*STRATUS*OCT™: Establishment of Normative Reference Values for Retinal Nerve Fiber Layer Thickness Measurements

Vincent Michael Patella
Carl Zeiss Meditec, Inc.
Dublin, CA

Abstract: A study was conducted to obtain clinical data from normal individuals to establish expected thickness and normal limits of the peripapillary retinal nerve fiber layer. The study was designed to support the development of a normative database for the *STRATUS*OCT, a high resolution tomographic device for imaging and measuring posterior ocular structures. Data from a statistically significant number of patients was gathered and analyzed. With this data, expected thickness and normal limits were determined.

Introduction: Proper diagnosis of ophthalmic disease involving the peripapillary retinal nerve fiber layer (RNFL) requires a substantive knowledge of the expected thickness and normal limits of the RNFL.[1,2,3] Prior to the study described in this paper, there have been few efforts to establish these reference values in a normal population using optical coherence tomography (OCT) in a protocol subject to regulations and guidance by the United States Food and Drug Administration (FDA).[4] The goal of the study detailed in this paper was to obtain clinical data from normal individuals in order to establish expected thickness and normal limits of the peripapillary RNFL. These normal limits have been included in an analysis package to allow *STRATUS*OCT users to identify patients with diagnostic results outside the normal range. The clinical study described herein was conducted in full conformance with regulations promulgated by FDA, specifically 21 C.F.R. Part 812, Investigational Device Exemptions.

Materials and methods: To collect the data, a prospective, multi-center, non-comparative study was designed, performed, and completed. The Clinical Study Plan was reviewed and approved by an independent Institutional Review Board.

Study candidates: In order to participate in the study, candidates had be to adults, 18 years or older, with no contraindicating ophthalmic conditions. Exclusion criteria included:

- Contraindications to dilation (including occludable anterior chamber angles), or intolerance or hypersensitivity to topical anesthetics or mydriatics in either eye.
- Ocular hypertension (IOP ≥ 22 mm Hg in either eye) or glaucoma in either eye.
- Evidence of reproducible visual field abnormality in either eye, defined as Pattern Standard Deviation significant at $p < 5\%$ level, or abnormal Glaucoma Hemifield test result, or any other pattern of loss which is consistent with ocular disease.
- Intraocular surgery in the study eye one year prior to enrollment.
- Best corrected visual acuity in the study eye worse than 20/32 on Early Treatment of Diabetic Retinopathy Study scale.
- Evidence of diabetic retinopathy, diabetic macular edema, or other vitreo-retinal disease in either eye.
- Evidence of optic nerve or retinal nerve fiber layer abnormality in either eye.
- History of diabetes.
- Current or recent (within the past 14 days) use of an agent with photosensitizing properties by any route.

There were no exclusions for race or gender. However, neither gender could comprise more than 60% of the study population.

Study measurements: Two types of measurements were completed for each subject in the study. The first type was three scans, each consisting of 256 test points measured along a nominal 1.73 mm radius circle on the peripapillary RNFL using a single alignment and capture. The thickness at each test point was determined by averaging the three measurements. The second type was a single scan consisting of 512 test points measured along a nominal 1.73 mm radius circle on the peripapillary RNFL. In order to distinguish between the two types of measurements, the first set of 256 test points was labeled as "Fast RNFL Thickness" and the second set of 512 test points was labeled as "RNFL Thickness."

For each subject, only one eye was measured. Right and left eyes were alternated on successive subjects. The goal, determined *a priori,* was to enroll a minimum of 350 normal subjects in the study to yield a minimum of 250 qualified scans, *i.e.,* the minimum required for establishing statistically significant normal limits. Each scan type was evaluated to determine whether that particular scan met the criteria for acceptance per the study protocol. Only qualified scans were used in the analyses.

Measurement equipment: All measurements were taken with the *STRATUS*OCT, a high resolution tomographic device (Carl Zeiss Meditec, Inc., Dublin, CA) using prerelease version 3.0 software.

Site Number	Site Name Investigator
1	Valley Eye Care Center, Inc. Jonathan Savell MD
2	Doheny Eye Institute USC Medical Center Rohit Varma MD
3	Department of Ophthalmology/Glaucoma Indiana University School of Medicine Louis Cantor MD
4	New England Eye Center Tufts University School of Medicine Joel Schuman MD
5	Bascom Palmer Eye Institute (Palm Beach) University of Miami School of Medicine David Greenfield MD
6	Bascom Palmer Eye Institute (Miami) University of Miami School of Medicine Donald Budenz MD

Table 1. Study sites and investigators.

Study locations: The study was conducted at six sites (Table 1) in the United States over a three-month period. Subject enrollment was completed in September 2002.

Results: A total of 410 subjects were enrolled across all sites to meet the goal of 350 qualifying normal subjects and 250 qualifying scans, per protocol, in each scan type category. In the end, 328 qualified scans were obtained using Fast RNFL Thickness measurements and 297 qualified scans were obtained using RNFL Thickness measurements.

The mean age of subjects producing qualified Fast RNFL Thickness scans was 47 years with a standard deviation of 15.8 years. The youngest subject was 18 years old and the oldest was 85 years old. The mean age of subjects producing qualified RNFL Thickness scans was 48 years with a standard deviation of 16.2 years. The youngest subject was 18 years old and the oldest was 86 years old. See Table 2 for a listing of the number of subjects in each age category by scan type.

Study subject gender was evenly divided. See Table 3 for a listing of the subject gender by scan type.

Age Category	Fast RNFL Thickness	RNFL Thickness
≤ 29	58	54
30 - 39	45	39
40 - 49	74	67
50 - 59	71	60
60 - 69	43	41
≥ 70	37	36
Total	328	297

Table 2. Subject distribution by age.

	Fast RNFL Thickness		RNFL Thickness	
Gender	Total	Percent	Total	Percent
Female	171	52	151	51
Male	155	47	144	49
Not recorded	2	< 1	2	< 1
Total	328		297	

Table 3. Subject distribution by gender.

OD or OS	Fast RNFL Thickness		RNFL Thickness	
Measured	Total	Percent	Total	Percent
OD (Right)	164	50	151	51
OS (Left)	164	50	146	49
Total	328		297	

Table 4. Subject distribution by eye measured.

The protocol required that the eye chosen to be scanned be alternated between right and left eyes on successive subjects. See Table 4 for a listing of the subject eye measured by scan type.

Subject ethnicity was recorded during the study, and a broad representation of various ethnic groups was observed in the study population.

Scan data: Each scan was initiated in the temporal region of the eye (denoted by T on Figure 1) and progressed in a Superior (S), Nasal (N), and then, Inferior (I) direction. For a Fast RNFL Thickness scan, *i.e.,* the average of three scans, the Expected Thickness of a normal individual was calculated at each of the 256 test points measured along the circle. For a RNFL Thickness scan, the Expected Thickness of a normal individual was calculated at each of the 512 test points measured along the circle. Figure 1 displays the resulting Expected Thickness of a normal individual, age corrected, with a mean age equivalent to that observed in the study population for a Fast RNFL Thickness scan. A similar graph was constructed for the RNFL Thickness scan.

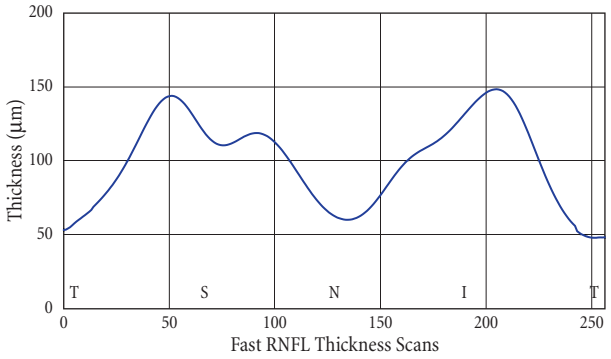

Figure 1. Expected Thickness of a normal individual, age corrected to the average of the study population.

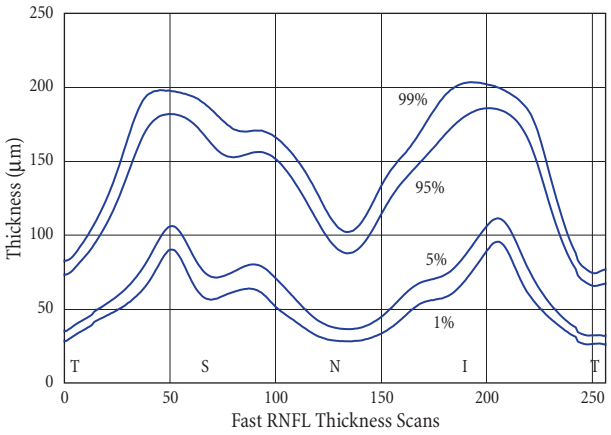

Figure 2. Normal Limits, age equals mean age, age corrected to the average of the study population.

Analysis: To be of value to the clinician, it is necessary to process the raw data collected to produce a series of values that can be used to determine if measurements of an individual patient fall within a "normal" range.[5,6]

Reference intervals for RNFL thickness measurements can be constructed if the distributions in normal subjects of the RNFL thickness measurements are known. The Expected Thickness data for a specific subject varies with sample point and subject characteristics. Statistical tests were performed to determine the dependence of the Expected Thickness on the following subject characteristics: age, right vs. left eye, and gender. It was determined that Expected Thickness was dependent upon age, but not significantly dependent upon the other variables. Age correction was therefore incorporated into the calculated results.

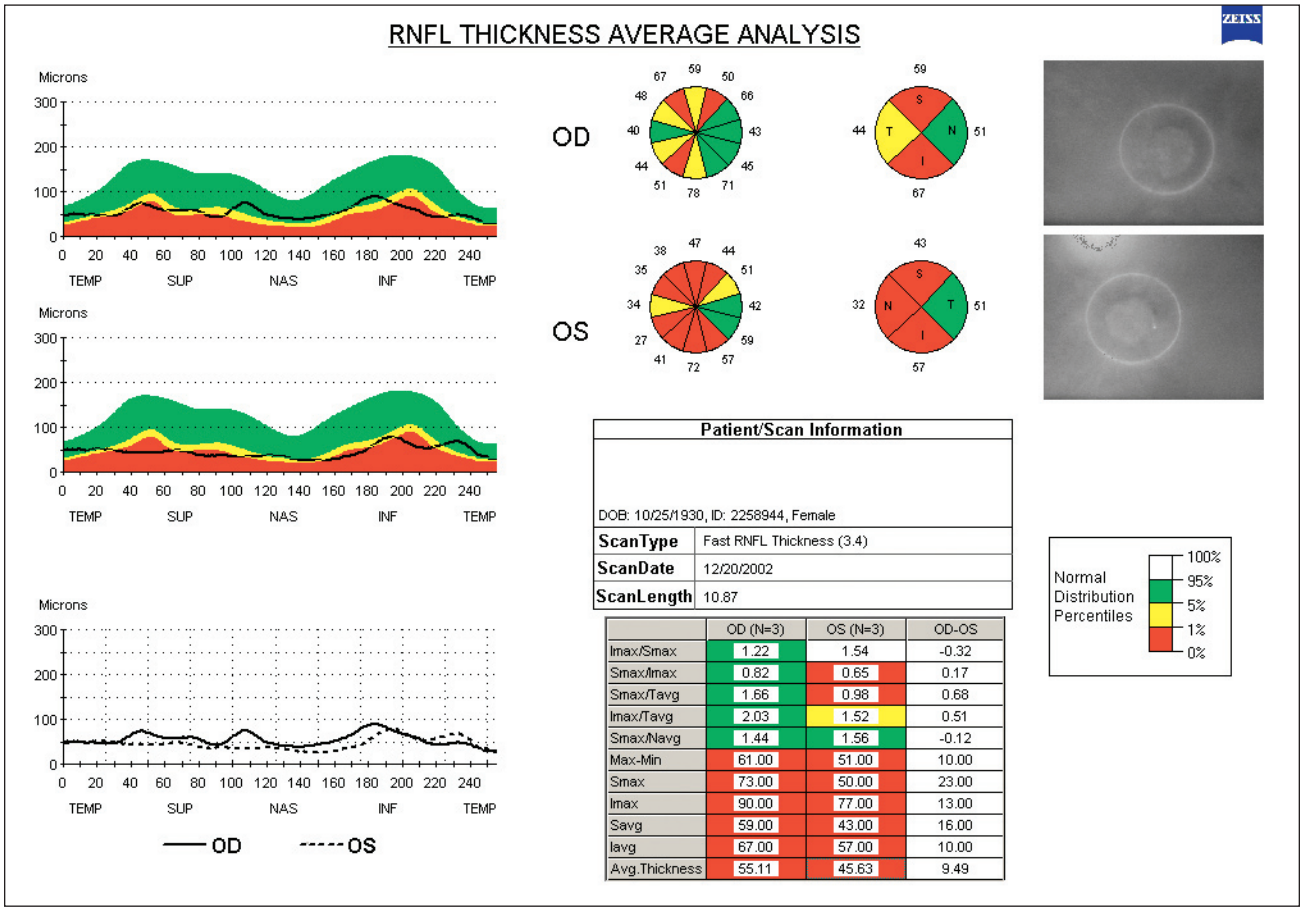

Figure 3. Sample *STRATUS*OCT display of patient data.

The Expected Thickness index can be estimated based upon scan type. Figure 1 displays the Expected Thickness, in microns (μm), of a normal individual, age corrected, for both Fast RNFL Thickness and RNFL Thickness scan types. Statistical determinations of confidence bands at 1%, 5%, 95%, and 99% were then calculated and applied to the Expected Thickness values. As illustrated in Figure 2, Normal Limits are then estimated for RNFL Expected Thickness indices at the 1%, 5%, 95%, and 99% levels for a Fast RNFL Thickness scan. A similar graph was constructed for the RNFL Thickness scan. Thus, age-matched normative data can be used to indicate whether an individual patient's RNFL thickness index falls within normal limits. A variety of *STRATUS*OCT analysis protocols, including application of the RNFL Normative Database, can be employed by the clinician to aid in the diagnosis of the condition of the patient's retinal structures.

Discussion: The reproducibility of retinal mapping has been well established in the clinical setting.[7,8] The *STRATUS*OCT with Retinal Nerve Fiber Layer (RNFL) Normative Database offers clinicians a quantitative tool for the comparison of the retinal nerve fiber layer in the human retina to a database of known normal subjects. The RNFL Normative Database is intended for use as a diagnostic aid in the detection and management of ocular diseases.

As presented in Figure 3, the *STRATUS*OCT displays the RNFL Normative Database information in the form of clinically useful graphs and charts. When applied to the appropriate scan type of an individual patient, the OD and OS graphics display colored bands, *i.e.,* red, yellow, green, and white, corresponding to the Normal Distribution Percentiles as estimated by the RNFL Normative Database. The same color scheme is applied to data presented in tabular format and to circular maps that describe segment and quadrant averages for each eye.

Comparing age-matched patient indices to those of the normal population offers the clinician increased possibilities for the detection and management of ocular diseases. Coupled with the precision and accuracy of *STRATUS*OCT imaging, the RNFL Normative Database allows for the possibility of early detection and management of ocular diseases involving the retina, especially glaucoma.

Additional areas for investigation and development of normative databases for the *STRATUS*OCT include: macular thickness analysis, intraocular comparisons, the shape of RNFL topography, correction for the size of eye or the diameter of the optic nerve, and, potentially, normative limits based upon racial background.

Conclusion: The study obtained clinical data from normal individuals and established Expected Thickness and Normal Limits of the peripapillary RNFL. These Normal Limits have been included in an analysis package for the *STRATUS*OCT as the RNFL Normative Database. Use of the data gathered in this study will facilitate proper diagnosis of ophthalmic disease involving the peripapillary retinal nerve fiber layer (RNFL) and assist clinicians in assessing whether patients' RNFL measurements fall outside the normal range.

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Carl Zeiss Meditec, Inc.
5160 Hacienda Drive
Dublin, CA 94568-7562
(877) 486-7473

Note: Results obtained from a previous version of the *STRATUS*OCT software require reanalysis using version 3.0, or higher, in order to apply the RNFL Normative Database.