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Reproducibility and repeatability of optical coherence tomography imaging of the optic nerve head in normal Beagle eyes

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Abstract

Objective—To assess the reproducibility and repeatability of an optical coherence tomography (OCT) device for imaging the optic nerve region of normal canines.

Animals studied—Twelve clinically healthy Beagles.

Procedures—All animals were anesthetized and an OCT device was used to image the optic disc region. Total disc area and retinal nerve fiber layer (NFL) thickness in eight segments were obtained from each image. Images were collected by two operators in succession and each operator took five scans, repositioning the device between measurements. B-scan segmentations and disc outlines were subsequently redrawn to obtain manual measurements, allowing for comparisons between automated measurements.

Results—Operator 1 had a mean ($\pm$ SD) NFL thickness (automatic) and disc area of $73.38 \pm 7.41 \mu\text{m}$ and $3.69 \pm 0.52 \text{ mm}^2$, while operator 2 had $74.27 \pm 7.33 \mu\text{m}$ and $3.67 \pm 0.51 \text{ mm}^2$, respectively. With manual corrections, operator 1 had a NFL thickness and disc area of $86.19 \pm 8.26 \mu\text{m}$ and $3.74 \pm 0.68 \text{ mm}^2$ while operator 2 had $86.85 \pm 6.91 \mu\text{m}$ and $3.81 \pm 0.56 \text{ mm}^2$, respectively. Operators did not obtain significantly different values for any region. Intra-class correlation ranged from 0.33–0.97 (average 0.78). Coefficient of variation ranged from 8.8–36.2%.

Conclusion—The OCT device demonstrated high inter-rater reliability. Intra-rater reliability was strong for disc area and total NFL; however the segmental regions demonstrated a higher degree of variability. Manual and automated measurements were significantly different for total NFL and some segmental regions therefore users should consistently use one method for longitudinal studies.

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Keywords

optical coherence tomography; optic nerve; canine; nerve fiber layer

Introduction

Optical coherence tomography (OCT) is a diagnostic tool that is able to obtain *in vivo* images of the anterior and posterior segments of the eye using a non-invasive and non-contact approach. Optical coherence tomography systems have become increasingly prevalent in human medical practices and are commonly employed to diagnose and monitor various retinal conditions including retinal detachment, macular degeneration, retinal atrophy, glaucoma, choroidal neovascularization, diabetic retinopathy, uveitis, vascular occlusive disease, and macular hole. (1)

Widespread use within veterinary medicine has been limited thus far, likely due to high equipment costs. However, less expensive models are becoming increasingly available, making the use of OCT in a veterinary setting more feasible. In veterinary medicine, OCT could be used to identify specific disease states, monitor the progression of disease and in assessment of the response to specific therapeutic interventions. Prior to its routine use, the validity of this imaging modality and its associated algorithms needs to be proven in species commonly seen in veterinary practice. While various OCT machines have been shown to produce highly repeatable and reproducible retinal thickness and optic nerve head measurements in physician based medicine, algorithms employed to generate these parameters are designed for use in humans. (2–5) Although many characteristics of the eye are similar across species, differences exist which may affect the ability of the software to capture and analyze images appropriately. (6) Such differences include variations in the size and shape of the globe and optic disc, variations in the photoreceptors, the presence of a tapetum lucidum, and the degree of myelination present within the NFL and/or optic nerve head. In a recent study, Hernandez-Merino et al. suggested that approximately 40% of the nerve fiber layer (NFL) was inadequately delineated by the algorithm employed for generating automatic thickness measurements in canine eyes. (7)

Several studies have successfully imaged the cornea of various species, including dogs and cats. (8,9) A study done by Alario et al. specifically assessed measurements of the anterior segment in dogs and found them to have very high repeatability and reproducibility (intra-class correlation of 0.975 and coefficient of variation 0.64–1.7%). (10) Additionally, studies that imaged the posterior segment in companion animals showed good proof of principle, however the equivalent of human studies calculating reproducibility and repeatability have not been conducted thus far. (7, 9, 11, 12, 13)

Glaucoma is a leading cause of blindness in dogs which results in the initial swelling of the optic disc and subsequent retinal neuronal damage and thinning of the nerve fiber layer. (5, 14) While thinning of the nerve fiber layer occurs throughout the entire retina, it is often localized. Since nerve fiber bundles all lead to the optic nerve head, imaging in the peripapillary area allows for the detection of changes that have occurred throughout the retina. (2) Therefore, the optic nerve head region was chosen for analysis in this study.

Glaucoma in humans can often be detected based on changes in disc shape and disc cup to area ratio. (6) However, in dogs, glaucoma often has to be very severe before fundusoscopic changes can be detected due to the degree of myelination present on the canine optic disc. (15) Furthermore, dogs have been reported to have highly variable shapes of their optic nerve heads, which could increase the difficulty in identifying abnormalities. Therefore, glaucoma in veterinary patients is often not recognized until more aggressive clinical signs are present and there is accompanying ocular pain. (12) Optical coherence tomography would allow veterinary ophthalmologists to have a more objective means of assessing the nerve fiber layer, documenting thinning in early stage glaucoma before clinically detectable vision loss has occurred. (16) This is particularly important as nerve fiber layer loss is irreversible once it has occurred. Furthermore, detection of changes over time in a glaucoma patient receiving medication might warrant alterations in treatments, translating into better vision preservation.

This study aims to describe the healthy canine optic nerve head and its associated nerve fiber thickness layer map, as well as establish the inter- and intra-rater reliability using a spectral domain optical coherence tomography (SD-OCT) device. Data generated herein could provide veterinarians confidence that this technology is appropriate for use in longitudinal studies and for monitoring disease progression.

Materials and Methods

Animals

Prior to enrollment in the study, 12 Beagles were given complete physical examinations by a veterinarian (CGP) to assess their general health. They also received a complete ophthalmic examination (CGP) which included Schirmer tear test I (Eagle Vision; Memphis, TN), fluorescein staining (Ful-Glo; Akorn Inc., LakeForest, IL), slit lamp biomicroscopy (Kowa SL – 15 Portable Slit-Lamp Biomicroscope; Kowa Co., Ltd, Tokyo, Japan), applanation tonometry (Tono-Pen Vet; iCare, Vantaa, Finland), and indirect ophthalmoscopy (Welch Allyn binocular indirect ophthalmoscope; Welch Allyn Distributors), to determine all were free of ocular disease. All procedures were in accordance with Tufts University Animal Care and Use Committee guidelines.

Anesthesia

Animals were anesthetized to reduce motion artifact and facilitate ease and quality of image acquisition. Twenty minutes prior to induction, all dogs were pre-medicated with butorphanol (0.2 mg/kg IM, Torbugesic; Pfizer, New York, NY). At this time, they also received one drop of topical tropicamide 1% ophthalmic solution (Tropicamide; Akorn Inc., LakeForest, IL) to dilate the pupil and aid in illumination of the retina during imaging. A 22 gauge sterile catheter was placed within the cephalic vein. All dogs were subsequently anesthetized with propofol (4 mg/kg IV, PropoFlow; Baxter Healthcare, Deerfield, IL). Once anesthetized, animals were intubated and received supplemental oxygen and isoflurane (0.5–2.5%). The percentage of isoflurane was adjusted throughout the procedure to achieve a safe and constant plane of anesthesia. All dogs were continuously monitored during the procedure, which included heart rate, respiratory rate, temperature, pulse oximetry and blood

pressure. Dogs were kept on a heating pad throughout the procedure to maintain their core body temperature.

Procedure

Dogs were placed in sternal recumbancy and a suction-pad body conformer (Vacu-positioner; Shor-Line, Kansas City, KS) was placed beneath the head to assist in appropriate head position. The eyelids were held open with a Barraquer lid speculum. A single stay suture was placed in the lateral bulbar conjunctiva using 5-0 nylon suture and gently anchored to the surgical table, providing globe centration. The cornea was continuously moistened with balanced salt solution (BSS; Alcon Laboratories Inc., Fort Worth, TX) between scans to prevent desiccation and maintain good image quality. The Optovue iVue OCT (iVue; Optovue Inc., Fremont, CA) was used for image acquisition. The OCT device remained in a table mounted position during imaging. Two operators (one novice and one experienced) each took 5 glaucoma optic nerve head scans (consisting of 13 concentric rings and 12 radial line scans, total of 13,812 A-scans per scan) and 5 glaucoma 3D scans (Fig. 1; 65,536 data points per scan) of each eye for a total of 40 scans per dog. In between each scan, the OCT device was drawn away from the globe, repositioned, and the re-centered onto the optic nerve head, as a means to simulate multiple follow-up image acquisitions and determine reproducibility.

Analysis

The first 3D glaucoma scan obtained was set as the baseline, and all scans acquired had quality index scores greater than 27 as recommended by the manufacturer. The glaucoma scan centers a 3.45 mm circle over the optic nerve head. The software calculates total retinal thickness and disc area and produces a retinal NFL thickness map in 8 sections: superior temporal (ST), temporal upper (TU), temporal lower (TL), inferior temporal (IT), inferior nasal (IN), nasal lower (NL), nasal upper (NU), superior nasal (SN). The 5 scans obtained were averaged to acquire automated NFL regional values for each eye of each dog for each operator. Manual adjustments were subsequently made to 3 out of the 5 scans for comparative purposes. The disc boundaries were redrawn in the 3-D view based on the onset of the retinal pigment epithelium. The segmentation lines were manually redrawn on all of the B scans to modify the NFL values. (Fig. 2) Intra-class correlation coefficient (ICC) and coefficients of variation $\times 100$ (CV) were determined for both automated and manual values. A mixed effects model was used to examine relationships between retinal NFL thickness and optic disc area with sex, globe, replicate, region, and manual/automated status. A P value <0.05 was considered statistically significant. All statistics were performed using a commercially available program. (SPSS v22.0; IBM Corporation, Armonk, NY).

Results

Twelve Beagles (24 eyes) with a mean ($\pm$ standard deviation; SD) age of 3.63 ± 0.4 years were included in this study. The study population consisted of 7 females and 5 males. Mean combined image quality score of all scans obtained was 76 ± 12 (all were greater than 27). Quality score was weakly but significantly correlated with disc area ($r = -0.350$, $p = 0.015$) and disc area was weakly correlated with overall NFL thickness ($r = 0.478$, $p < 0.001$).

Operator 1 had a mean ($\pm$ SD; N = 120) NFL thickness (automatic) of $73.38 \pm 7.41 \mu\text{m}$ and operator 2 had a mean of $74.27 \pm 7.33 \mu\text{m}$. The mean total optic nerve head disc area was $3.69 \pm 0.52 \text{ mm}^2$ for operator 1 and $3.67 \pm 0.51 \text{ mm}^2$ for operator 2. With manual corrections (N=72), operator 1 had an NFL of $86.19 \pm 8.26 \mu\text{m}$ and disc area of $3.74 \pm 0.68 \text{ mm}^2$ while operator 2 had $86.85 \pm 6.91 \mu\text{m}$ and $3.81 \pm 0.56 \text{ mm}^2$ respectively.

The ICC for NFL was 0.97 and 0.96 for disc area. CV was 10.0% for automated NFL (N=240) and 8.8% manually (N=144). Disc area had a CV (N= 240) of 14% automatically and 16.4% manually. Figure 3 uses Bland-Altman plots to demonstrate inter-rater agreement for total NFL and disc area.

In addition to total NFL, the program automatically calculates the NFL in 8 regions centered on the optic nerve head (ST, TU, TL, IT, IN, NL, NU, and SN). Table 1 includes average NFL thickness ($\pm$ SD), CV and ICC between operators for each region. Measurements of these smaller regions were more variable when repeated than total NFL thickness regardless of operator. The CV of automated measurements in individual regions ranged from 15.2–36.2% (mean = 19.8%; median = 17.7%) while manual CVs ranged from 15.4–24.9% (mean = 20.7%; median = 20.9%). For disc area, total NFL, and most regions, ICC was excellent (>0.75) between operators. The exceptions were moderate agreement (0.6–0.74) for automated and manual IT, fair agreement (0.4–0.59) for automated and manual NU, and poor agreement (<0.4) for automated TU.

A mixed ANOVA was conducted to assess if there were sex, globe, imager, and regional differences between replicate measurements. There was no significant main effect of replication or imager on NFL thickness and disc area confirming reproducibility and repeatability of these measures. There were sex differences between dogs such that IN was thicker in females with a mean difference of $7.84 \mu\text{m}$ ($F(1, 79) = 8.95$, $p < 0.01$) while NU and TL were thinner in females with a mean difference of 8.57 and $9.95 \mu\text{m}$, respectively ($F(1,79) = 9.54$, 8.86 , $p < 0.01$ for all). Automated and manually adjusted measurements were not significantly different from each other for disc area ($p=0.44$), however overall NFL as well as NFL in the NL, NU, TL and TU regions were significantly different ($p < 0.01$) between these methods. In all of these areas, manual measurements had greater thickness values than the automated algorithm with mean differences of 13.3, 38.7, 22.3, 29.8, and $15.6 \mu\text{m}$ respectively. (Fig. 5)

Nerve fiber layer measurements were significantly different by region. In the pairwise comparison of the mixed model ANOVA, ST had a significantly thicker NFL than all other regions ($p < 0.02$). SN was the second thickest region having a mean difference of $-5.74 \mu\text{m}$ from ST but otherwise significantly thicker than the remaining regions ($p < 0.01$). IT was the third largest, then IN, NL and TL were not significantly different from each other, TL was significantly larger than TU. TU and NU were significantly smaller than all other regions ($p < 0.01$).

Discussion

Despite anatomical differences between the canine and human eye, we obtained high quality images of the canine posterior segment. Furthermore, the operators did not have statistically different quality scores from each other (mean difference = 0.68, $p = 0.66$) despite varying levels of experience, reaffirming the user-friendliness of this device. While it has been shown that quality can have a significant impact on NFL thickness measurements, the quality of our images was weakly correlated with disc area ($r = -0.35$, $p = 0.02$) but not NFL measurements in any region. This is likely related to the consistent high quality of the images obtained and previous studies may have been affected by more variation in quality. Users should consider that disc area might be affected by image quality in addition to NFL. The weak correlation ($r = 0.48$, $p < 0.01$) between disc area and overall NFL thickness present in this study has been seen by others and led to the hypothesis that canines with larger optic discs often have more nerve fibers going to the disc (6, 17) though this was not found to be true in all studies. (14)

An ICC over 0.9 is considered excellent, so total NFL and disc area was highly reproducible by different operators with both automated and manual measurements. ICCs this high were especially promising as one operator was experienced with this technology and the other was a first-time user. Furthermore, CVs tended to be very similar between operators, regardless of method of measurement, showing that in addition to having high inter-rater agreement; operators also had similar variability (Table 1). CVs have been variable when reported in the literature for SD-OCT and appear to be different for different brands and models. A CV of 10% for total NFL seems to be fairly average with some studies achieving less than 10% CV and others finding worse reproducibility indices. (17) Reproducibility studies in humans have shown SDs of measurements of the NFL to be 10–20 μm in normal and glaucomatous eyes (14) which was comparable to our findings (SD of total NFL automated 7.37 μm (CV = 10%) and manual 7.59 μm (CV = 8.8%). In general, method of measurement did not seem to make a consistent difference in CV or ICC and thus consistent use of one method of measurement is recommended.

Smaller regional segments had lower ICCs and higher CVs particularly in the temporal upper, inferior temporal, and nasal upper regions. Thus, longitudinal changes in these areas should be interpreted with caution. The inferior region is thought to be the region with highest sensitivity to glaucomatous changes, corresponding with superior visual field loss (18) though Bowd et al. were able to show NFL thickness changes in all quadrants in a comparison of normal and glaucomatous human eyes. (14) The automated and manual total NFL values obtained were statistically significantly different, with an average difference of 12.7 μm . However, this difference is considered to be clinically insignificant as scans using this machine are only accurate to 5 μm (Optovue iVue manual).

The values reported in the literature for NFL thickness are variable and there appears to be some degree of overlap between normal and glaucomatous eyes. (14) Scanning laser polarimetry is considered to be a less sensitive imaging modality, with one study measuring mean NFL thickness at $141.6 \mu\text{m} \pm 18$ in normal eyes and $105.1 \pm 23.9 \mu\text{m}$ in glaucomatous eyes of cocker spaniels. (15) However, Hernandez-Merino et al. used SD-OCT (Heidelberg

Engineering Spectralis OCT) to perform a peripapillary circular scan around the optic nerve in canines and many of their values fall in the same range as ours (superior quadrant $91.3 \pm 7.0 \mu\text{m}$, temporal quadrant $55.8 \pm 11.5 \mu\text{m}$, inferior quadrant $76.42 \pm 9.2 \mu\text{m}$, nasal quadrant $78.8 \pm 15.7 \mu\text{m}$). (7) In most studies of normal human eyes, nasal and temporal quadrants are thinner than the superior and inferior regions. (7, 14, 19, 20) Hood et al., created NFL thickness profiles from OCT scans in humans, showing thickness maxima in the ST and IT regions corresponded with major retinal blood vessels. (21) The authors suggest this association may be due to the fact that blood vessel thickness directly contributes to the NFL thickness as it is included by the algorithm. In addition, the development of blood vessels is influenced by the axonal distribution such that they are present in the areas of greatest NFL thickness. (21) The ST region might also be thickest as a result of the high acuity area centralis in canines, which is located dorso-temporal to the optic disc, providing more axons from this region. (6)

When manually adjusted, the NL, NU, TL, TU regions increased in thickness significantly from both a statistical and clinical perspective (mean difference (95% confidence interval): $38.70 \mu\text{m}$ ($30.45\text{--}46.95 \mu\text{m}$), $22.26 \mu\text{m}$ ($16.74\text{--}27.78 \mu\text{m}$), $29.75 \mu\text{m}$ ($23.10\text{--}36.40 \mu\text{m}$), $15.61 \mu\text{m}$ ($10.87\text{--}20.35 \mu\text{m}$) respectively). With these changes, NL was significantly thicker than every region except ST ($F(8, 351) = 25.65$, $p < 0.01$). While we cannot say whether automated or manual measurements are a better reflection of actual NFL thickness, the automated measurements seem to better reflect the characteristic double hump pattern of the neuroretinal rim with peaks in the superior and inferior regions reported in humans and canines. (5, 7, 14, 16, 20, 21) We believe these regional differences between the automated and manual measurements may relate to the ability of the software algorithm to detect smaller peripapillary retinal vessels (i.e. cilioretinal arteries) and their integration into the NFL thickness map.

Rosolen et al. states that the NFL, ganglion cell layer, inner plexiform layer, inner nuclear layer and outer nuclear layer were clearly identifiable in their images. (9) In this study, the ganglion cell and inner plexiform layers were difficult to distinguish from NFL using the glaucoma scan and these layers were likely included in the measurement of NFL especially when making manual adjustments. This could have contributed to the differences between manual and automated measurements. However, NFL correlates with the distribution of retinal ganglion cells (6) which are destroyed by glaucoma so including this layer in our measurements should not significantly affect the ability to detect glaucomatous change over time. However, as this study was conducted in normal dogs, future studies will be required to evaluate the sensitivity of this system and its capacity to detect early retinal changes in dogs with glaucoma.

In humans, a sex difference has been noted in mean retinal thickness, with men reportedly having thicker retinas by $5\text{--}15 \mu\text{m}$ depending on the region. (22) Sex seemed to have played a role in some of our NFL values, with males showing an increased NFL compared to females in the NU and TL regions (mean difference of 8.57 and $9.95 \mu\text{m}$ respectively, $p < 0.01$). However, females had IN NFL thicknesses that were greater than males by $7.84 \mu\text{m}$. The low number of dogs involved in this study makes it difficult to further evaluate sex differences.

In conclusion, the SD-OCT device employed herein generated high quality images with consistent values regardless of operator. Intra-rater reliability of disc area and average NFL is high enough that these values would offer meaningful information when looking at a patient's scans over time; however the values of the 8 individual segments should be interpreted with caution as they were highly variable. Users should try to be consistent in whether they use the computer generated values or manually adjust them, as this may result in significantly different measurements for some regions.

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Figure 1.

Fundus photograph from the left eye of a 3 year old, female spayed Beagle depicting the canine optic nerve head (a). Optical coherence tomography scans were taken centered on this region to obtain cross-sectional images for optic nerve head and retinal nerve fiber layer analysis. Three-dimensional NFL thickness map produced by the OCT 3-D glaucoma scan of the same canine eye (b).

Figure 2.

Cross sectional B-scans obtained using the glaucoma scan in a healthy canine eye.

Automatically generated segmentation produced automatically (a). The three segmentation lines should run over the inner retinal surface, beneath the nerve fiber layer and at the level of retinal pigment epithelium. Manual segmentation of the same B-scan (b).

Figure 3.

Bland-Altman plots demonstrating inter-rater agreement for automated total NFL (a), manual total NFL (b), automated disc area (c) and manual disc area (d). Mean difference (continuous line) and the upper and lower limits of agreement (dashed lines) are represented.

Figure 4.

Nerve fiber layer (NFL) thickness map divided into eight regions produced by the glaucoma scan obtained from a normal 3 year old female canine. Images (a) and (b) are the retinal thickness maps automatically generated by the SD-OCT software while images (c) and (d) are the thickness maps from the same eye after manual corrections (modification of disc boundaries, RPE tips, and B scans). Nerve fiber layer thickness of individual segments varied to a greater degree following adjustments than overall NFL thickness.

Comparison of automated and manual measurements across regions

Figure 5.

Automated and manual measurements were significantly different from each other in the NL, NU, TL and TU regions resulting in a significantly different total NFL. Manual adjustments resulted in thicker NFL measurements of these regions. The asterisks denotes significant differences between automated and manual measurements.

Table 1

A summary of mean NFL thicknesses (μm) by region around the optic nerve head. Intra-class correlation coefficient (ICC) is a measure of inter-rater reliability while coefficient of variation (CV) is a measure of intra-rater reliability.

	Automated				Manual			
	Operator 1		Operator 2		Operator 1		Operator 2	
	Mean $\pm$ SD	CV	Mean $\pm$ SD	CV	Mean $\pm$ SD	CV	Mean $\pm$ SD	CV
ST	95.40 $\pm$ 16.47	17.3%	96.86 $\pm$ 16.73	17.3%	99.28 $\pm$ 18.27	18.4%	98.89 $\pm$ 15.20	15.4%
TU	55.66 $\pm$ 10.55	18.9%	59.82 $\pm$ 21.68	36.2%	71.27 $\pm$ 13.31	18.7%	73.13 $\pm$ 13.94	19.1%
TL	63.58 $\pm$ 14.26	22.4%	65.01 $\pm$ 15.68	24.1%	93.75 $\pm$ 22.36	23.8%	93.08 $\pm$ 19.53	21.0%
IT	80.05 $\pm$ 13.15	16.4%	81.23 $\pm$ 12.34	15.2%	75.99 $\pm$ 15.83	20.8%	76.79 $\pm$ 13.92	18.1%
IN	75.48 $\pm$ 11.79	15.6%	76.06 $\pm$ 12.73	16.7%	72.35 $\pm$ 17.98	24.9%	71.44 $\pm$ 14.94	20.9%
NL	68.71 $\pm$ 16.04	23.3%	65.74 $\pm$ 15.45	23.5%	105.78 $\pm$ 25.97	24.6%	105.46 $\pm$ 24.11	22.9%
NU	59.77 $\pm$ 11.03	18.5%	59.26 $\pm$ 9.92	16.7%	79.83 $\pm$ 18.09	22.7%	82.08 $\pm$ 19.73	24.0%
SN	88.99 $\pm$ 15.42	17.3%	90.00 $\pm$ 16.25	18.1%	91.04 $\pm$ 15.66	17.2%	93.40 $\pm$ 18.15	19.4%
								0.805