Influence of the Eye-Tracking–Based Follow-Up Function in Retinal Nerve Fiber Layer Thickness Using Fourier-Domain Optical Coherence Tomography

Alinne M. C. Costa,[1] *Rogerio A. Costa,*[2,3] *Luiz A. S. Melo Jr,*[2] *Daniela Calucci,*[2] *Juliana L. Orefice,*[2] *and Sebastião Cronemberger*[1]

Purpose. To evaluate the eye-tracking–based follow-up (EBF) function in the reproducibility of the peripapillary retinal nerve fiber layer (RNFL) thickness measurements obtained with Fourier-domain optical coherence tomography (Fd-OCT).

Methods. Thirty healthy subjects were imaged on an Fd-OCT device at the same visit by two examiners. Peripapillary circular scans in "high-speed" (HS) mode with the "automatic real time" (ART) set at 16 and in "high-resolution" (HR) mode with the ART off were obtained without and with the EBF function activated.

Results. Mean (±SD) global RNFL thickness was 105.1 (±9.5) μm on HS mode and 105.4 (±9.6) μm on HR mode. Interobserver analysis for global RNFL thickness revealed an intraclass correlation coefficient (ICC) greater than or equal to 0.96 for all but the HR mode without the use of EBF function (ICC = 0.73). Intraobserver analysis for global RNFL thickness revealed an ICC greater than 0.98 for all but the HR mode without the use of EBF function (ICC = 0.86). The interobserver and intraobserver analyses revealed the lowest ICC values for the temporal region on both HS and HR modes. Higher ICC values were obtained with the HS mode and when the EBF function was activated, particularly when using the HR mode.

Conclusions. The EBF function had no influence in the reproducibility of the global peripapillary RNFL thickness measurements in healthy subjects on HS mode with ART on. However, reproducibility of the global RNFL thickness measurements on HR mode as well as of the temporal and temporal superior regions in both HS and HR modes was greater with the EBF function. (*Invest Ophthalmol Vis Sci.* 2013;54:1958–1963) DOI:10.1167/iovs.12-10884

From the [1]Department of Ophthalmology, Faculdade de Medicina, Universidade Federal de Minas Gerais, Belo Horizonte, Minas Gerais, Brazil; [2]Division of Macula, Centro Brasileiro de Ciências Visuais, Belo Horizonte, Minas Gerais, Brazil; and [3]Department of Ophthalmology, Otolaryngology and Head and Neck Surgery, Faculdade de Medicina de Ribeirão Preto, Universidade de São Paulo, Ribeirão Preto, São Paulo, Brazil.

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Corresponding author: Alinne M. C. Costa, Rua Pernambuco 881 apto 801, Belo Horizonte, MG 30.130-151, Brazil; alinnecamargos@gmail.com.

Retinal nerve fiber layer (RNFL) evaluation in glaucoma has been recorded as early as 1972, when Hoyt and Newman described RNFL atrophy in patients with glaucoma and suggested RNFL thinning as a possible sensitive indicator of glaucomatous damage.[1,2] Severe loss of retinal ganglion cells, in general associated with advanced disease, causes dark slits or grooves among arcuate bundles approaching the optic disc that may be identified on careful clinical examination. Notwithstanding, early disease often produces subtle attenuation of light reflectance from RNFL, which can be overlooked on clinical examination, being highly dependent on the experience of the observer.[3]

Optical coherence tomography (OCT) is a noninvasive cross-sectional imaging technique of the human retina that enables quite reliable and reproducible RNFL measurements in vivo.[4,5] First generations of OCT used time-domain technology for image acquisition.[5,6] In this technology, a beam of light is scanned across the tissue sample and reflected from a reference mirror, which is positioned at a known distance from each retinal layer.[1,6] By comparing the two light beams, time-domain OCT measures the optical backscattering of light to generate a cross-sectional image of the tested tissue.[6] Significant progress in the field of OCT retinal imaging has occurred in the past decade, such as the development and implementation of a novel high-speed OCT technique called spectral OCT.[7–9] Spectral OCT is based on Fourier-domain (Fd) detection, which significantly improves the speed and sensitivity of OCT instruments.[10–13] Moreover, the application of new broad bandwidth light sources to this new spectral OCT technology has enabled the combination of high-speed imaging with superior axial resolution. These advances have been associated with an overall improvement of the capability of OCT systems to resolve individual retinal layers in vivo.

Several Fd-OCT devices have been made commercially available in the past few years. One such device is Spectralis OCT (Heidelberg Engineering, Inc., Heidelberg, Germany). This Fd-OCT instrument, in addition to high-speed (HS), high-resolution (HR) retinal imaging capability, features an eye-tracking system that corrects for eye movements during the scanning process.[14,15] This particular Spectralis feature enables the acquisition of B-scans of superior quality by means of real averaging (Automatic Real Time [ART] software tool) as well as true follow-up imaging of previously scanned fundus regions (which is automatically acquired after selection of the baseline scanned region by the examiner).

The purpose of this study was to evaluate the influence of the eye-tracking–based follow-up (EBF) function in the reproducibility of the peripapillary RNFL thickness measurements in healthy subjects obtained with Fd-OCT.

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METHODS

Thirty-two healthy volunteers were prospectively recruited within the staff members of the Faculdade de Medicina, Universidade Federal de Minas Gerais, and of the Centro Brasileiro de Ciências Visuais, Belo Horizonte, Minas Gerais, Brazil. The research followed the tenets of the Declaration of Helsinki and was approved by the local institutional review board (#ETIC103/08). A written informed consent was obtained from the participants before their inclusion in the study, and after explanation of the nature and possible consequences of the study.

The eligibility criteria included subjects aged between 18 and 60 years old and who had a best-corrected visual acuity (BCVA) equal to or better than 20/25, a spherical equivalent refractive error limited to ±2.00 diopters, and had no clinical evidence of retinal or optic nerve eye disease (see below). Eligible subjects received a detailed ophthalmologic examination, including measurement of BCVA according to a standardized refraction protocol using a retroilluminated Lighthouse for the Blind distance visual acuity test chart, slit lamp biomicroscopy, applanation tonometry, and dilated biomicroscopic and indirect fundus examination. Standard automated perimetry was also performed (Humphrey field analyzer; Carl Zeiss Meditec, Dublin, CA) using Swedish interactive threshold algorithm (SITA) standard test, program Central 24-2. Subjects were considered to have no retinal or optic nerve disease if they had no history of either ocular or neurologic disease or surgery, had a reliable and normal SITA standard test (<33% fixation losses, false-positives, false-negatives, glaucoma hemifield test results within normal limits, and mean and pattern SD with a probability level of more than 5%), had IOPs less than 21 mm Hg, and had no evidence of any optic nerve or retinal disease based on binocular biomicroscopic and indirect fundus examination performed by two different retinal specialists (RAC, JLO) and a glaucoma specialist (AMC).

For those subjects meeting the eligible criteria, 30° and 20° stereoscopic pairs of the optic disc as well as of the macula were obtained on both blue-light reflectance and near-infrared reflectance. Blue-light autofluorescence of the optic disc and macula was also documented. For all the above photographic documentation, the HR mode with the ART mean module set at 25 frames and "normalized" function activated was utilized.

Fd-OCT Scanning Protocol

All subjects underwent Fd-OCT imaging on a commercially available device (Spectralis HRA+OCT; Heidelberg Engineering, Inc., Heidelberg, Germany) 30 minutes after instillation of mydriatic (1% tropicamide) eyedrops. This Fd-OCT instrument features a dual-beam active eye-tracking technology (coined TruTrack) that simultaneously images the eye with two beams of light.[16] One beam maps more than 1000 points of the retina and creates a detailed retinal map each time a patient is imaged. Using the mapped image as a reference, the second beam is directed to the desired location despite blinks or saccadic eye movements. This mapped retinal image also serves as the base to automatic placement of follow-up scans in precisely the same location as the baseline scan (coined AutoRescan feature).[16] Once a scan is marked for follow-up (i.e., "baseline"), the AutoRescan tool automatically finds the desired location in subsequent examinations, thus eliminating subjective operator placement.

Initially, the first examiner (DC) used the built-in scan acquisition function named "circular" to acquire two peripapillary B-scans (first B-scan: HS mode, ART set at 16; second B-scan: HR mode, ART function off) in a circular (12° [approximately 3.4 mm] in diameter) pattern centered at the optic disc. Both peripapillary circular B-scans were then selected for automatic follow-up scanning. At the end of the first scanning session, subjects were asked to leave the Fd-OCT examining room. After 5 to 10 minutes, subjects were brought back to the examining room and a second Fd-OCT scanning session was performed by the same (first) examiner. For the second session, the built-in scan acquisition function "circular" was used to acquire four additional peripapillary B-scans in a circular (12° [approximately 3.4 mm] in diameter) pattern centered at the optic disc; two B-scans with the EBF function off (first B-scan: HS mode, ART set at 16; second B-scan: HR mode, ART function deactivated), and the other two with the EBF function activated (third B-scan: HS mode, ART set at 16; fourth B-scan: HR mode, ART function deactivated). Subjects were then asked to leave the Fd-OCT examining room again. After 30 minutes, the same Fd-OCT imaging procedures performed by the first examiner (DC) were carried out by the second examiner (AMC). For all the above Fd-OCT imaging procedures, internal fixation (set to "nasal" position) was used. Both eyes of each subject were imaged on Fd-OCT, provided that no evidence of retinal or optic nerve disease was present in both eyes.

Validation and Analysis of Fd-OCT Data

The Fd-OCT data were processed by the built-in RNFL segmentation algorithm (software serial number H2E-10,488-002-002; HRA/Spectralis viewing module version 5.4.6.0, HEYEX version 1.7.0.0; Heidelberg Engineering). The RNFL algorithm determines automatically the inner and outer boundary of the RNFL along the circular peripapillary B-scan in micrometers. Initially, each circular B-scan was displayed separately using the "Thickness Profile–Layer RNFL" function to verify the automatic RNFL delineation/segmentation, as well as its quality score (signal-to-noise ratio [SNR]) and the position in respect to the optic disc. If any B-scan of the series presented an apparent error of the automatic RNFL delineation or an SNR value of less than 15 (as suggested by the manufacturer), or was poorly centered at the optic disc, the patient was excluded.

The built-in analysis software plots the RNFL thickness (average values) in a pie chart diagram representing six regions of the optic disc (temporal superior [TS], temporal [T], temporal inferior [TI], nasal superior [NS], nasal [N], and nasal inferior [NI]), together with an average overall RNFL thickness (global [G]).

Statistical Analysis

The statistical package PASW Statistics 18 (SPSS, Inc., Chicago, IL) was used to analyze the data. Only the measurements obtained from the right eyes of the subjects were used in the analysis. Intraclass correlation coefficient (ICC) and repeatability coefficient (RC) were calculated to evaluate the measurement error.[17] For interobserver reproducibility, measurements obtained from the first (i.e., baseline) scan as well as follow-up scans with and without EBF performed by each observer on the same subject were used. For intraobserver reproducibility, measurements from the scans performed on the same subject by the same observer were analyzed. To evaluate the influence of the quality of the scans on the reproducibility, the Spearman rank correlation test was performed to obtain the correlation between SNR (quality score) and interobserver as well as intraobserver (measurements taken by one of the examiners [DC]) reproducibility (within-subject SD was used as measurement of reproducibility).

RESULTS

Thirty-two subjects were examined. Two subjects had to be excluded because an apparent error of the automatic RNFL delineation was observed in at least one B-scan during the validation process. The remaining 30 subjects were included in the study. There were 19 (63.3%) females and 11 (36.7%) males with a mean (±SD) age of 37.4 (±11.2) years (range: 19–60 years). Six peripapillary B-scans per patient (only right eye) per examiner were selected: three acquired in HS mode with the ART set at 16, and three acquired in HR mode with the ART function off. For all peripapillary B-scans acquired in HS mode and ART set at 16, 7.8% (14 of 180 B-scans) presented an ART value different from 16 (seven B-scans per examiner). With

respect to the influence of the quality score, there was no significant correlation between SNR values and reproducibility (see Supplementary Material and Supplementary Table S1, http://www.iovs.org/lookup/suppl/doi:10.1167/iovs.12-10884/-/DCSupplemental).

The mean RNFL thickness measurements for global as well as peripapillary regions on Fd-OCT are shown in Table 1. The mean global peripapillary RNFL thickness was 105.1 μm on HS mode and 105.4 μm on HR mode. The mean peripapillary RNFL thickness for individual regions on HS and HR modes, respectively, was 145.1 μm and 146.6 μm (TS), 71.7 μm and 72.9 μm (T), 155.3 μm and 154.8 μm (TI), 110.3 μm and 109.9 μm (NS), 81.8 μm and 80.5 μm (N), and 122.1 μm and 124.1 μm (NI). Interobserver and intraobserver analyses are shown in Tables 2 and 3. Interobserver analysis for global RNFL thickness revealed an ICC greater than or equal to 0.96 for all but the HR mode without the use of EBF function (ICC = 0.73). Intraobserver analysis for global RNFL thickness revealed an ICC greater than or equal to 0.98 for all but the HR mode without the use of EBF function (ICC = 0.86). For separate peripapillary regions, in general the interobserver and intraobserver analysis revealed the lowest ICC values for the temporal region on both HS and HR modes. Higher ICC values were generally obtained with the HS mode and when the EBF function was activated, especially when considering scans acquired in the HR mode (Tables 2, 3).

Discussion

OCT is a noninvasive technology capable of producing high-resolution cross-sectional images of the retina, which enables the assessment of the peripapillary RNFL and macular retinal thicknesses in micrometric scale.[18,19] Fd-OCT represents the latest commercially available generation of OCT. In addition to HS and HR retinal imaging capability, the Fd-OCT instrument used in the present study features an eye-tracking system (EBF function) that automatically aligns the patient's current fundus image with a preselected reference image.[14] This particular EBF function enables active follow-up imaging of previously scanned fundus regions. In theory, this virtually true automatic alignment carries the potential to minimize bias related to mispositioning of the scans in subsequent Fd-OCT examinations. Given that longitudinal quantitative assessment of RNFL thickness may be a good indicator of glaucomatous damage,[20,21] this particular Fd-OCT feature would be useful in the diagnosis and management of glaucoma.

From a clinical perspective, the reproducibility of any new technology must be verified, as it influences the diagnostic accuracy and the ability to monitor disease progression.[22] The reproducibility values (i.e., ICC) for peripapillary (global and per region) RNFL thickness measurements using some of the commercially available Fd-OCT instruments (Cirrus HD-OCT; Carl Zeiss Meditec, Inc.; 3D OCT-1000; Topcon, Tokyo, Japan; and RTVue OCT; Optovue Corporation, Fremont, CA) ranged from 0.74 to 0.99.[22–26] Two studies have also investigated the reproducibility of Spectralis for peripapillary RNFL thickness measurements performed by a single examiner.[27,28] In the study by Langenegger et al.,[27] the reproducibility values (i.e., ICC) for peripapillary RNFL thickness measurements in healthy subjects using the Spectralis device *without* the EBF function were 0.98 (global), 0.86 (TS), 0.83 (T), 0.91 (TI), 0.91 (NI), 0.83 (N), and 0.91 (NS). With the EBF function activated, ICC values increase to 0.99 in all but TI (0.97) and NI (0.98) regions.[27] Wu et al.[28] also obtained similar results about the reproducibility of Spectralis with the EBF function activated for peripapillary RNFL measurements, with ICC values ranging from 0.97 (T) to 0.99 (global, NS, and NI). The singularity of the present study relies on the evaluation of the Spectralis reproducibility for peripapillary RNFL thickness measurements obtained by two different examiners with the use of two distinct acquisition modes (HS and HR), thus providing supplementary information about the use of this Fd-OCT instrument for this purpose. Our results showed that interobserver reproducibility (with and without the EBF function) of the peripapillary RNFL thickness measurements on HS mode was very good for all (ICC > 0.80) but the temporal region, which was good. Interestingly, the ICC values for interobserver reproducibility on HR mode were generally lower than those on HS mode. The intraobserver ICC values for peripapillary RNFL thickness measurements on HS mode with the EBF function activated were greater than or equal to 0.98 in all but TS (0.97) and T (0.93) regions. Serbecic et al.[29] compared the detection of RNFL measurements using either HS or HR mode and found the highest coefficient of variation in the temporal sector with no significant differences between the modes of image acquisition. The high reproducibility of peripapillary RNFL thickness measurements with the Spectralis can be attributable to different technical factors, such as improved image resolution, imaging speed (40,000 A-scans/s) or retinal segmentation algorithms. However, given the results of the current study, it is likely that its improved reproducibility has also been positively influenced by the EBF function.

Table 1. Peripapillary RNFL Thickness in Right Eyes of Healthy Subjects Obtained at First Scanning Session (Baseline) Using Fd-OCT (Circular, 12° [~3.4 mm] in Diameter B-Scan Centered at the Optic Disc) in HS and in HR Modes

Mode	Region	Mean	SD	Minimum	Maximum
HS (ART set at 16)	Global	105.1	9.5	88.0	130.5
	Temporal superior	145.1	14.7	104.0	170.0
	Temporal	71.7	6.9	55.5	86.0
	Temporal inferior	155.3	17.9	112.5	189.5
	Nasal superior	110.3	19.6	77.5	171.0
	Nasal	81.8	13.9	48.5	117.0
	Nasal inferior	122.1	25.8	68.0	169.0
HR (ART function off)	Global	105.4	9.6	88.5	129.5
	Temporal superior	146.6	15.3	102.5	175.5
	Temporal	72.9	6.5	60.5	84.5
	Temporal inferior	154.8	18.1	118.0	191.5
	Nasal superior	109.9	21.3	73.0	172.5
	Nasal	80.5	13.0	50.0	116.0
	Nasal inferior	124.1	25.8	66.5	161.0

Data are expressed in micrometers.

Table 2. ICC and RC (with and without the EBF Function) for RNFL Measurements in Right Eyes of Healthy Subjects Using Fd-OCT (Circular, 12° [~3.4 mm] in Diameter B-Scan, HS Mode, ART Set at 16, and Centered at the Optic Disc)

HS Mode	Interobserver						Intraobserver			
	Baseline Scan		Without EBF*		EBF Activated†		Without EBF*		EBF Activated†	
Region	ICC	RC	ICC	RC	ICC	RC	ICC	RC	ICC	RC
Global	0.98	3.4	0.98	3.5	0.97	4.8	0.98	4.1	0.99	3.0
Temporal superior	0.91	12.8	0.88	14.6	0.91	12.4	0.87	15.3	0.97	6.8
Temporal	0.74	10.4	0.75	10.3	0.78	9.7	0.75	10.3	0.93	5.7
Temporal inferior	0.97	8.9	0.95	11.2	0.94	12.4	0.95	11.5	0.98	7.8
Nasal superior	0.94	13.9	0.94	14.4	0.91	16.9	0.95	12.5	0.99	7.0
Nasal	0.92	10.8	0.96	7.9	0.93	10.3	0.96	8.3	0.99	3.4
Nasal inferior	0.96	13.9	0.97	12.9	0.96	15.2	0.96	14.5	0.99	7.1

RC is expressed in micrometers.
* Without using information from previous scan (baseline).
† Using information from previous scan (baseline).

Moreover, the overall greater ICC values obtained for HS scanning may be related, at least in part, to the fact that the ART tool was not used during HR scanning.

In the current study, the intraobserver reproducibility for peripapillary RNFL measurements in normal eyes was consistently greater with the EBF function activated (Tables 2, 3). Similar observations have been recently reported in eyes with glaucoma imaged with Spectralis Fd-OCT.[27,28] An excellent reproducibility of RNFL measurements was observed for both glaucomatous and normal eyes, which improved significantly when the EBF function (i.e., AutoRescan) was activated. The gain of reproducibility with the EBF activated was significantly greater in glaucomatous eyes than in normal eyes.[27,28] In addition, one should bear in mind that, in the current study, all scans were acquired under mydriasis, and RNFL measurements before and after mydriasis might not be interchangeable for some Fd-OCT devices.[30,31]

Whereas other commercially available Fd-OCT instruments provide an axial resolution and scanning speed comparable to the one used in the current study and may also include a "test–retest" software function, the Spectralis was the first Fd-OCT to integrate active real-time eye tracking (EBF function). This dual–beam eye-tracking technology mitigates eye motion artifacts and ensures point-to-point correlations between OCT and fundus images without postprocessing of the data. Moreover, the EBF function (i.e., AutoRescan tool) automatically finds the desired location, thus eliminating subjective operator placement and increasing clinician ability to observe "true" change over time rather than change resulting from alignment error. The precision of this tool for macular thickness measurements was documented by Wolf-Schnurrbusch et al.,[32] who showed that the Spectralis system offered 1-μm measurement reproducibility.

In the present study, the lowest ICC values for peripapillary RNFL measurements were generally observed for the temporal and temporal superior regions, and without the EBF function. Rather similar intraobserver (one examiner only) results were obtained by Langenegger et al.,[27] with the lowest ICC values also observed for the temporal (0.83) and temporal superior (0.86) regions, and without the EBF function. In both studies, however, ICC values increase to greater than or equal to 0.93 in each region with the activation of the EBF function. The actual reasons for the greater variability in the temporal region in OCT studies remain unknown. One possible explanation for the several studies that showed higher variability in the temporal and/or nasal regions might be due to normal anatomy, in that the temporal and nasal RNFL are usually thinner than the superior and inferior RNFL.[33]

Past studies have related threshold values for reduction in peripapillary RNFL thickness that were associated with clinically relevant glaucomatous structural changes.[34,35] Lee et al.[34] reported a decrease of 4.3 (±6.5) μm in global peripapillary RNFL thickness between the baseline and follow-up examinations in patients with progressive RNFL atrophy. In monkeys, the response to exposure to elevated IOP varied widely throughout the group, with an overall trend of RNFL

Table 3. ICC and RC (with and without the EBF Function) for RNFL Measurements in Right Eyes of Healthy Subjects Using Fd-OCT (Circular, 12° [~3.4 mm] in Diameter B-Scan, HR Mode, ART Function Off, and Centered at the Optic Disc)

HR Mode	Interobserver						Intraobserver			
	Baseline Scan		Without EBF*		EBF Activated†		Without EBF*		EBF Activated†	
Region	ICC	RC	ICC	RC	ICC	RC	ICC	RC	ICC	RC
Global	0.97	4.8	0.73	14.5	0.96	5.5	0.86	10.7	0.99	3.2
Temporal superior	0.83	18.2	0.56	32.9	0.74	22.1	0.71	25.3	0.96	8.5
Temporal	0.75	9.5	0.43	15.7	0.52	13.6	0.66	11.3	0.88	6.7
Temporal inferior	0.81	22.9	0.73	27.4	0.88	18.0	0.80	24.2	0.95	12.2
Nasal superior	0.86	22.7	0.65	35.8	0.83	25.9	0.79	28.6	0.93	15.8
Nasal	0.89	12.4	0.61	22.6	0.55	29.9	0.71	20.6	0.84	19.0
Nasal inferior	0.91	21.7	0.82	33.3	0.92	21.2	0.89	25.0	0.98	10.4

RC is expressed in micrometers.
* Without using information from previous scan (baseline).
† Using information from previous scan (baseline).

thinning at the rate of 3.77 μm per week.[33] In the current study, the ICC (and RC) values for interobserver (baseline, without and with the EBF function) and intraobserver (without and with the EBF function) for the global peripapillary RNFL thickness measurements were 0.98 (3.4 μm), 0.98 (3.5 μm), 0.97 (4.8 μm), 0.98 (4.1 μm), and 0.99 (3.0 μm), respectively. In this sense, it is reasonable to assume that the Fd-OCT device used in the current study carries potential for the longitudinal evaluation of glaucomatous RNFL thinning, independent of the use of the EBF function, as long the HS mode is selected and the ART is set at 16.

In conclusion, we evaluated the influence of the EBF function in the reproducibility of the peripapillary RNFL thickness measurements in healthy subjects obtained with Fd-OCT. Our results suggest that the Fd-OCT device used in this study provides excellent reproducibility of peripapillary RNFL thickness measurements on HS mode with ART mode on (with and without EBF function), and HR with EBF function. In HS mode with ART mode enabled, the EBF function had no significant influence in the reproducibility of the global peripapillary RNFL thickness measurements in healthy subjects. However, the reproducibility of the peripapillary RNFL thickness measurements on HS mode in the temporal and temporal superior regions, as well as for practically all measurements on HR mode, was greater when the EBF function was activated. Additional studies are needed to evaluate the clinical relevance of our findings and possible influence of the EBF function in the detection and monitoring of glaucoma and other optic neuropathies.

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