprietary software included with the Heidelberg system has been continually updated and allowed for intra retinal segmentation from version 6[1]. However, the software had limitations which made its use difficult. For example, currently the Spectralis software does not perform choroido-scleral interface segmentation automatically, in normal volume scans. This requires manual segmentation of individual B-scans. Additionally, although commercial OCT devices have on-board proprietary segmentation software which are fast and designed to give reliable values for interpretation by clinicians, the definition of the retinal boundaries varies between manufacturers and this makes quantitative retinal thickness comparisons difficult. Proprietary software is almost always limited to images captured by the parent device and cannot be applied to images from other OCT devices[4]. The algorithms are normally not accessible due to their proprietary nature, forcing the development of independent custom-built software. The initial iteration of Heidelberg proprietary segmentation software was considered inaccurate for segmenting retinal pathology[5]. However, the software has recently been updated.

Modern cross-platform softwares offer to overcome some of these drawbacks[6]. One cross platform system, accessible using a subscription model, has been developed by Voxeleron (Voxeleron LLC, Pleasanton, CA, USA). Their Orion software is reported to provide device independent eight layer retinal segmentation. Orion software has recently been used for a number of retinal disease studies including longitudinal measurement of retinal layer volumes in AMD[7], layer segmentation in retinitis pigmentosa[8], glaucoma and retinal manifestations of neurological disease[9,10,11]. This software measures retinal layer volumes with distinct boundaries which include the Retinal Nerve Fiber Layer (RNFL), Ganglion Cell-Inner Plexiform Layer (GCIPL), Inner Nuclear Layer (INL), Outer Plexiform Layer (OPL), Outer Nuclear Layer (ONL), Photoreceptors (PR) and Retinal Pigment Epithelium–Bruch's Membrane complex (RPE-BM). Additionally, the software is able to rapidly add new layer segmentations, such as the choroidal scleral interface with a semi-automated input. It measures the retinal volumes in the central macular area (6 mm diameter) automatically centered on the fovea, thereby supporting longitudinal analysis. This software has already proven to be reliable in the retinal layer segmentations captured using the Topcon 3D OCT-2000 imaging system[12]. However, there has been little study of retinal segmentation using this Orion software on images captured using Heidelberg HRA + OCT systems.

In this study, we compare retinal layer segmentation performed by Orion software and Heidelberg Spectralis software using scans from normal eyes and eyes with pathology. First, we qualitatively assess how well both software are able to segment retina compared to gold standard manual segmentation and then try to understand how retinal segmentation is different between the software by quantitatively comparing differences in measurements of retinal layer volumes between the software in normal and diseased retina.

Methods

Institutional Review Board (IRB) approval was acquired from the University of California San Diego for the review and analysis of patients' data. Patient's consent was obtained as per institutional protocol and all data and images were anonymized for patient's safety. This retrospective cross sectional study was conducted according to the principles of the Declaration of Helsinki. The study complied with the Health Insurance Portability and Accountability Act of 1996. Lists of patients who presented to Jacobs Retina Centre (JRC), University of California San Diego between 1st January 2019 and 31st December 2020 with diagnosis of normal eyes, eyes with intermediate dry Age-related Macular Degeneration (iAMD), representing an outer retinal pathology commonly seen in the clinical setting, and diabetic retinopathy with Diabetic Macular Edema (DME), representing an inner retinal pathology commonly seen in clinic, were identified from the retinal imaging report database. Pathology was confirmed by a retinal specialist (WRF). iAMD was confirmed if patients had at least one druse > 125μm in the eyes being classified with iAMD. Images were captured using a standard protocol for imaging all patients attending the Jacobs Retina Center involving forty-nine line volume scans. The Spectralis SD-OCT with the HRA + OCT protocol was used, and in each eye, a macular area (6×6 mm^2) cube centered on the fovea was scanned with an automated real time (ART) of 16 as part of the routine protocol. Retinal layer segmentations were then performed using Heidelberg Spectralis HRA + OCT (Heidelberg engineering software, HEE, Germany, version 1.10.4.0) and automated OCT layer segmentation software Orion (Voxeleron, Version 3.0.0). Images from 45 normal eyes, 33 iAMD eyes and 30 eyes with DME were used for the assessment (Fig. 1).

Exclusion criteria included images which were incomplete or unclear in one or more B scans and images with values more than 3 sigma from reference mean population values. Additionally, enhanced depth imaging (EDI) volume scans in Heidelberg were also excluded from the study because they could not be segmented and exported as raw data to the Orion software. In normal patients, fourteen eyes were excluded as outliers because their segmented layer volume data was beyond 3 sigma from mean population values. In eyes with iAMD, nine images were excluded due to the incomplete layer volume data in B scans. Of the DME patients, three patients were excluded because they had only EDI scans and four patients with segmented layer volume data beyond 3 sigma from mean population values were discarded as outliers.

Retinal layer segmentations of the ETDRS zone were first performed using Heidelberg Engineering software (version 1.10.4.0). The segmented layer volumes were then analyzed. The images used were exported as raw data from the Heidelberg Spectralis software and retinal layer segmentation of the exported images were performed using Orion software (Voxeleron, Version 3.0.0). Retinal layer thickness was measured in microns and the volume in mm^3. Quantitative comparisons were made between the different retinal layer volumes measured with Heidelberg and Orion. Volumes of NFL, GCL, IPL, INL, OPL, ONL, INLY (Inner retinal layer in total) and OUTLY (Outer retinal layer in total) were obtained in Heidelberg. The INLY was defined as the volume lying between the ILM and ELM (interior border) and OUTLY was defined as the volume lying between the ELM (outer border) and the RPE-Bruch's membrane complex. NFL, GCL_IPL, INL, OPL, ONL, PR and RPE_BRUCHS layer volumes in mm^3 were obtained from the exported (csv) files in the Orion software. The GCL and IPL layers in Orion software were considered a single layer and hence the two-layer volumes in Heidelberg were added to match the Orion. Similarly, PR and RPE_BRUCHS layer volumes were considered a single layer in Orion. Total retinal layer volumes (TRV) were also obtained from both Heidelberg Spectralis and Orion.

For qualitative analyses of the images, the degree of segmentation error in Heidelberg Spectralis and Orion were graded as good, mild, moderate or severely deranged from manual segmentation by two masked ophthalmologists VA and TM using reference images (Supplementary Figs. 1, 2 and 3). A total of 8 surfaces (per one foveal B-scan) for 108 eyes, adding up to 864 surfaces were checked and manually outlined by each grader. The qualitative grading was based on the difference from the expected manual segmentation of images. Intergrader agreement was calculated using a Kappa statistic. In instances where there was disagreement between the two graders a third masked grader and senior retinal specialist (SB) made a final decisive grading.

During the course of this study, the Spectralis software had been updated once further. To ensure that the segmentation had not changed with the update, a sample set of 10 images of normal, intermediate dry AMD and DME eyes each of which were previously segmented with the old software version were then segmented with the new version of the Heidelberg software (6.15.7.0). A Wilcoxon analysis was then used to compare the automated segmentation gradings between the two softwares. For statistical analyses, Microsoft Excel 2016 and statistical software R (3.4.2, September 2017) were used. A p value of < 0.05 was taken as indicating statistical significance. The correlation strength for the layer volumes was classified based on the Pearson correlation coefficient values into weakly positive (Pearson coefficient, $r < 0.4$), moderately positive (Pearson coefficient, $r >= 0.4$ - <0.7) and strongly positive (Pearson coefficient, $r >= 0.7$).

Results

Retinal layer segmentation in Normal eyes

In order to investigate how well the two software systems were able to segment images generated by the Heidelberg HRA + OCT machine, we first compared the retinal layer segmentations of images obtained from normal eyes with manual segmentation. A total of forty-five normal eyes were

analyzed.

In normal eyes, retinal segmentation using Spectralis software found, 82% to have good, 17% mild and 0.9% to have moderate segmentation error (Table 1), while Orion software was found to have 97% good, 2% mild and 0.4% moderate segmentation error compared with manual segmentation. Comparison of layer segmentation between the softwares and manual segmentation found that Orion was significantly better than Heidelberg Spectralis in the segmentation of NFL and INL layers ($p < 0.05$) (Table 2). For all other layers, there was no significant difference between the two softwares. In normal eyes, the NFL demonstrated the least agreement in the qualitative analysis (Supplementary Table 1). In Heidelberg, the intergrader agreement for all layers was 76%, with a kappa statistic of 0.32. For Orion images, the intergrader agreement for all layers was 90% with a kappa statistic of 0.38 (Supplementary Table 2). The test-retest kappa average for normal eyes was 0.91.

Table 1
Percentage of segmentation error gradings in Normal eyes, in iAMD eyes and in eyes with DME

NORMAL eyes	**GOOD**	**GOOD**	**MILD**	**MILD**	**MODERATE**	**MODERATE**	**SEVERE**	**SEVERE**
	HB	OR	HB	OR	HB	OR	HB	OR
PERCENTAGE	81.78	97.33	17.33	2.22	0.89	0.44	0.00	0.00
AMD eyes	**GOOD**	**GOOD**	**MILD**	**MILD**	**MODERATE**	**MODERATE**	**SEVERE**	**SEVERE**
	HB	OR	HB	OR	HB	OR	HB	OR
PERCENTAGE	70.91	79.39	24.24	15.76	4.85	4.85	0.00	0.00

Table 2
Wilcoxon test comparing different layers in Heidelberg Spectralis and Orion

DME eyes	**GOOD**	**GOOD**	**MILD**	**MILD**	**MODERATE**	**MODERATE**	**SEVERE**	**SEVERE**
	HB	OR	HB	OR	HB	OR	HB	OR
PERCENTAGE	41.33	61.33	25.33	26.00	19.33	12.67	14.00	0.00

To better understand where the differences between the software existed in retinal segmentation in normal eyes, we performed a quantitative comparison of Heidelberg and Orion retinal layer segmentation. This analysis showed that the NFL, ONL, INLY and TRV layers had strongly positive correlation ($r >= 0.7$), GCL_IPL, INL, OPL layers had a moderately positive correlation ($r >= 0.4$ - <0.7) and only OUTLY had a weakly positive correlation ($r < 0.4$) (Table 3). Paired t-test comparisons showed that there was no significant difference between Heidelberg and Orion in the GCL_IPL and OUTLY volumes. However, there was a significant difference in all other layers ($p = < 0.05$) (Table 3)(Fig. 3). In summary, although there was a general correlation between the softwares in retinal segmentation in normal eyes there were significant differences in measurement of layer volumes suggesting that different landmarks were used by the softwares for segmentation.

Table 3
Layer volume data in normal eyes.

Qualitative comparison of grading of retinal segmentation using Orion and Spectralis software to manual, in normal, intermediate dry AMD and diabetic macular edema eyes						
		NFL	GCIPL	INL	OPL	ONL
Normal eyes (N = 45)	Mean +/-SD (Heidelberg)	1.422+/-0.499	1.156+/-0.367	1.222+/-0.471	1.111+/-0.383	1.044+/-0.208
	Mean +/-SD (Orion)	1.089+/-0.358	1.022+/-0.149	1.000+/-0.000	1.000+/-0.000	1.044+/-0.208
	p-value	0.001	0.070	0.004	0.125	1.000
Intermediate dry AMD eyes (N = 33)	Mean +/-SD (Heidelberg)	1.394+/-0.556	1.212+/-0.545	1.303+/-0.529	1.333+/-0.595	1.455+/-0.617
	Mean +/-SD (Orion)	1.152+/-0.442	1.182+/-0.528	1.152+/-0.442	1.212+/-0.485	1.576+/-0.663
	p-value	0.035	0.883	0.180	0.406	0.485
Diabetic Macular Edema eyes (N = 30)	Mean +/-SD (Heidelberg)	2.000+/-1.083	1.833+/-1.085	2.433+/-0.971	2.200+/-1.186	1.833+/-1.020
	Mean +/-SD (Orion)	1.633+/-0.809	1.433+/-0.679	1.267+/-0.640	1.533+/-0.681	1.700+/-0.702
	p-value	0.109	0.052	0.000	0.003	0.487

Segmentation quality grading scheme: 1 = Good, 2 = Mild, 3 = Moderate, 4 = Severe

Vol. of the retinal layers in ETDRS zone	NFL	GCL_IPL	INL	OPL	ONL	INLY	OUTLY	TRV
MEAN+/-SD (Heidelberg)	0.95+/-0.120	1.95+/-0.136	0.97+/-0.048	0.82+/-0.084	1.72+/-0.138	6.41+/-0.280	2.27+/-0.066	8.68+/-0.290
MEAN+/-SD (Orion)	1.14+/-0.134	1.97+/-0.153	0.87+/-0.042	0.78+/-0.062	2.08+/-0.107	6.85+/-0.276	2.27+/-0.101	9.12+/-0.308
Pearson Correlation	0.743	0.623	0.403	0.669	0.738	0.943	0.289	0.950
P value (paired t-test)	< 0.001	0.253	< 0.001	< 0.001	< 0.001	< 0.001	0.682	< 0.001

Segmentation in eyes with intermediate dry AMD

Having compared retinal layer segmentation between the softwares in normal eyes, we then compared layer segmentation in eyes with pathology. iAMD was chosen as a prototypical disease for outer retinal disease as it mainly affects the outer retina leaving the inner retina predominantly unaffected[4]. Thirty-three eyes with iAMD were analyzed. Out of 33 eyes, 71% were reported as good, 24% were reported mild and 4.9% were reported as moderate segmentation error by Spectralis while Orion reported 79% segmentation as good, 15.8% as mild and 4.9% were reported as moderate segmentation error (Table 1). The qualitative analysis comparison performed using Wilcoxon test (Table 2) showed that Orion segmented retina in iAMD eyes are significantly better than Heidelberg Spectralis segmentation only for the NFL layer (p = < 0.05). For all other layers, the comparison did not find significant differences between the softwares. When comparing Spectralis and Orion software with manual grading in iAMD eyes, the average level of agreement between Spectralis & Orion for all layers was 73% and the kappa statistic average of all layers was 0.36. In iAMD eyes, ONL was the layer with most discrepancy in the qualitative analysis (Supplementary Table 1). In Heidelberg, the intergrader agreement for all layers was 56% and the kappa statistic average of all layers was 0.29. For Orion images, the intergrader agreement for all layers was 74% and the kappa statistic average of all layers was 0.51 (Supplementary Table 2). The test-retest kappa average for eyes with dry AMD was 0.87.

Using a quantitative analysis, the retinal layer volume segmentation was compared between the softwares in iAMD eyes to better understand differences between segmentation. The OPL layer volume measurement was found to have an extremely weak correlation between softwares. The NFL and OUTLY layers had a weakly positive correlation (r < 0.4), the GCL_IPL, INL and ONL layers had a moderate correlation (r > = 0.4 - <0.7) while the INLY and TRV layers were strongly correlated (r > 0.7) (Table 4)(Fig. 4). Using a paired t-test it was found that in GCL_IPL, OPL and OUTLY layers there was no significant difference between the Spectralis and Orion softwares. In all other layers, the differences were statistically significant, p = < 0.05.

Table 4
Layer volume data in eyes with iAMD

Vol. of the retinal layers in ETDRS zone	NFL	GCL_IPL	INL	OPL	ONL	INLY	OUTLY	TRV
Mean +/- SD (Heidelberg)	0.94 +/- 0.112	1.80 +/-0.159	0.94 +/-0.103	0.82 +/-0.068	1.69 +/-0.167	6.18 +/ 0.283	2.34 +/- 0.124	8.52+/- 0.296
Mean +/- SD (Orion)	1.12 +/-0.114	1.85 +/-0.161	0.82 +/-0.048	0.79 +/-0.053	2.04 +/-0.115	6.63 +/-0.276	2.35 +/-0.112	8.98+/-0.307
Pearson Correlation	0.361	0.589	0.495	0.041	0.564	0.780	0.345	0.694
P value (paired t-test)	< 0.001	0.125	< 0.001	0.168	< 0.001	< 0.001	0.588	< 0.001

Figure 4: Comparison of retinal layer volumes between Heidelberg Spectralis and Orion in eyes with iAMD

Retinal layer segmentations in eyes with DME

Eyes with diabetic macular edema(DME) were chosen to test retinal layer segmentation in eyes with inner retinal pathology. Thirty DME eyes were analyzed. On comparing both softwares to manual segmentation, 41.3% images were reported to have good segmentation, 25.3% were reported to have a mild error and 19.3% were reported as moderate segmentation error using Spectralis while Orion reported 61.3% segmentation as good, 26% as mild error and 12.7% as moderate segmentation error (Table 1). A comparison performed using Wilcoxon test (Table 2) found that Orion was significantly better at segmenting GCIPL, INL and OPL layers (p = < 0.05). For other layers, there was no statistically significant difference. When comparing Spectralis and Orion software with manual grading in DME eyes, the average level of agreement between Spectralis & Orion for all layers was 37% and the kappa statistic average of all layers was 0.19. In eyes with DME, INL was the layer with most relative discrepancy in the qualitative analysis (Supplementary Table 1). For Spectralis images, the level of intergrader agreement for all layers was 53% with a kappa statistic average for all layers of 0.55 while for Orion images, the degree of intergrader agreement for all layers was 58% with a kappa statistic average of all layers of 0.30 (Supplementary Table 2). The test-retest kappa average for eyes with DME was 0.83.

To better understand how the two software were different in segmenting DME eyes, a quantitative analysis was performed comparing retinal layer volumes after segmentation. The NFL, OPL and OUTLY layers showed moderate correlation (r > = 0.4 - <0.7), while the GCL_IPL, INL, ONL, INLY and TRV layers showed strong correlation (r > = 0.7) between Spectralis and Orion (Table 5). Paired t-tests found a significant difference between all layer volumes except OPL and OUTLY layers (Table 5)(Fig. 5).

Table 5
Layer volume data in eyes with Diabetic Macular Edema (DME)

Vol. of the retinal layers in ETDRS zone	NFL	GCL_IPL	INL	OPL	ONL	INLY	OUTLY	TRV
Mean +/- SD (Heidelberg)	1.01 +/- 0.233	1.76 +/-0.284	1.08 +/-0.163	0.88 +/-0.095	1.90 +/-0.324	6.62 +/ 0.712	2.22 +/- 0.111	8.83+/- 0.788
Mean +/- SD (Orion)	1.21 +/-0.228	1.95 +/-0.298	0.88 +/-0.104	0.84 +/-0.117	2.16 +/-0.250	7.02 +/-0.716	2.28 +/-0.181	9.30+/-0.819
Pearson Correlation	0.415	0.805	0.699	0.484	0.732	0.990	0.433	0.985
P value (paired t-test)	< 0.001	< 0.001	< 0.001	0.038	< 0.001	< 0.001	0.072	< 0.000

Comparison of previous version and latest update of Heidelberg Spectralis software

The quantitative findings (layer volume values) from the image segmentation with old Spectralis software (1.10.4.0) and new Spectralis software (6.15.7.0), were compared for 10 eyes each of type normal, iAMD and DME. The volume of each retinal layer analyzed by the new software was found to be identical to the previous analysis by the old software version. For the same 30 eyes, a qualitative comparison was done by looking at the images with layer segmentation by the new software versus the old one. The layer segmentation done by both the versions of software were found to be identical to each other.

Discussion

The aim of the present study was to compare automated retinal segmentation to manual segmentation using the cross-platform Orion software segmented retinal layers compared with the proprietary Spectralis software segmented retinal layers for normal and diseased eyes using images captured by the Heidelberg HRA + OCT machine. In addition, we aimed to understand how the different softwares, segmented layers differently.

We compared the two softwares to the gold standard of manual grading using two manual graders. This method of validation has been used frequently to assess intraretinal segmentation software[13–17]. We found that Orion software was subjectively better at segmenting retinal layers than Heidelberg in

both normal and particularly in diseased eyes. The Orion software has previously been compared to manual grading in normal retina using images acquired by the Heidelberg HRA + OCT machine[14]. In this previous study, 24 volume scans were both automatically segmented into 7 retinal layers and manually segmented by two experts. The study found that the mean differences and ranges between the software and manual raters were all within the axial resolution (~ 5μm) of the device. We similarly found that the Orion software grading agreed well with manual grading as Orion software was found to have 97% good, 2% mild and 0.4% moderate segmentation in normal eyes.

We used a modified grading system utilizing reference images to grade how deviated the automated segmentation was from manual segmentation performed by two graders and a further senior grader casting a decision in cases where there was disagreement. To our knowledge, our paper is the first to compare Orion software intraretinal segmentation in diseased retina to manual segmentation. We found that the agreement in retinal segmentation fell to 79% good, 15.8% mild and 4.9% moderate segmentation error in eyes with outer retinal pathology and fell even further to 61.3% good, 26% mild and 12.7% moderate segmentation error in eyes with intra-retinal pathology when Orion software was compared with manual grading.

A number of research softwares are now available for cross platform segmentation of the retina[18–21]. A previous study tried comparing five automated intra-retinal segmentation software, which did not include Orion software. This previous study used 610 B-scans with a size of 768×496 pixels from only 10 eyes of mild non-proliferative diabetic retinopathy patients. The software compared included Heidelberg Spectralis (version 6), IOWA Reference Algorithm, Automated Retinal Analysis tools (AURA), Dufour's Algorithm, OCTRIM3D[1,2]. The 'ground truth' was set as manual grading from macular SD-OCT volume data. Two experienced graders labeled 5 retinal surfaces in representative images of the SD-OCT volume dataset. The inter-observer differences were used to investigate the accuracy of software. Therefore, a total of 250 (5 surfaces per B-scan) were checked and manually outlined by each grader in the pathologic dataset. The inner retinal layers appear to be well delineated using the Heidelberg Engineering and IOWA software in normal human retina[2]. The softwares were compared for the capability to detect the different layer surfaces, the accuracy of segmentation, as well as the presence and ease of use of the input and output formats of the image data and segmented layers.

Other research software has previously looked and found changes in inner retinal layers in early AMD[22]. However, no comparison was made to manual grading and additionally no validation was performed in disease. Another study compared intraretinal segmentation of images obtained by Zeiss Cirrus HD-OCT machine (Carl Zeiss Meditec, Inc., Dublin, CA). The study compared segmentation by custom software and the Iowa Reference Algorithm OCT-Explorer (version 3.5) with 2 manual graders in normal, early/intermediate AMD and advanced AMD eye[23]. A third masked grader was used to grade the images using a 4 point ordinal score, similar to the one used in the present study, and found that both the new software and the Iowa Reference Algorithm OCT-Explorer performed intraretinal segmentation well in normal and early/intermediate AMD but the accuracy dropped off in advanced AMD. Interestingly, the new algorithm performed better, although not significantly, than both manual graders in advanced disease as judged by the third masked grader. This was thought to be due to the relatively limited normal images used to train the Iowa Reference Algorithm. This again highlights the importance of using more real world clinical data to enable the algorithms to correctly segment OCT images in diseased retina.

We found that the retinal layer segmentations correlated well between the two advanced softwares. However, in general, the layer volumes were significantly different. ONL was the layer with maximum difference (in measured layer volume) between Heidelberg and Orion in both normal and diseased eye. Inner and outer retinal layers in general and the total retinal volume also matched very well (within 0 to 10%) between Heidelberg and Orion softwares for all types of eyes, normal and with pathology, as shown in Fig. 2. In normal eyes, the layers GCL_IPL, INL and OPL matched very well (within 1 to 10%) between Heidelberg and Orion. NFL and ONL layers matched moderately well (within 10 to 20%). In dry AMD eyes for retinal segmentations, the GCL_IPL and OPL layers matched very well (within 1 to 10%) between Heidelberg and Orion while NFL, INL and ONL layers matched moderately well (within 10 to 20%). In the eyes with DME, ONL layer had the maximum difference in measured layer volume between Heidelberg and Orion. In terms of percentage difference of measured layer volume in DME eyes, INL layer had the maximum difference between the softwares. In DME eyes for retinal segmentations, the GCL_IPL and OPL layers matched very well (within 1 to 10%) between Heidelberg and Orion while NFL, INL and ONL layers matched moderately well (within 10 to 20%) as shown in Fig. 2.

The Orion software has been used previously in cross platform comparisons[25]. Zeiss Cirrus and Heidelberg HRA + OCT images were compared using Orion software but only for retinal segmentation. Using the Orion software, there was good compatibility of total retinal thickness measurements when analyzing images from the same subject using both devices. To our knowledge, there are no studies which compared Orion software to other softwares in diseased eyes. In the present study, a finding of significant differences in the retinal layer segmentation between the two automated softwares is likely due to differences in the method used by the algorithm to segment the retina. Research groups have used a variety of algorithms to perform intra-retinal segmentation including using graph-based[26–29], active contour [30] and texture models[31].

One of the limitations of the present study was that although an attempt was made to mask the graders by using the same sections to avoid software identifiable images, Spectralis and Orion retinal segmentation appears different and as a result the graders would likely be able to identify which software was used for grading. Additionally, for the studies comparing automated segmentation to manual grading, only horizontal B scans crossing the fovea were tested, as it was felt that this was the most common scan used by physicians in clinic. The findings of comparison may have been different in other areas of the macula beyond the fovea. A limitation of making conclusions about which software was better in the clinical setting was that we used only DME retina to represent inner retinal pathology and iAMD to represent outer retinal pathology. In the clinical setting there is a far larger variety of types of retinal pathology. Future studies should ideally look at comparing automated intra-retinal segmentation in a wider spectrum of retinal diseases to provide a better representation of how the software performs in the real-world clinic setting.

In summary, this paper adds to the knowledge regarding how different software platforms perform in intraretinal segmentation in normal and in particular the diseased eyes. The findings will be useful to inform the use of intraretinal segmentation not only in the clinic but also in clinical trials. We found that although Orion and Heidelberg software measurement of retinal layer volumes were correlated, they were found to be significantly different suggesting that different retinal landmarks were chosen for identifying retinal layers. Both software performed well in normal retina when compared with manual segmentation. However, our findings suggest caution in using the software for intra-retinal segmentation in disease. The Orion software offers a reliable alternative for intraretinal segmentation which may be useful particularly for research studies and clinical trials.

Declarations

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AUTHOR CONTRIBUTION STATEMENT

VA was responsible for the design of the work, acquisition of data and drafting of the paper.

TM helped as a grader for qualitative analysis of the images.

WRF helped with proof reading and overall guidance.

JAJ helped with the data and statistical analysis.

DUB helped with purchase of the Orion software and provided technical advice.

SB was responsible for the concept and design of the work, image grading, drafting and literature search.

References

[1] Tian, J. et al. Performance evaluation of automated segmentation software on optical coherence tomography volume data. *J. biophot.* 9.5 : 478-489 (2016).

[2] Dysli, C., Enzmann, V., Sznitman, R., & Zinkernagel, M. S. Quantitative analysis of mouse retinal layers using automated segmentation of spectral domain optical coherence tomography images. *Transl Vision Sci & Tech August*. 4, 9 (2015).

[3] Tan, J. et al. The measurement repeatability using different partition methods of intraretinal tomographic thickness maps in healthy human subjects. *Clin Ophthalmol.*;10 : 2403–15. 16 (2016).

[4] Terry, L. et al. Automated retinal layer segmentation using spectral domain optical coherence tomography: evaluation of inter-session repeatability and agreement between devices. *PLoS ONE.* ; 11: e0162001 (2016).

[5] GmbH HE. Spectralis HRA+OCT User Manual Software Version 6.0. (2014).

[6] Oakley, J. D., Andorra, M., Martinez-Lapiscina, E. H., Russakoff, D., & Villoslada, P. Comparison of automated retinal segmentation across OCT devices using independent analysis software. *Invest. Ophthalmol. & Vis. Sci.* 57.12 : 5956-5956 (2016).

[7] Lamin, A., Oakley J. D., Dubis, A. M., Russakoff, D. B. & Sivaprasad, S. Changes in volume of various retinal layers over time in early and intermediate age-related macular degeneration. *Eye* ; 33:428-434 (2019).

[8] Jolly, J. K. et al. Inner retinal thickening affects microperimetry thresholds in the presence of photoreceptor thinning in patients with RPGR retinitis pigmentosa. *Br J Ophthalmol.* (2020).

[9] Monteiro, M. L., Mello, L. G. M., Bissoli, L. B., Maia, R. D. P. D. & Saraiva, F. P. OCT findings in Patients with Parkinson's Disease with or Without Pramipexole Treatment. *Invest. Ophthalmol. & Vis. Sci.*; 61: 5107-5107 (2020).

[10] Behbehani, R., Adnan, H., Al-Hassan, A. A., Al-Salahat, A. & Alroughani, R. Predictors of retinal atrophy in multiple sclerosis: A longitudinal study using spectral domain optical coherence tomography with segmentation analysis. *Mult Scler & Relat Disord*; 21: 56-62 (2018).

[11] Gameiro, G. R. et al. Retinal tissue hypoperfusion in patients with clinical Alzheimer's disease. *Eye and Vision* ; 5:1(2018).

[12] Lamin, A., El Nokrashy, A., Chandra, S. & Sivaprasad, S. Association of longitudinal changes in drusen characteristics and retinal layer volumes with subsequent subtype of choroidal neovascularisation. *Ophthal Res.*; 63(4): 375-382 (2020).

[13] Garvin, M. K. et al. Automated 3-D intraretinal layer segmentation of macular spectral-domain optical coherence tomography images. *IEEE Tran on Med Imag*; 28: 1436-1447 (2009).

[14] Oakley, J. D. et al. Assessing manual versus automated segmentation of the macula using optical coherence tomography. *Invest. Ophthalmol. & Vis. Sci*; 55: 4790-4790 (2014).

[15] DeBuc, D. C. et al. Reliability and reproducibility of macular segmentation using a custom-built optical coherence tomography retinal image analysis software. *J Biomed Opt*; 14: 064023 (2009).

[16] Kafieh, R., Rabbani, H., Abramoff, M. D. & Sonka, M. Intra-retinal layer segmentation of 3D optical coherence tomography using coarse grained diffusion map. *Med Image Anal*; 17: 907-928 (2013).

[17] Droby, A. et al. A novel automated segmentation method for retinal layers in OCT images proves retinal degeneration after optic neuritis. *Br. J. Ophthalmo*l. 100.4 : 484-490 (2016).

[18] Lee, K. A. M, Garvin, M., Sonka, M., The Iowa Reference Algorithms (Retinal Image Analysis Lab, Iowa Institute for Biomedical Imaging, Iowa City, IA)

[19] A. L. AURA tools : AUtomated Retinal Analysis tools: Tool/Resource.

[20] PA D. OCT Segmentation Application.

[21] Tian, J. et al. Performance evaluation of automated segmentation software on optical coherence tomography volume data. *J Biophot.*; 9: 478-489 (2016).

[22] Savastano, M. C. et al. Differential vulnerability of retinal layers to early age-related macular degeneration: evidence by sd-oct segmentation analysis. *Invest. Ophthalmol. & Vis. Sci.;* 55: 560-566 (2014).

[23] de Sisternes, L. et al. Automated intraretinal segmentation of SD-OCT images in normal and age-related macular degeneration eyes. *Biomed optics express*; 8: 1926-1949 (2017).

[24] Dysli, C., Enzmann, V., Sznitman, R. & Zinkernagel, M. S., Quantitative analysis of mouse retinal layers using automated segmentation of spectral domain optical coherence tomography images. *Transl. Vision. Sci & tech.*; 4:9-9 (2016).

[25] Oakley, J. D., Andorra, M., Martinez-Lapiscina, E. H., Russakoff, D. & Villoslada, P. Comparison of automated retinal segmentation across oct devices using independent analysis software. *Invest. Ophthalmol. & Vis. Sci.*; 57: 5956-5956 (2016).

[26] Chiu, S. J. et al. Automatic segmentation of seven retinal layers in SDOCT images congruent with expert manual segmentation. *Optics express*. Aug 30;18(18): 19413-28 (2010).

[27] Garvin, M. K. et al. Automated 3-D intraretinal layer segmentation of macular spectral-domain optical coherence tomography images. *IEEE Tran on med imaging*. Mar 10;28(9): 1436-47 (2009).

[28] Lang, A. et al. Retinal layer segmentation of macular OCT images using boundary classification. *Biomed optics express*. 1;4(7): 1133-52 (2013).

[29] Dufour, P. A. et al. Graph-based multi-surface segmentation of OCT data using trained hard and soft constraints. *IEEE Tran on med imaging.* 18;32(3): 531-43 (2012).

[30] Yazdanpanah, A., Hamarneh, G., Smith, B. R. & Sarunic, M. V. Segmentation of intra-retinal layers from optical coherence tomography images using an active contour approach. *IEEE Tran on med imaging*. 14;30(2): 484-96 (2010).

[31] Kajić, V. et al. Robust segmentation of intraretinal layers in the normal human fovea using a novel statistical model based on texture and shape analysis. *Optics express*. 5;18(14): 14730-44 (2010).

Tables

Table 1: Percentage of segmentation error gradings in Normal eyes, in iAMD eyes and in eyes with DME

NORMAL eyes	GOOD	GOOD	MILD	MILD	MODERATE	MODERATE	SEVERE	SEVERE
	HB	OR	HB	OR	HB	OR	HB	OR
PERCENTAGE	81.78	97.33	17.33	2.22	0.89	0.44	0.00	0.00

AMD eyes	GOOD	GOOD	MILD	MILD	MODERATE	MODERATE	SEVERE	SEVERE
	HB	OR	HB	OR	HB	OR	HB	OR
PERCENTAGE	70.91	79.39	24.24	15.76	4.85	4.85	0.00	0.00

DME eyes	GOOD	GOOD	MILD	MILD	MODERATE	MODERATE	SEVERE	SEVERE
	HB	OR	HB	OR	HB	OR	HB	OR
PERCENTAGE	41.33	61.33	25.33	26.00	19.33	12.67	14.00	0.00

Table 2: Wilcoxon test comparing different layers in Heidelberg Spectralis and Orion

Qualitative comparison of grading of retinal segmentation using Orion and Spectralis software to manual, in normal, intermediate dry AMD and diabetic macular edema eyes						
		NFL	GCIPL	INL	OPL	ONL
Normal eyes (N = 45)	Mean +/-SD (Heidelberg)	1.422+/- 0.499	1.156+/-0.367	1.222+/-0.471	1.111+/-0.383	1.044+/-0.208
	Mean +/-SD (Orion)	1.089+/-0.358	1.022+/-0.149	1.000+/-0.000	1.000+/-0.000	1.044+/-0.208
	p-value	0.001	0.070	0.004	0.125	1.000
Intermediate dry AMD eyes (N = 33)	Mean +/-SD (Heidelberg)	1.394+/- 0.556	1.212+/-0.545	1.303+/-0.529	1.333+/-0.595	1.455+/-0.617
	Mean +/-SD (Orion)	1.152+/-0.442	1.182+/-0.528	1.152+/-0.442	1.212+/-0.485	1.576+/-0.663
	p-value	0.035	0.883	0.180	0.406	0.485
Diabetic Macular Edema eyes (N = 30)	Mean +/-SD (Heidelberg)	2.000+/- 1.083	1.833+/-1.085	2.433+/-0.971	2.200+/-1.186	1.833+/-1.020
	Mean +/-SD (Orion)	1.633+/-0.809	1.433+/-0.679	1.267+/-0.640	1.533+/-0.681	1.700+/-0.702
	p-value	0.109	0.052	0.000	0.003	0.487
Segmentation quality grading scheme: 1 = Good, 2 = Mild, 3 = Moderate, 4 = Severe						

Table 3: Layer volume data in normal eyes.

Vol. of the retinal layers in ETDRS zone	NFL	GCL_ IPL	INL	OPL	ONL	INLY	OUTLY	TRV
MEAN+/-SD (Heidelberg)	0.95+/-0.120	1.95+/-0.136	0.97+/-0.048	0.82+/-0.084	1.72+/-0.138	6.41+/-0.280	2.27+/-0.066	8.68+/-0.290
MEAN+/-SD (Orion)	1.14+/-0.134	1.97+/-0.153	0.87+/-0.042	0.78+/-0.062	2.08+/-0.107	6.85+/-0.276	2.27+/-0.101	9.12+/-0.308
Pearson Correlation	0.743	0.623	0.403	0.669	0.738	0.943	0.289	0.950
P value (paired t-test)	< 0.001	0.253	< 0.001	< 0.001	< 0.001	< 0.001	0.682	< 0.001

Table 4: Layer volume data in eyes with iAMD

Vol. of the retinal layers in ETDRS zone	NFL	GCL_ IPL	INL	OPL	ONL	INLY	OUTLY	TRV
Mean +/- SD (Heidelberg)	0.94 +/-0.112	1.80 +/-0.159	0.94 +/-0.103	0.82 +/-0.068	1.69 +/-0.167	6.18 +/-0.283	2.34 +/-0.124	8.52+/-0.296
Mean +/- SD (Orion)	1.12 +/-0.114	1.85 +/-0.161	0.82 +/-0.048	0.79 +/-0.053	2.04 +/-0.115	6.63 +/-0.276	2.35 +/-0.112	8.98+/-0.307
Pearson Correlation	0.361	0.589	0.495	0.041	0.564	0.780	0.345	0.694
P value (paired t-test)	< 0.001	0.125	< 0.001	0.168	< 0.001	< 0.001	0.588	< 0.001

Table 5: Layer volume data in eyes with Diabetic Macular Edema (DME)

Vol. of the retinal layers in ETDRS zone	NFL	GCL_ IPL	INL	OPL	ONL	INLY	OUTLY	TRV
Mean +/- SD (Heidelberg)	1.01 +/-0.233	1.76 +/-0.284	1.08 +/-0.163	0.88 +/-0.095	1.90 +/-0.324	6.62 +/-0.712	2.22 +/-0.111	8.83+/-0.788
Mean +/- SD (Orion)	1.21 +/-0.228	1.95 +/-0.298	0.88 +/-0.104	0.84 +/-0.117	2.16 +/-0.250	7.02 +/-0.716	2.28 +/-0.181	9.30+/-0.819
Pearson Correlation	0.415	0.805	0.699	0.484	0.732	0.990	0.433	0.985
P value (paired t-test)	< 0.001	< 0.001	< 0.001	0.038	< 0.001	< 0.001	0.072	< 0.000

Figures

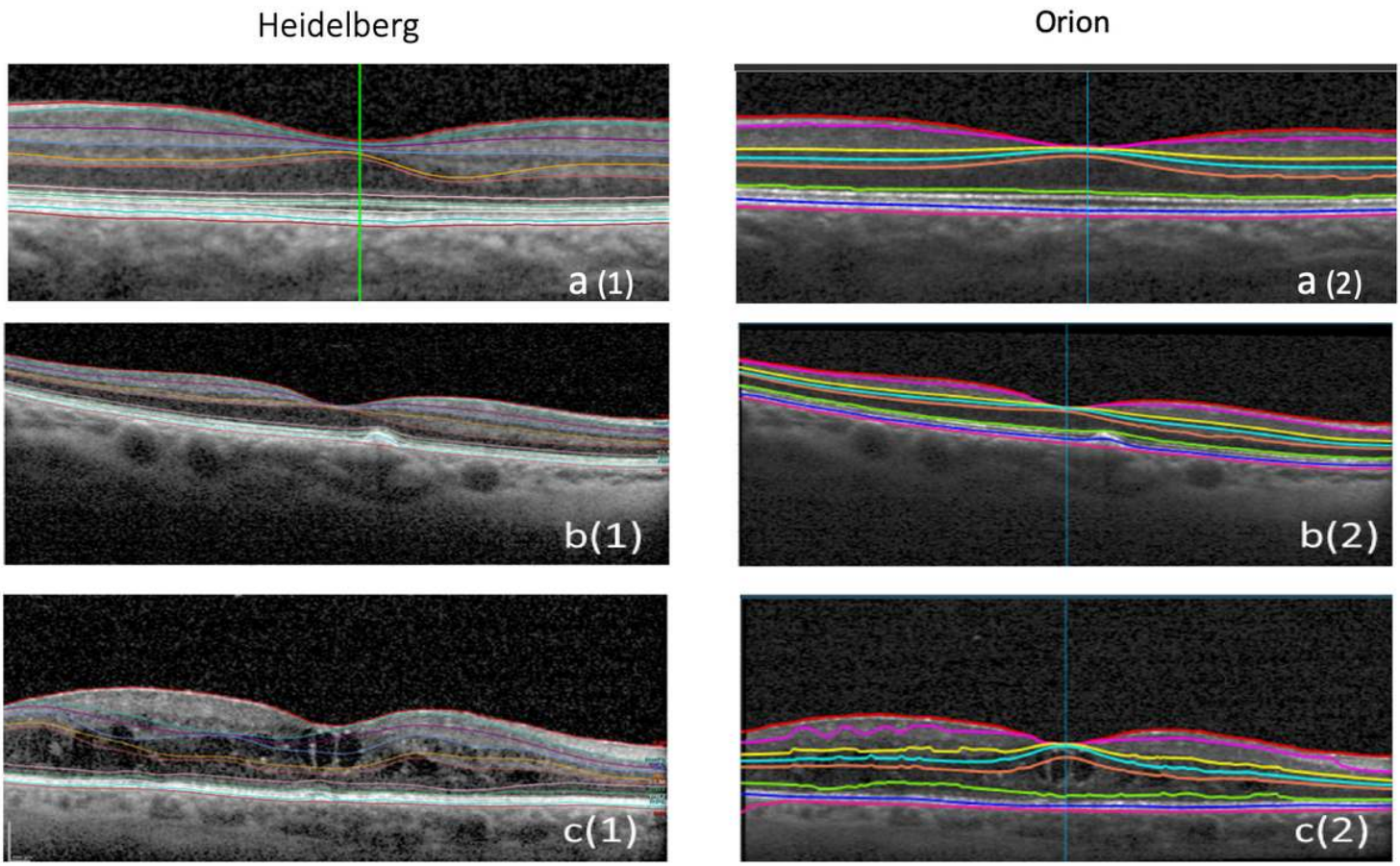

Figure 1

Representative images of retinal segmentation of Normal, Intermediate dry AMD and DME eyes using Heidelberg Spectralis HRA+OCT (Heidelberg engineering software, HEE, Germany, version 1.10.4.0) and automated OCT layer segmentation software Orion (Voxeleron, Version 3.0.0). (a1) Normal eyes Heidelberg segmentation, a(2) Normal eyes Orion segmentation, b(1) iAMD Heidelberg segmentation, b(2) iAMD Orion segmentation, c(1) DME Heidelberg segmentation, c(2) DME Orion segmentation.

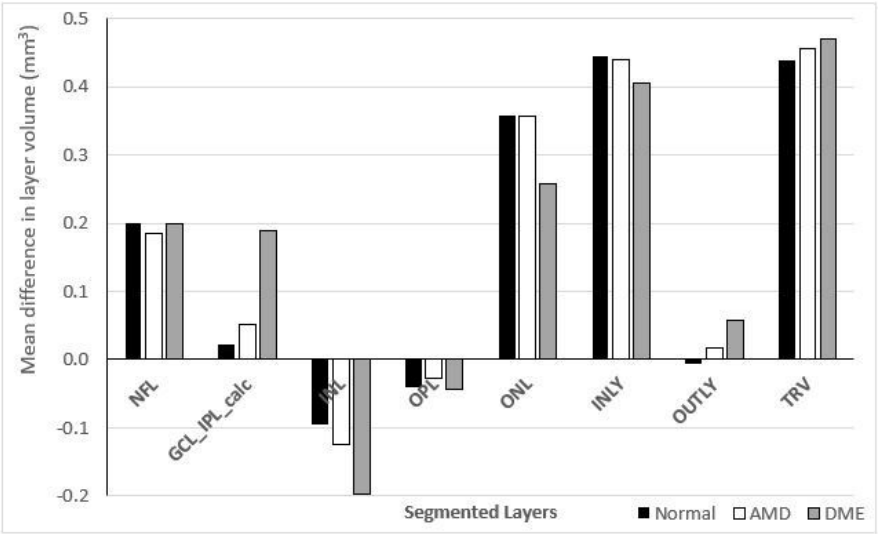

Figure 2

Mean difference between layer volumes measured by Heidelberg Spectralis and Orion expressed in mm3 for different types of patients across different segmented layers

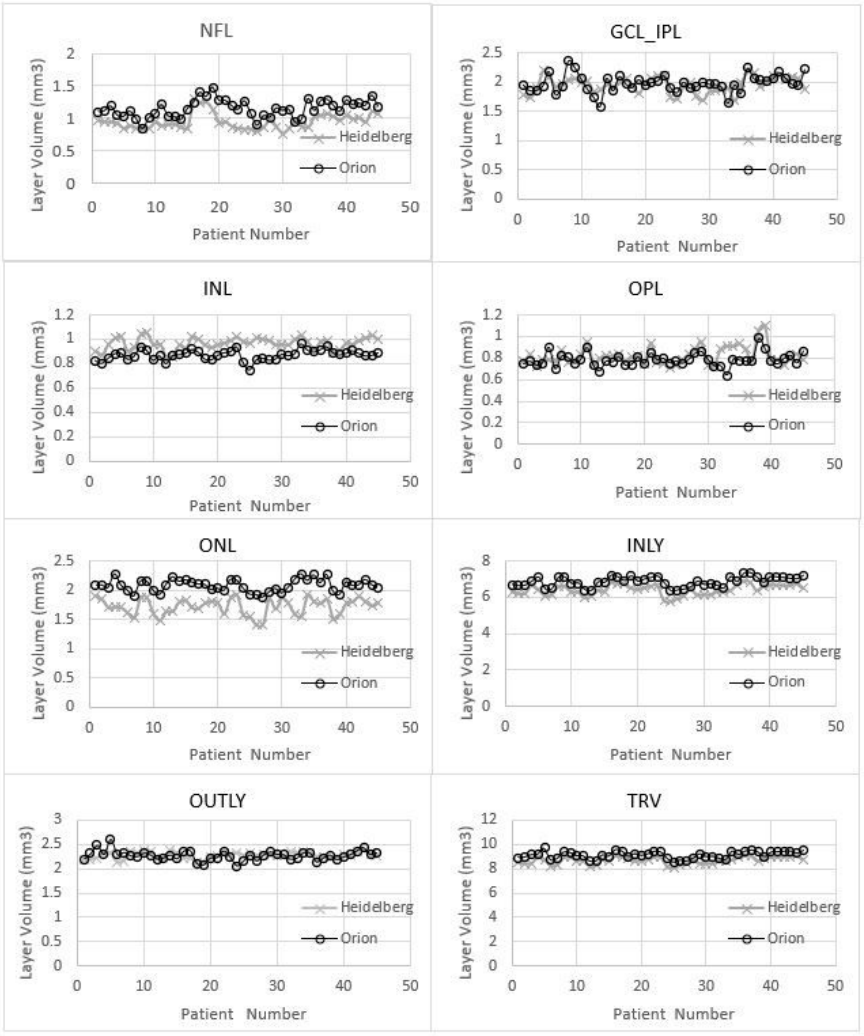

Figure 3

Comparison of retinal layer volumes between Heidelberg Spectralis and Orion in normal eyes

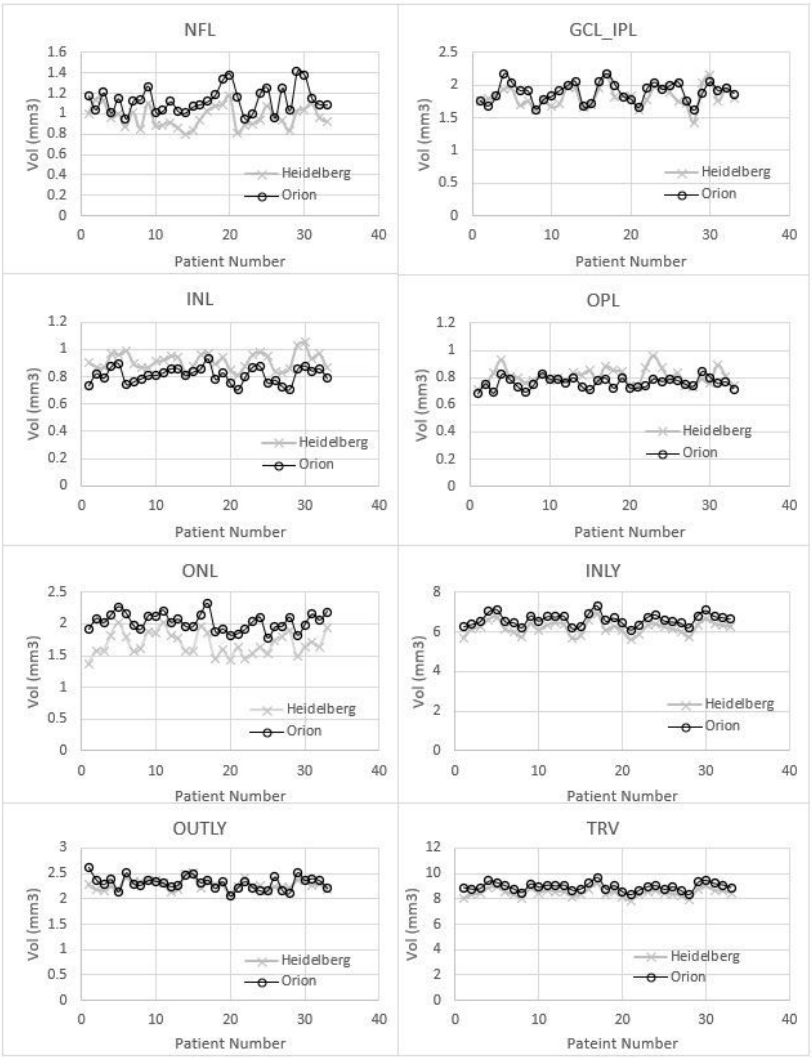

Figure 4

Comparison of retinal layer volumes between Heidelberg Spectralis and Orion in eyes with iAMD

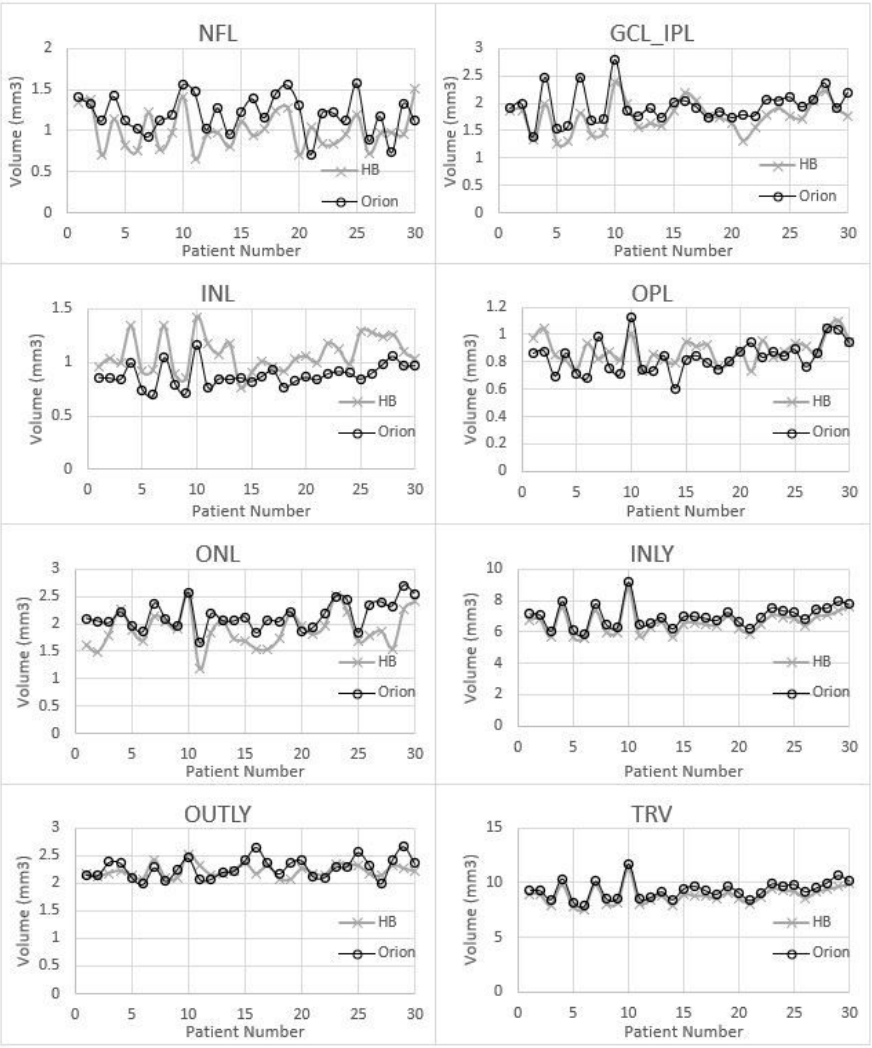

Figure 5

Comparison of retinal layer volumes between Heidelberg Spectralis and Orion in eyes with DME

Supplementary Files

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