Published in final edited form as:

Curr Eye Res. 2018 March ; 43(3): 415–423. doi:10.1080/02713683.2017.1406526.

Analysis of agreement of retinal layer thickness measures derived from segmentation of horizontal and vertical Spectralis OCT macular scans.

Natalia Gonzalez Caldito, MD¹, Bhavna Antony, PhD², Yufan He, BS², Andrew Lang, PhD², James Nguyen, BS¹, Alissa Rothman, BS¹, Esther Ogbuokiri, BS¹, Ama Avornu, BS¹, Laura Balcer, MD³, Elliot Frohman, MD, PhD⁴, Teresa C. Frohman, PA-C⁴, Pavan Bhargava, MD¹, Jerry Prince, PhD², Peter A. Calabresi, MD¹, and Shiv Saidha, MBBCh, MD, MRCP¹

¹Department of Neurology, Johns Hopkins University School of Medicine, Baltimore, MD, USA

²Department of Electrical and Computer Engineering, Johns Hopkins University, Baltimore, MD, USA

³Department of Neurology, New York University Langone Medical Center, New York, NY, USA

⁴Department of Neurology and Ophthalmology, University of Texas Austin Dell Medical School, Austin TX, USA

Abstract

Purpose: Optical coherence tomography (OCT) is a reliable method used to quantify discrete layers of the retina. Spectralis OCT is a device used for this purpose. Spectralis OCT macular scan imaging acquisition can be obtained either on the horizontal or vertical plane. The vertical protocol has been proposed as favorable, due to postulated reduction in confound of Henle's fibers on segmentation derived metrics. Yet, agreement of segmentation measures of horizontal and vertical macular scans remains unexplored. Our aim was to determine this agreement.

Materials and methods: Horizontal and vertical macular scans on Spectralis OCT were acquired in 20 healthy controls (HCs) and 20 multiple sclerosis (MS) patients. All scans were segmented using Heidelberg software and a Johns Hopkins University (JHU) developed method. Agreement was analyzed using Bland Altman analyses and intra-class correlation coefficients (ICCs).

Address reprint requests & correspondence to: Dr. Shiv Saidha, Associate Professor of Neurology, Division of Neuroimmunology and Neurological Infections, Johns Hopkins Hospital, 600 N. Wolfe St., Baltimore, MD 21287, USA, Tel: (410) 614-1522, Fax: 410-502-6736, ssaidha2@jhmi.edu.

Declaration of Interests

Laura Balcer has received consulting fees from Biogen.

Elliot Frohman has received speaker and consulting fees from Genzyme, Acorda, Novartis, and TEVA.

Teresa Frohman has received speaker and consulting fees from Acorda, Genzyme, and Novartis

Peter Calabresi has received personal honorariums for consulting from Biogen and Vertex. He is PI on research grants to Johns Hopkins from Novartis, Teva, MedImmune, Annexon, and Biogen.

Shiv Saidha has received consulting fees from Medical Logix for the development of CME programs in neurology and served on scientific advisory boards for Biogen-Idec, Genzyme, Genentech, EMD Serono & Novartis. He has received equity compensation for consulting from JuneBrain LLC, a retinal imaging device developer. He receives research support from Genentech Corporation and the National MS Society, and received support from the Race to Erase MS foundation. He serves on the working committee of the International MS Visual System (IMSVISUAL) consortium.

NGC, BA, YH, TF, JN, AR, EO, AA, PB, and JP have no conflicts of interest

Results: Using both segmentation techniques, mean differences (agreement at the cohort level) in thicknesses of all macular layers derived from both acquisition protocols in MS patients and HCs were narrow ($<1\mu\text{m}$), while limits of agreement (LOA) (agreement at the individual level) were wider. Using JHU segmentation mean differences (and LOA) for the macular retinal nerve fiber layer (RNFL) and ganglion cell layer + inner plexiform layer (GCIP) in MS were $0.21\mu\text{m}$ (-1.57 – $1.99\mu\text{m}$) and $-0.36\mu\text{m}$ (-1.44 – $1.37\mu\text{m}$) respectively.

Conclusions: OCT segmentation measures of discrete retinal layer thicknesses derived from both vertical and horizontal protocols on Spectralis OCT agree excellently at the cohort level (narrow mean differences), but only moderately at the individual level (wide LOA). This suggests patients scanned using either protocol, should continue to be scanned with the same protocol. However, due to excellent agreement at the cohort level, measures derived from both acquisitions can be pooled for outcome purposes in clinical trials.

Keywords

Optical coherence tomography (OCT); multiple sclerosis (MS); segmentation; retinal layers; analysis of agreement

Introduction

Multiple sclerosis (MS) is a chronic, autoimmune, inflammatory demyelinating disease of the central nervous system (CNS) that results in axonal and neuronal degeneration[1], the principal substrates underlying permanent disability in MS[2]. While magnetic resonance imaging (MRI) and in particular non-conventional MRI techniques such as volumetric, diffusion tensor imaging (DTI), and magnetization transfer imaging (MTI) may be used to assess neurodegeneration[3] [4] [5] [6], these techniques are expensive, may lack sensitivity at the individual patient level or in small cohorts of patients, and pose challenges with respect to implementation into routine clinical practice. Optical Coherence Tomography (OCT) is a non-invasive, reliable, reproducible, well-tolerated technique, that acquires cross-sectional three dimensional images of tissue such as the retina using low-coherence near infrared light [7]. OCT has revolutionized the clinical practice of Ophthalmology. Disease stage analysis of age-related macular degeneration, central serous chorioretinopathy and diabetic retinopathy among other pathologies have been successfully achieved using OCT [8][9]. Furthermore, OCT has been increasingly utilized as a complementary tool to MRI in neurology, particularly in patients with MS [10]. There is an abundance of evidence supporting the potential utility of OCT measures, in particular peripapillary-RNFL (pRNFL) and GCIP thicknesses, to monitor neurodegeneration and neuroprotection *in-vivo* in MS[11] [12][13]. OCT derived GCIP thickness has several advantages over conventional pRNFL thickness including excellent reliability and reproducibility [14] [10], and superior structure-function relationships in MS (with global disability and visual function). GCIP thinning mirrors global brain atrophy over time in MS, and is accelerated in patients exhibiting inflammatory disease activity as well as disability progression[15] [16]. Collectively, these findings provide support for OCT measures as complementary outcomes for monitoring and tracking neurodegeneration, and accordingly neuroprotection, in MS. While pRNFL and GCIP thinning related to optic neuropathy in MS may occur relatively slowly, rapid and

substantial reductions in pRNFL and GCIP thicknesses occur within weeks of acute optic neuritis (ON)[11] making the ON model (using OCT outcomes) a potentially rapid throughput screening model for testing neuroprotection and/or neurorestoration in MS [14].

Modern spectral-domain OCT has very high resolution ($<5\mu\text{m}$) allowing virtually all retinal layers to be discerned and segmented at the macula[17]. For this purpose, several segmentation methods have been developed[18][19]. The ganglion cell (GCL) and inner plexiform (IPL) layers can be difficult to distinguish and therefore the composite of these two measures (GCIP) is often used as an estimate of GCL integrity[11]. The GCL contains ganglion cell neurons from which RNFL axons are derived, which ultimately coalesce at the optic discs to form the optic nerves[20]. Automated OCT macular segmentation techniques enable determination of macular GCIP, inner nuclear layer (INL) and outer nuclear layer (ONL) thicknesses [16] [17] [23] [18], and have been transitioning into clinical practice. An open source OCT segmentation technique developed at Johns Hopkins University (JHU) can be consistently and uniformly applied across different SD-OCT platforms enabling the determination of retinal layer thickness measures from different devices that can be potentially pooled for evaluating outcomes [24].

Acquisition protocols differ substantially between the different OCT platforms [25] Nonetheless, analyses comparing automated, semi-automated and manual segmentation techniques within and across SD-OCT platforms demonstrate congruency in intra-retinal segmentation derived measures across segmentation techniques in both healthy controls and MS patients[26][27][28] [29][30]. Cross-sectional and longitudinal comparisons of retinal layer measures derived from segmentation methods across OCT platforms have shown an excellent agreement at the cohort level [24] [31], suggesting data derived using a number of different segmentation techniques applied to different SD-OCT images can be pooled for the purpose of assessing outcomes. At the individual level however, this agreement is modest, therefore not supporting inter-changeability across segmentation techniques at the patient level[24]. In assessing OCT segmentation measures and their utility as outcomes in clinical trials, assessment not only across devices is important, but also across acquisition techniques/protocols within and across devices.[25][32]

Spectralis (Heidelberg Engineering, Heidelberg, Germany) is one of the most commonly employed OCT platforms in practice. Traditionally, Spectralis macular scans were acquired using a horizontal acquisition protocol (Figure 1a) [33]. More recently, a vertical macular acquisition protocol has been implemented (Figure 1b) and proposed as potentially favorable due to postulated reduction in confound of segmentation derived metrics from Henle's fiber layer (HFL)[34]. HFL is formed by photoreceptor axons within the outer plexiform layer (OPL). The OPL comprises synapses between the dendrites of horizontal cells in the INL and the fibers of photoreceptors located in the ONL[20]. The first synapses occur approximately $350\mu\text{m}$ from the fovea center, being obliquely centered. In addition, HFL has birefringence properties. Due to these characteristics, the angle at the time of scan acquisition could theoretically lead to changes in HFL identification and accordingly ONL and OPL thickness measurements[35]. Despite the theoretical advantage of vertically acquired macular scans and increasing utilization of this acquisition protocol on Spectralis OCT, the degree to which OCT segmentation derived measures from horizontal and

vertically acquired macular scans agree remains unclear, which has important implications for clinical trial design. The primary objective of this cross-sectional study was to determine the agreement of different retinal layers thickness measurements derived from segmentation of horizontally and vertically acquired Spectralis macular scans.

Materials and methods

Participants.

We recruited 20 healthy controls (HC) among JHU staff and 20 MS patients from the Johns Hopkins Multiple Sclerosis Center. The study was approved by the Institutional Review Board of JHU in accordance with the Declaration of Helsinki and written informed consent was obtained from all study participants. MS diagnosis and subtype classification were confirmed by the treating neurologist, based on the revised McDonald criteria[36]. Individuals with refractive errors of greater than ± 6 diopters, history of ocular surgery, glaucoma, hypertension, diabetes or other neurological or ophthalmological pathology were excluded.

OCT.

Retinal imaging was performed by experienced technicians using Spectralis OCT, software version 6.4.7.0, as described in detail elsewhere[37], [38]. Macular scans were obtained using Posterior Pole Horizontal and Posterior Pole Vertical macular scans protocols. Each of these protocols includes the acquisition of 61 b-scans. The images included in this study had a signal strength >20 dB, an automated real time (ART) for horizontal and vertical macular scan of 9 and 15 respectively and were devoid of artifact, in accordance with the OSCAR-IB criteria[39]. Horizontal and vertical scans from each participant were acquired in random order and consecutively on the same day.

OCT segmentation.

Segmentation of the different layers of the retina was performed using two different validated algorithms - JHU segmentation and Heidelberg segmentation (Heidelberg Eye Explorer 1.9.11), both of which are described in detail elsewhere [16], [17], [18]. JHU segmentation calculates macular RNFL (mRNFL), GCIP (does not identify GCL and IPL separately), INL, OPL, ONL, inner photoreceptor segment (IPS), outer photoreceptor segment (OPS) and retinal pigment epithelium (RPE) thicknesses (Figure 2). Heidelberg segmentation identifies the following macular layers (figure 3): mRNFL, GCL, IPL, INL, OPL, ONL, IPS, OPS and RPE. Since GCL thickness cannot be reliably determined across OCT platforms or OCT segmentation techniques, for consistency, GCL and IPL thicknesses were combined to determine GCIP thickness. Automated retinal layer boundary segmentations from both algorithms were visually examined for all scans and as best as can be determined by visual inspection alone, all retinal layer boundary delineations appeared to be accurate and did not require manual correction. The JHU segmentation area is 5mm, while the Heidelberg segmentation area is 6mm.

Statistical analysis.

Stata version 13 (Stata Corp, College Station, TX) was used for statistical analyses. Bland Altman analyses and intra-class correlation coefficients (ICCs) were used to assess agreement of retinal layer thickness measures derived from segmentation of horizontally and vertically acquired macular OCT scans. Bland Altman plots illustrate differences in measures between techniques (y-axis) against the average of measures from the same techniques (x-axis). Bland Altman limits of agreement (LOA) demonstrate where 95% of the data points of should lie within ± 2 standard deviations of the mean difference. The mean difference is the average difference between the methods assessed[40]. 95% confidence intervals (CIs) of each LOA as well as the 95% CI of the mean difference were calculated. Absolute agreement two-way mixed effects ICCs were utilized. This index has been previously used to compare OCT measures derived from different scanners [14], [18]. If X_a is the measurement on machine a , and X_b is the measurement on machine b , then the inter scanner agreement is defined as follows:

$$1 - \frac{|X_a - X_b|}{\frac{X_a + X_b}{2}}$$

In this study, ICCs were used to assess the consistency of measurements. A higher ICC (maximum: 1.0) represents better agreement. In general, an ICC with a lower limit of the 95% CI >0.75 is considered to be consistent with excellent reproducibility[41]. Each target/participant was rated by the same number of raters, in this case, the horizontal and vertical macular scan protocols, with the rater being a fixed effect and the target a random effect. The two-way mode was utilized in order to measure the differences of interest, with absolute agreement allowing measurement of exact agreement.

Results

The study cohort was comprised of 20 MS patients and 20 HCs. A summary of demographics and clinical characteristics is outlined in Table 1.

Cross-sectional agreement of retinal layer thicknesses derived from horizontal and vertical macular scans calculated using JHU segmentation

Bland-Altman analyses—Similar results between both cohorts were found for all the layers except for the INL and ONL layer.

Results of Bland Altman analyses were similar between the HC and MS cohorts (Figure 3). Overall agreement was similar to that observed when assessing Heidelberg segmentation derived measures.

In both groups, mean differences (difference between the averages obtained by the two methods) were excellent being $<1\mu\text{m}$ for all retinal layers. For example, mean difference in GCIP thickness in HCs and MS patients was $-0.12\mu\text{m}$ and $-0.36\mu\text{m}$ respectively. There was a modest discrepancy in mean differences of INL thickness between HCs and MS patients

(0.3 μ m vs. 0.08 μ m). LOA were generally wide for most retinal layer thicknesses across both cohorts, but particularly for the OPL and ONL layers in MS (>4 μ m for each). The mean differences and pertinent LOA are listed in Table 2.

ICCs—Overall, results were similar in the MS and HC cohorts, with ICCs of retinal layer thicknesses across vertical and horizontal macular scans excellent and generally greater than 0.90 (Table 4). In particular, the ICC for GCIP thickness in HCs and MS patients was 0.99 (95% CI: 0.98–0.995) and 0.99 (95% CI: 0.997–0.999) respectively. On the other hand, ICCs for OPL thickness were generally lower, being 0.85 (95% CI: 0.73–0.92) and 0.71 (95% CI: 0.51–0.83) in HCs and MS patients respectively.

Cross-sectional agreement of retinal layer thicknesses derived from horizontal and vertical macular scans calculated using Heidelberg segmentation

Bland-Altman analyses—Results of Bland Altman analyses were similar between the HC and MS cohorts (Figure 4). Average retinal layer thicknesses are presented in table 3.

In both cohorts, mean differences (measure of agreement at the cohort level) were excellent being <1 μ m for all retinal layers, with the exception of the mRNFL which was close to 1 μ m. For example, mean difference in GCIP thickness in HCs and MS patients was 0.18 μ m and 0.08 μ m respectively. However, the limits of agreement (LOA; measure of agreement at the individual patient level) were generally wide for most retinal layer thicknesses across both cohorts, and in particular for the OPL and ONL layers (>7 μ m for both). The mean differences and their pertinent LOA are listed in Table 3.

ICCs.—Again, results were similar in the MS and HC cohorts, with ICCs of retinal layer thicknesses across vertical and horizontal macular scans excellent and generally greater than 0.9 (Table 4). In particular, it is worth noting that the ICC for GCIP thickness in HCs and MS patients was 0.98 (95% CI: 0.97–0.99) and 0.99 (95% CI: 0.99–0.997) respectively. On the other hand, ICCs for OPL thickness were generally lower, being 0.77 (95% CI: 0.61–0.87) and 0.70 (95% CI: 0.5–0.83) in HCs and MS patients, respectively.

Discussion.

The results of this study reveal excellent agreement at the cohort level of retinal layer thickness measures derived from vertically and horizontally acquired Spectralis OCT macular scans. The cohort wide agreement is supported by the comparable results obtained regardless of which (Heidelberg or JHU) segmentation protocol was utilized. Moreover, no major discrepancies in agreement were noted with similar results obtained across the MS and HC cohorts. The collective study findings of excellent agreement at the cohort level strongly suggest that Spectralis OCT measures derived from segmentation of vertically and horizontally acquired macular scans can be pooled as outcomes in clinical trials. However, the more modest agreement observed at the individual patient level suggest that patients should not be switched from horizontal to vertical macular scanning acquisitions, or vice versa, either during clinical tracking/monitoring or participation in clinical trials.

In terms of interpreting Bland Altman assessments of agreement, quantitative Bland Altman determined mean differences (cohort level agreement) and LOA (individual/subject level agreement) need to be interpreted within the context of the variables being assessed. With respect to agreement in the thickness measures of individual retinal layers derived from segmentation of vertically and horizontally acquired macular scans, mean differences and LOA pertaining to each layer therefore need to be interpreted according to the average thickness of each layer of interest. The results of this cross-sectional comparison show excellent agreement at the cohort level for GCIP, INL, OPL, ONL and RPE layer measurements between vertically and horizontally acquired macular scans, regardless of segmentation technique. Since the mRNFL is relatively thin, mean differences of approximately 1 μ m for mRNFL thicknesses derived from segmentation of vertically and horizontally acquired macular scans (in MS and HCs) with the Heidelberg technique indicates sub-optimal cohort wide agreement for this measurement. Within the context of retinal layer thicknesses, moderate agreement at the individual level for mRNFL, GCIP, INL, and RPE, and poor agreement for OPL and ONL (especially using the Heidelberg technique with which the LOA were over 7 μ m for each of these layers in MS and HCs) thicknesses derived from segmentation of vertically and horizontally acquired macular scans were observed. ICCs generally demonstrated excellent agreement for all layers, consistent with excellent reproducibility. OPL thickness had the lowest ICCs (<0.75) indicating less agreement for this measure. GCIP thickness had the highest ICC across methods and cohorts. These ICCs reinforce and corroborate the primary study findings.

While prior studies have analyzed agreement of retinal thickness measures across OCT platforms[25], [24] and segmentation techniques (including automated, semi-automated and manual methods) [17], there has been a lack of assessment of the agreement between retinal layer thickness measures derived from horizontal and vertical macular scans acquired on Spectralis OCT. In part, this relates to the more recent advent and routine availability of vertical macular acquisition protocols on up-to-date commercially available Spectralis OCT devices and an according shift towards vertical from horizontal macular acquisitions. Moreover, the momentum contributing to the switch from horizontal to vertical macular acquisition protocols partially relates to the proposed benefit of reducing HFL confound upon segmentation measures, particularly of the OPL and ONL layers. In light of this shifting practice it is relevant to determine the agreement between retinal layer thickness measures derived from horizontal and vertical macular scans acquired on Spectralis OCT. This is important not only from a clinical monitoring perspective but also in terms of outcomes in clinical trials, particularly across centers that may be using either horizontal or vertical macular acquisition protocols or considering changing from horizontal to vertical macular acquisition protocols. Our study findings of excellent agreement at the cohort level for almost all retinal layer thickness measures (with the exception of perhaps the mRNFL) and only poor-modest subject level agreement of all retinal layer thickness measures derived from segmentation of horizontally and vertically acquired macular scans suggests measures from both acquisition protocols can be pooled as outcomes in clinical trials. However, patients should continue to remain undergoing imaging using the same consistent imaging acquisition and processing protocols.

This study has several limitations. Agreement between retinal layer thickness measures derived from segmentation of horizontally and vertically acquired Spectralis OCT macular scans was only performed cross-sectionally and not longitudinally, since our center only recently acquired vertical macular acquisition capabilities on our Spectralis OCT device. However, longitudinal agreement should also be assessed in future studies. Our study findings are not generalizable across OCT devices or segmentation techniques, since only two segmentation techniques on the same single OCT platform were assessed. Moreover, our study findings can also not be extended to all disease states, and especially diseases in which morphologic or qualitative abnormalities also occur within the macula, since agreement in the current study was only assessed in HCs and MS patients (in whom retinal neurodegeneration occurs typically without frank morphologic/anatomic distortion). Although further OCT technology, including Swept Source (SS) OCT, have been developed, this study was executed with SD-OCT. The technology of SS-OCT acquires scans with higher speed and provides the ability to obtain clear images of deep ocular structures such as the choroid and lamina cribrosa[42] [43].

In conclusion, this study shows excellent cohort wide agreement across the majority of retinal layer thicknesses (other than the mRNFL) derived from segmentation of horizontally and vertically acquired macular scans obtained on Spectralis OCT, indicating that measures obtained from both acquisition protocols can be pooled for assessing outcome in trials. However, individual or subject level agreement across the majority of retinal layer thicknesses derived from segmentation of horizontally and vertically acquired macular scans obtained on Spectralis OCT was poor-modest. The sub-optimal agreement at the patient level highlights the importance of maintaining consistent acquisition protocols for individual patients.

Acknowledgments

We gratefully acknowledge to Prof. Jerry Prince (Department of Electrical and Computer Engineering, Johns Hopkins University, Baltimore, MD, USA) and his team for providing the Segmentation algorithm used in this study. We thank Norah Cowley for helping with the image adjustments.

Funding

This study was funded by Race to Erase MS (to S.S.), NIH (5R01NS082347-02 [to PAC and SS]), National MS Society (RG-1606-08768 to SS) and Walters Foundation (to EMF, LJB and PAC)

REFERENCES

- [1]. Compston A and Coles A . Multiple sclerosis. Lancet 2008 372; 1502–1517. [PubMed: 18970977]
- [2]. Dutta R and Trapp BD. Mechanisms of neuronal dysfunction and degeneration in multiple sclerosis. Prog. Neurobiol 2011 93; 1–12. [PubMed: 20946934]
- [3]. Van Waesberghe JHTM , Kamphorst W , De Groot CJA , Van Walderveen MAA , Castelijns JA , Ravid R et al. Axonal loss in multiple sclerosis lesions: magnetic resonance imaging insights into substrates of disability. Ann. Neurol 1999 46; 747–754. [PubMed: 10553992]
- [4]. Miller DH , Grossman RI , Reingold SC and McFarland HF . The role of magnetic resonance techniques in understanding and managing multiple sclerosis. Brain 1998 121; 3–24. [PubMed: 9549485]

- [5]. Filippi M, Campi A, Dousset V, Baratti C, Martinelli V, Canal N, Scotti G et al. Magnetization transfer imaging study of normal appearing white matter in multiple sclerosis. *Neurology* 1995 45; 478–482. [PubMed: 7898700]
- [6]. Grazioli E, Zivadinov R, Weinstock-Guttman B, Lincoff N, Baier M, Wong JR et al. Retinal nerve fiber layer thickness is associated with brain MRI outcomes in multiple sclerosis. *J Neurol Sci* 2008 268 (1–2). 12–17. [PubMed: 18054962]
- [7]. Huang D, Swanson EA, Lin CP, Schuman JS, Stinson WG, Chang W et al. Optical coherence tomography. *Science* 1991 254(5035); 1178–1181. [PubMed: 1957169]
- [8]. Mehreen A and Duke JS. Optical coherence tomography – current and future applications. *Curr Opin Ophthalmol* 2013 24 (3); 213–221.
- [9]. Mrejen S and Spaide RF. Optical coherence tomography: Imaging of the choroid and beyond. *Surv. Ophthalmol* 2013 58 (5); 387–429. [PubMed: 23916620]
- [10]. Saidha S, Syc BS, Durbin MK, Eckstein C, Oakley JD, Meyer SA et al. Visual dysfunction in multiple sclerosis correlates better with optical coherence tomography derived estimates of macular ganglion cell layer thickness than peripapillary retinal nerve fiber layer thickness. *Mult. Scler* 2011 17; 1449–1463. [PubMed: 21865411]
- [11]. Syc BC, Saidha S, Newsome S, Ratchford JN, Levy M, Ford ET et al. Optical coherence tomography segmentation reveals ganglion cell layer pathology after optic neuritis. *Brain* 2012 135; 521–533. [PubMed: 22006982]
- [12]. Ratchford JN, Saidha S, Sotirchos ES, Oh JA, Seigo MA, Eckstein C et al. Active MS is associated with accelerated retinal ganglion cell/inner plexiform layer thinning. *Neurology* 2013 80; 47–54. [PubMed: 23267030]
- [13]. Varga BE, Gao W, Laurik KL, Tatrai E, Simo M, Somfai GM et al. Investigating tissue optical properties and texture descriptors of the retina in patients with multiple sclerosis. *Plos one* 2015 10 (11). 1–20.
- [14]. Saidha S and Calabresi PA. Optical coherence tomography should be part of the routine monitoring of patients with multiple sclerosis: Yes. *Mult. Scler* 2014; 1296–1298.
- [15]. Saidha, Al-Louzi O, Ratchford JN, Bhargava P, Oh J, Newsome SD et al. Optical coherence tomography reflects brain atrophy in multiple sclerosis: a four-year study. *Ann. Neurol*, 2016 78; 801–813.
- [16]. Green AJ, McQuaid S., Hauser SL, Allen IV and Lyness R. Ocular pathology in multiple sclerosis: retinal atrophy and inflammation irrespective of disease duration. *Brain* 2010 133; 1591–1601. [PubMed: 20410146]
- [17]. Seigo MA, Sotirchos ES, Newsome S, Babiarz A, Eckstein C, Ford ET et al. In vivo assessment of retinal neuronal layers in multiple sclerosis with manual and automated optical coherence tomography segmentation techniques. *J. Neurol* 2012 259; 2119–2130. [PubMed: 22418995]
- [18]. Kafieh R, Rabbani H. and Kermani S. A Review of algorithms for segmentation of optical coherence tomography from retina. *J. Med. Signals Sens* 2013 3(1). 45–60. [PubMed: 24083137]
- [19]. Tian J, Varga B, Tatrai E, Fanni P, Somfai GM, Smiddy WE et al. Performance evaluation of automated segmentation software on optical coherence tomography volume data. *J Biophotonics* 2016 9 (5). 478–489. [PubMed: 27159849]
- [20]. Kolb H. Simple anatomy of the retina. 2005 May 1 [Updated 2012 Jan 31]. In: Kolb H, Fernandez E, Nelson R, eds. *Webvision: The organization of the retina and visual system* [online book] Salt Lake City (UT): University of Utah Health Sciences Center 1995; 1–24.
- [21]. Lang A, Carass A, Hauser M, Sotirchos ES, Calabresi PA, Ying HS et al. Retinal layer segmentation of macular OCT images using boundary classification. *Biomed. Opt. Express* 2013 4; 518–533.
- [22]. Garcia-martin E, Polo V, Larrosa JM, Marques ML, Herrero R, Martin J et al. Retinal layer segmentation in patients with multiple sclerosis using spectral domain optical coherence tomography. *ophthalmology* 2014 121; 573–579. [PubMed: 24268855]
- [23]. Tian J, Varga B, Somfai GM, Lee W and Smiddy WE. Real-time automatic segmentation of optical coherence tomography volume data of the macular region. *Plos one* 2015 10 (8). 1–20.

- [24]. Bhargava P , Lang A , Al-Louzi O , Carass A , Prince J , Calabresi PA et al. Applying an open-source segmentation algorithm to different oct devices in multiple sclerosis patients and healthy controls: implications for clinical trials. *Mult. Scler. Int* 2015 vol. 2015; 1–10.
- [25]. Wolf-schnurbusch UEK , Ceklic L , Brinkmann CK , Iliev ME , Frey M , Rothenbuehler SP et al. Macular thickness measurements in healthy eyes using six different tomography instruments. *IOVS* 2017 50 (7). 3432–3437.
- [26]. Chiu SJ , Li XT , Nicholas P , Toth CA , Izatt JA and Farsiu S . Automatic segmentation of seven retinal layers in SDOCT images congruent with expert manual segmentation. *Opt Express* 2010 18 (18). 19413–19428. [PubMed: 20940837]
- [27]. Sotirchos ES , Seigo MA , Calabresi PA and Saidha S . Comparison of point estimates and average thicknesses of retinal layers measured using manual optical coherence tomography segmentation for quantification of retinal neurodegeneration in multiple sclerosis. *Curr Eye Res* 2013 38 (1). 224–228. [PubMed: 22954302]
- [28]. de Sisternes L , Jonna G , Moss J , Marmor MF , Leng T , Rubin DL . Automated intraretinal segmentation of SD- OCT images in normal and age-related macular degeneration eyes. *Biomed Opt Express* 2017 8(3). 1178–1181.
- [29]. Oberwahrenbrock T , Weinhold M , Mikolajczak J , Zimmermann H , Paul F , Beckers I et al. Reliability of intra-retinal layer thickness estimates. *Plos one* 2015 10 (15).1–16.
- [30]. Liu X , Shen M , Huang S , Leng L , Zhu D and Lu F . Repeatability and reproducibility of eight macular intra- retinal layer thicknesses determined by an automated segmentation algorithm using two SD-OCT instruments. *Plos one* 2014 9(2).
- [31]. Matlach J , Wagner M , Malzahn U and Winfried G . Repeatability of peripapillary retinal nerve fiber layer and inner retinal thickness among two spectral domain optical coherence tomography devices. *Invest Ophthalmol Vis Sci* 2014 55 (10). 6536–6546. [PubMed: 25228545]
- [32]. Ho J , Sull AC , Vuong LN , Chen Y , Liu J , Fujimoto JG et al. Assessment of artifacts and reproducibility across spectral and time domain optical coherence tomography devices. *Ophthalmology* 2009 116 (10). 1960–1970. [PubMed: 19592109]
- [33]. Menke MN , Dabov S , Knecht P , Sturm V . Reproducibility of retinal thickness measurements in healthy subjects using spectralis optical coherence tomography. *Am. J. Ophthalmol* 2009 147; 467–472. [PubMed: 19026403]
- [34]. Mrejen S , Gallego-Pinazo R , Freund KB , Paques M . Recognition of Henle’s fiber layer on OCT images. *Ophthalmology* 2013 120; 32–34.
- [35]. Lujan BJ , Roorda A , Knighton RW , Carroll J . Revealing Henle’s fiber layer using spectral domain optical coherence tomography. *Invest. Ophthalmol. Vis. Sci* 2011 vol. 52; 1486–92. [PubMed: 21071737]
- [36]. Polman CH , Reingold SC , Banwell B , Clanet M , Cohen JA , Filippi M et al. Diagnostic criteria for multiple sclerosis: 2010 Revisions to the McDonald Criteria. *Ann. Neurol* 2011 69:292–302. [PubMed: 21387374]
- [37]. Syc BS , Warner CV , Hiremath GS , Farrell SH , Ratchford JN , Conger A et al. Reproducibility of high-resolution optical coherence tomography in multiple sclerosis. *Mult. Scler* 2010 16; 829–839. [PubMed: 20530512]
- [38]. Warner CV , Syc SB , Stankiewicz AM , Hiremath G , Farrel SK , Crainiceanu CM et al. The impact of utilizing different optical coherence tomography devices for clinical purposes and in multiple sclerosis trials. *Plos One* 2011 6, issue 8.
- [39]. Tewarie P , Balk L , Costello F , Green A , Martin R , Schippling S et al. The OSCAR-IB consensus criteria for retinal OCT quality assessment. *PLoS One* 20127; issue 4.
- [40]. Giavarina D . Understanding Bland Altman analysis. *Biochem. Medica* 2015 25; 141–151.
- [41]. Lee J , Koh D , Ong CN . Statistical evaluation of agreement between two methods for measuring a quantitative variable. *Comput. Biol. Med* 1989 19; 61–70. [PubMed: 2917462]
- [42]. Miller AR , Roisman L , Zhang Q , Zheng F , Oliveira Dias JF , Yehoshua Z et al. Comparison between spectral-domain and swept-source optical coherence tomography angiographic imaging of choroidal neovascularization. *Invest Ophthalmol Vis Sci* 2017 58 (3). 1499–1505. [PubMed: 28273316]

- [43]. Press D , .Comparison of choroidal thickness measurements between spectral-domain OCT and swept-source OCT in normal and diseased eyes. Clin Ophthalmol 2016 10 2271–2276. [PubMed: 27881909]

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

Figure 1. Spectralis OCT horizontal (**A**) and vertical (**B**) macular scan acquisition protocols (right eye of same MS patient in each panel). Both macular scan protocols acquire 61 b-scans.

Figure 2.

(A) Spectralis macular B scan showing the different layers identified using the JHU segmentation technique: retinal nerve fiber layer (RNFL), ganglion cell inner plexiform layer (GCIP, inner nuclear layer (INL), outer plexiform layer (OPL), outer nuclear layer (ONL), inner photoreceptor segment (IPS), outer photoreceptor segment (OPS), retinal pigment epithelium (RPE) (B) The automated segmentation algorithm from the Heidelberg Eye Explorer segments 11 different retinal boundaries: the inner limiting membrane (ILM), the boundaries between the retinal nerve fiber layer (RNFL) and the ganglion cell layer (GCL), between the GCL and the inner plexiform layer (IPL), between the IPL and the inner nuclear layer (INL), between the INL and the outer plexiform layer (OPL), between the OPL and the outer nuclear layer (ONL), the external limiting membrane (ELM), two photoreceptor layers (PR1/2), the RPE, and the basement membrane (BM) with the underlying choroid.

Figure 3.

Bland Altman plots of cross-sectional comparison in retinal layer thickness measures derived from JHU segmentation of vertically and horizontally acquired Spectralis macular scans of the MS patient cohort. The red lines represent the limits of agreement (**A**) Ganglion cell inner layer (GCIP) (**B**) Inner nuclear layer (INL) (**C**) Outer plexiform layer (OPL) (**D**) Outer nuclear layer (ONL).

Figure 4. Bland Altman plots of cross-sectional comparison in retinal layer thickness measures derived from Heidelberg segmentation of vertically and horizontally acquired Spectralis macular scans of the MS patient cohort. The red lines represent the limits of agreement (**A**) Ganglion cell inner layer (GCIP) (**B**) Inner nuclear layer (INL) (**C**) Outer plexiform layer (OPL) (**D**) Outer nuclear layer (ONL).

Table 1:

Demographics and clinic characteristics of Study Participants

	Healthy control cohort	Multiple sclerosis cohort	p
No. (eyes)	20 (40)	20 (40)	
Mean age (SD)	27.75 (6.46)	42.35 (11.16)	<0.001 ^a
Female (%)	7 (35)	14(70)	0.03 ^b
RRMS (%)	NA	16(80)	
Eyes with previous optic neuritis (%)	NA	11(27.5)	
Disease duration (SD)	NA	9.15 (6.06)	

^aWilcoxon rank-sum test;^bChi2 test; SD= standard deviation; RRMS= relapsing remitting multiple sclerosis

Table 2:

Agreement of retinal layer thickness measures derived from JHU segmentation of horizontally and vertically acquired Spectralis OCT macular scans.

Average thickness(SD)(μm)		Mean difference(95% CI)(μm)		Lower LOA (95% CI)(μm)		Upper LOA (95% CI) (μm)	
HC cohort	MS patient cohort	HC cohort	MS patient cohort	HC cohort	MS patient cohort	HC cohort	MS patient cohort
mRNFL	26.47 (2.2)	23.74 (4.09)	0.14 (-0.15 to 0.43)	0.21 (-0.07 to 0.49)	-1.67 (-2.17 to -1.18)	-1.57 (-2.06 to -1.08)	1.96 (1.46 to 2.45)
GCIP	80.95 (3.98)	69.03 (12.11)	-0.12 (-0.29 to 0.04)	-0.03 (-0.26 to 0.19)	-1.16 (-1.44 to -0.87)	-1.44 (-1.82 to -1.05)	0.91 (0.63 to 1.19)
INL	40.63 (3.04)	39.09 (3.97)	0.3 (0.1 to 0.51)	-0.08 (-0.22 to 0.05)	-0.99 (-1.34 to -0.64)	-0.93 (-1.17 to -0.7)	1.95 (1.6 to 2.31)
OPL	21.02 (1.39)	20.72 (1.31)	-0.34 (-0.59 to -0.09)	-0.36 (-0.69 to -0.02)	-1.92 (-2.36 to -1.49)	-2.48 (-3.06 to -1.9)	1.24 (0.81 to 1.67)
ONL	72.66 (8.01)	69.3 (5.6)	-0.14 (-0.33 to 0.06)	-0.07 (-0.44 to 0.3)	-1.34 (-1.68 to -1.02)	-2.38 (-3.02 to -1.75)	1.07 (0.74 to 1.4)
RPE	36.24 (1.86)	33.82 (2.45)	-0.1 (-0.38 to 0.18)	0.003 (-0.34 to 0.35)	-1.88 (-2.37 to -1.4)	-2.17 (-2.77 to -1.58)	1.68 (1.19 to 2.16)
							2.18 (1.58 to 2.78)

SD =standard deviation; CI =confidence interval); LOA=limit of agreement

mRNFL =macular retinal nerve fiber layer; GCIP=ganglion cell inner plexiform layer; INL=inner nuclear layer; OPL=outer plexiform layer; ONL=outer nuclear layer; RPE=retinal pigment epithelium

Agreement of retinal layer thickness measures derived from Heidelberg segmentation of horizontally and vertically acquired Spectralis OCT macular scans.

Table 3.

	Average thickness(SD)(μ m)		Mean difference(95% CI)(μ m)		Lower LOA (95% CI)(μ m))		Upper LOA (95% CI) (μ m)	
	HC cohort	MS patient cohort	HC cohort	MS patient cohort	HC cohort	MS patient cohort	HC cohort	MS patient cohort
mRNFL	26.6 (2.56)	24.64(4.25)	0.98 (0.61 to 1.35)	0.96 (0.59 to 1.33)	-1.35 (-1.99 to -0.71)	-1.38 (-2.02 to -0.74)	3.32 (2.68 to 3.96)	3.3 (2.66 to 3.95)
GCIP	75.94 (3.8)	64.34 (11.29)	0.18(-0.05 to 0.4)	-0.08 (-0.24 to 0.4)	-1.26 (-1.65 to -0.86)	-1.93 (-2.48 to -1.38)	1.61(1.22 to 2)	2.09 (1.54 to 2.64)
INL	35.28 (2.3)	34.38 (3.39)	0.03 (-0.24 to 0.3)	-0.19 (-0.42 to 0.05)	-1.7 (-2.18 to -1.23)	-1.69 (-2.1 to -1.28)	2.62 (2.14 to 3.09)	2.27 (1.86 to 2.69)
OPL	29.01 (2.67)	28.15 (2.32)	-0.3 (-0.89 to 0.3)	-0.3 (-0.92 to 0.3)	-4.05 (-5.08 to -3.02)	-4.15 (-5.2 to -3.1)	3.46 (2.43 to 4.49)	3.53 (2.48 to 4.58)
ONL	70.41 (8.63)	65.91 (6.76)	0.12 (-0.45 to 0.7)	0.25 (-0.32 to 0.83)	-3.51 (-4.5 to -2.5)	-3.37 (-4.36 to -2.38)	3.75 (2.76 to 4.75)	3.88 (2.88 to 4.87)
RPE	14.59 (0.92)	14.06 (1.4)	-0.38 (-0.55 to -0.21)	-0.32 (-0.49 to -0.14)	-1.45 (-1.74 to -1.15)	-1.41 (-1.71 to -1.11)	0.68 (0.39 to 0.97)	0.78 (0.48 to 1.08)

SD =standard deviation; CI =confidence interval); LOA=limit of agreement

mRNFL =macular retinal nerve fiber layer; GCIP=ganglion cell inner plexiform layer; INL=inner nuclear layer; OPL=outer plexiform layer; ONL=outer nuclear layer; RPE=retinal pigment epithelium

Table 4:

Summary of the intra-class correlation coefficients (ICCs) (95% CI) of retinal layer thickness measures derived from Heidelberg and JHU segmentation of vertically and horizontally acquired Spectralis OCT macular scans.

		mRNFL	GCIP	INL	OPL	ONL	RPE
Heidelberg segmentation	Healthy controls	0.75(0.58;0.86)	0.98(0.97;0.99)	0.93(0.87;0.96)	0.77(0.61;0.87)	0.98(0.96;0.98)	0.85(0.74;0.91)
	MS patients	0.96(0.93;0.989)	0.995(0.99;0.997)	0.98(0.95;0.99)	0.7(0.5;0.83)	0.96(0.93;0.98)	0.92(0.86;0.96)
JHU segmentation	Healthy controls	0.92(0.85;0.95)	0.99(0.98;0.995)	0.98(0.96;0.99)	0.85(0.73;0.92)	0.997(0.994;0.998)	0.89(0.8;0.94)
	MS patients	0.98(0.95;0.99)	0.998(0.997;0.999)	0.99(0.99;0.996)	0.71(0.51;0.83)	0.98(0.96;0.99)	0.9(0.82;0.95)

mRNFL =macular retinal nerve fiber layer; GCIP=ganglion cell inner plexiform layer; INL=inner nuclear layer; OPL=outer plexiform layer; ONL=outer nuclear layer; RPE=retinal pigment epithelium.