Reference Database of Inner Retinal Layer Thickness and Thickness Asymmetry in Healthy Thai Adults as Measured by the Spectralis Spectral-Domain Optical Coherence Tomography

Janejit Choovuthayakorn a, Susama Chokesuwattanaskul a, b, Phichayut Phinyo c, d, e, Linda Hansapinyo a, Kessara Pathanapitoon a, Voraporn Chaikitmongkol a, Nawat Watanachai a, Paradee Kunavisarut a, Direk Patikulsila a

a Department of Ophthalmology, Faculty of Medicine, Chiang Mai University, Chiang Mai, Thailand; b Cornea and Refractive Surgery Unit, Department of Ophthalmology, Faculty of Medicine, Chulalongkorn University and King Chulalongkorn Memorial Hospital, Bangkok, Thailand; c Department of Family Medicine, Faculty of Medicine, Chiang Mai University, Chiang Mai, Thailand; d Musculoskeletal Science and Translational Research (MSTR), Chiang Mai University, Chiang Mai, Thailand; e Clinical Epidemiology and Clinical Statistics Center, Faculty of Medicine, Chiang Mai University, Chiang Mai, Thailand

Keywords
Reference database · Retinal thickness · Inner retinal thickness · Posterior pole asymmetry

Abstract

Introduction: The study aimed to determine a reference database of the thickness and intraocular thickness asymmetry of total retina, retinal nerve fiber layer (RNFL), ganglion cell layer (GCL), and inner plexiform layer (IPL) in healthy Thai subjects measured by the Spectralis spectral-domain optical coherence tomography. Methods: This cross-sectional study recruited the healthy subjects age ≥18 years, having spherical refraction within ±6 diopters and cylindrical refraction ±3 diopters, from a hospital’s personnel and the people visiting the ophthalmology department. Only 1 eye of each subject was randomly selected for an analysis. Macular images were obtained using posterior pole thickness scan protocol over a 24° × 24° area at the center of the fovea. The automated retinal thickness segmentation values of total retina and three inner retinal layers were calculated for the mean and the mean intraocular thickness difference between superior and inferior retinal hemispheres. The influence of age, gender, and axial length on thickness and thickness asymmetry of individualized retinal layer was evaluated. Results: 252 subjects were included in study with a mean (SD) age of 46.7 (15.8) years, and 120 (47.6%) were males. According to the Early Treatment Diabetic Retinopathy Study map, the inner ring area was the thickest location of the total retina (range; 326.0–341.5 µm), GCL (range; 47.7–52.7 µm), and IPL (range; 39.9–42.1 µm), whereas the thickest location of RNFL was at the outer ring area (range; 18.8–47.5 µm). For posterior pole intraocular thickness asymmetry, the greatest mean ± SD difference was observed for total retina (9.0 ± 2.2 µm), followed by RNFL (9.9 ± 3.2 µm) and GCL (2.7 ± 0.6 µm), and the lowest mean difference was noted for IPL (2.4 ± 0.5 µm). The thickness and thickness asymmetry of each retinal layer were variably influenced by age, gender, and axial length; however, these factors had a minimal influence on the thickness asymmetry maps of GCL and RNFL. Conclusion: The reference database of the macular thickness and thickness asymmetry from this study would be beneficial in determining physiologic variations of the OCT parameters in the healthy Thai population.

© 2022 The Author(s).
Published by S. Karger AG, Basel

Correspondence to:
Janejit Choovuthayakorn, janejit.c@cmu.ac.th
Introduction

Spectral-domain optical coherence tomography (SD-OCT) is an innovative imaging technology that generates high-resolution cross-sectional retinal images using a fast acquisition [1–4]. The retinal thickness at the macular location and around the optic nerve head could be measured. The technology is promising to improve the detection and monitor many ophthalmic and neuro-ophthalmic diseases in adult and pediatric populations [5–12]. Among currently available SD-OCT, Spectralis® is one of the platforms that incorporates retinal segmentation software and provides automated total and individual retinal layer thickness values. In addition, the algorithm also provides an intraocular and interocular macular thickness asymmetry analysis map. Combining these OCT retinal microstructural parameters, clinicians could predict early alterations, make a more precise decision in clinical practice, and perhaps understand the pathological process. Additionally, excellent repeatability and reproducibility of OCT thickness measurements have been well demonstrated in several studies [13–17].

However, in the OCT structural assessment, the normal physiologic deviation should be carefully determined, as several potential confounders such as age, gender, axial length (AXL), OCT scanning pattern and platform, and race could considerably impact the thickness of macular and peripapillary RNFLs [18–23]. Therefore, the reference database of each specific population is mandatory to establish the interpretation of the OCT findings. This study aimed to explore the distribution of retinal thickness, intraocular thickness asymmetry of the total retina, and the three inner retinal layers including RNFL, ganglion cell layer (GCL), and inner plexiform layer (IPL), in healthy Thai adults as measured by the Spectralis SD-OCT. This reference database could be used in clinical practice for the assessment of any structural abnormalities in determining and understanding pathological conditions.

Materials and Methods

This cross-sectional study was performed in accordance with the Declaration of Helsinki. The study protocol was approved by the Chiang Mai University Ethics Committee and registered in the Thai clinical trial registration (TCTR ID Number: 20190919002). Healthy subjects, mainly the hospital staff and the people accompanying the patients at the outpatient ophthalmology clinic, aged >18 years and having no prior ophthalmic diseases, were recruited between September 2019 and August 2020. After the study objectives and the procedure were thoroughly explained by the blinded on-site clinicians, who were not involved in the data analysis, only volunteer subjects with informed consent were included for eligibility screening. Each subject underwent complete ophthalmic examinations including Snellen best-corrected visual acuity, automated refraction by KR-8100 auto-refractometer (Topcon, Japan), anterior segment examination by a slit-lamp biomicroscopy, intraocular pressure (IOP) measurement by the Goldmann applanation tonometry, posterior segment examination by 90 diopters (D) lens, AXL measurement by IOL master (Carl Zeiss Meditec, Inc., Dublin, CA, USA), and retinal OCT scan by Spectralis SD-OCT (Heidelberg Engineering, Heidelberg, Germany). The subjects who fulfilled the following inclusion criteria were enrolled: (1) Snellen best-corrected visual acuity of 20/40 and better; (2) spherical refraction within ±6 D and cylindrical refraction within ±3 D; and (3) IOP less than 21 mm Hg and no reported history of high IOP. The exclusion criteria were (1) having a current or history of any ophthalmic or systemic conditions, for example, retinal and macular disorders, optic neuropathies, glaucoma, intraocular inflammation and uveitis, diabetes mellitus, and neurologic diseases, that might affect macular thickness or the visual field; (2) having unhealthy optic disk appearances including cup to disk ratio >0.6, asymmetrical cup to disk of >0.2 between eyes, disk pallor, disk hemorrhage, and neuroretinal rim thinning (determined by SC and JC, and disagreement resolved by LH); (3) receiving prior intraocular surgeries; or (4) having prior ocular trauma.

Optical Coherence Tomography Retinal Imaging

The OCT scans were obtained using the Spectralis HRA SD-OCT by experienced operators with enabling of eye tracking system. The peripapillary RNFL scan and a macular volume scan protocol were performed. Briefly, the macular volume scan comprised 61 B-scan lines (768 A-scans per one B-scan line, 120 µm apart, over 30º × 25° area) aligned parallel to the fovea to optic disk center axis. One B-scan contains an average of nine real-time images. Before the scan, the focus was adjusted in accordance with the subject’s refractive error using a dial knob adjustment provided with the machine. Heidelberg HRA/Spectralis viewing module version 6.9.5.0 and eye explorer version 1.10.2.0 were used to obtain OCT images. All OCT scans were checked for signal quality, decentration, and segmentation by a retinal specialist (J.C.). Images with decentration were corrected. Eyes having images with signal strength <15 decibels and segmentation error were excluded. If both eyes of a subject fulfilled the eligible criteria, only one eye was randomly chosen for OCT image analysis.

Macular thickness values of total retinal layer, RNFL, GCL, and IPL were automatically segmented and measured by the machine’s software. All thickness values were collected based on two output maps: the Early Treatment Diabetic Retinopathy Study (ETDRS) map and the posterior pole retinal thickness map. The ETDRS map contained 9 sectors within 3 rings, one central 1-mm diameter ring (defined as central subfield or 1 sector), 3-mm inner diameter ring (containing 4 sectors of inner superior, inner nasal, inner inferior (II), and inner temporal), and 6-mm outer diameter ring (containing 4 sectors of outer superior, outer nasal, outer inferior, and outer temporal). The posterior retinal thickness map is composed of 64 grid cells (32 each for superior and inferior hemisphere) and 3º × 3º for each grid cell (covering 24º central area). Figure 1 illustrates the ETDRS sectors and numbering of correspondence grid cells of the posterior pole asymmetry map.
Statistical Analysis

The continuous data were described as mean and standard deviation (SD) and categorical data as percentage. The mean (95% confidence interval [CI]) macular thickness of total retina, RNFL, GCL, and IPL across nine sectors of ETDRS map and 64 grid cells of posterior pole thickness maps were analyzed. For posterior pole asymmetry analysis, an absolute mean difference (95% CI) in thickness of 32 corresponding superior and inferior hemisphere pairs of each retinal layer was calculated. For each retinal layer, multivariable linear regression analysis for association between mean retinal thickness of each ETDRS sector and mean thickness asymmetry of each of the 32 corresponding posterior pole grid cells with the independent variables (gender, age, and AXL) was performed. value <0.05 was considered significant. All statistical analyses were performed using STATA software version 16.0.

Results

Among 275 subjects screened, 23 were excluded due to vitreo-retinal interface and macular abnormalities (10), suspected optic disk appearance (8), low signal strength (3), and segmentation error (2). Overall, 252 eligible subjects remained which 120 (47.6%, 120/252) were males. The mean age of the subjects was 46.7 (SD = 15.8; range = 20–82) years. Demographics and characteristics of studied subjects are described in Table 1.

The mean (SD) thickness of total retina, RNFL, GCL, and IPL by the ETDRS map is shown in Table 2. Overall, the RNFL thickness was maximum at the outer ring sector (range = 18.8–47.5 µm), while the greatest thickness of total retina (range = 326.0–341.5 µm), GCL (range = 47.7–52.7 µm), and IPL (range = 39.9–42.1 µm) was in the inner ring sectors. Patterns of the association between thickness of each ETDRS sector (by individualized retinal layer) and gender, age, and AXL are illustrated in online supplementary Tables 1–4 (for all online suppl. material, see www.karger.com/doi/10.1159/000525512). Of note, several ETDRS sectors of total retina, GCL, and IPL showed a decrease in thickness as the age increased (by decade); however, the opposite trend was observed in some sectors of the RNFL. For gender, most sectors of the four retinal layers were thicker in males compared to females. For AXL, the longer eye was associated with the thinner of outer ring but the thicker of inner ring in some sectors of the total retina, GCL, and IPL.

The mean (95% CI) posterior pole thickness (micrometers) for total retinal layer, RNFL, GCL, and IPL by each grid cell is illustrated in Figure 2. The global mean intraocular thickness asymmetry±SD (95% CI) (micrometers) between superior and inferior hemispheres was 9.0 ± 2.2 (8.7–9.3) for total retinal layer, 9.9 ± 3.2 (9.5–10.3) for RNFL, 2.7 ± 0.6 (2.6–2.8) for GCL, and 2.4 ± 0.5 (2.3–2.5) for IPL. Figure 3 shows the mean absolute difference of the 32 intraocular corresponding superior and inferior hemispheres of the total retina, RNFL, GCL, and IPL. An increase in thickness asymmetry was observed toward the peripheral-nasal side of macular area, corresponding to

### Table 1. Characteristics of eligible healthy subjects

| Characteristics                        | Male     | Female   |
|----------------------------------------|----------|----------|
| Mean age (SD), year                    | 47.1 (15.4) | 46.4 (16.2) |
| Mean AXL (SD), mm                      | 23.7 (0.9)  | 23.4 (0.78) |
| Mean spherical equivalence (SD), dpt    | −0.2 (1.1)  | 0.3 (1.6) |
| Right eye laterality, n (%)            | 62 (51.7)   | 65 (49.2) |

SD, standard deviation.

Fig. 1. Retinal thickness map based on Spectralis posterior pole scan protocol including the ETDRS map (a) and 64 grid cells’ posterior pole asymmetry map (b). IS, inner superior; IN, inner nasal; II, inner inferior; IT, inner temporal; OS, outer superior; ON, outer nasal; OI, outer inferior; OT, outer temporal; U, upper hemisphere; L, lower hemisphere.
major vascular arcade, for total retinal layer and RNFL, with a maximum mean difference (95% CI) of 23.4 (21.3–25.5) µm and 34.2 (32.0, 36.4) µm, respectively. For GCL, the maximum thickness asymmetry was observed on the temporal side of the macula with a mean difference (95% CI) of 4.9 (4.6, 5.2) µm for GCL and 4.0 (3.7, 4.3) µm for IPL. Gender, age, and AXL impacted a small number of corresponding thickness asymmetry grid cells of GCL and IPL compared to total retina and RNFL (online suppl. Fig. 1–4).

### Discussion

This study defined a reference database of posterior pole retinal thickness, as well as thickness asymmetry of total retina, and inner retinal layers as measured by automated retinal segmentation algorithm of Spectralis SD-OCT in healthy Thai adults. For retinal thickness and its intraocular thickness asymmetry, each individual retinal layer was distributed in distinct patterns. The RNFL had the maximum thickness in the peripheral macular area and slightly declined toward the foveal center, whereas, for the total retina, GCL, and IPL, the maximum thickness was at the parafoveal location and gradually declined toward both the foveal center and the peripheral macular area. For intraocular thickness asymmetry, total retina and RNFL attained more intraocular variations than GCL and IPL. In this study, age, gender, and AXL had a greater influence on the quantitative OCT retinal thickness than on the intraocular retinal thickness asymmetry.

Spectralis is one of the commercially available SD-OCT machines that is commonly used in clinical practice. The machine contains a software algorithm that enables a fast acquisitional cross-sectional retinal scan and generates a highly reproducible automated retinal thickness measurement for total and segmented individual retinal layers [14, 16, 17]. In Spectralis, the retinal thickness values were presented as either the ETDRS (covering a central 10-degree area) or posterior pole (covering a central 24-degree area) analysis formats. However, a reference thickness database is an essential component to determine physiologic variations from pathological conditions. Previous studies reported that scan protocol, race, age, gender, body mass index, and other systemic factors may alter the retinal thickness reference database [22, 24–26]. Moreover, data derived from a homogeneous population are necessary to implement a more precise reference database for early detection of abnormalities [25, 27].

There have been several reported factors that possibly impact the quantitative retinal thickness measurement; however, the evidence has been inconsistent. A study by Invernizzi et al. [25] reported that, by dividing retinal thickness displayed on the ETDRS map into three areas (center, inner ring, and outer ring), none of the three inner retinal layers (RNFL, GCL, and IPL) were impacted by aging. However, the number of corresponding thickness asymmetry grid cells of IPL was affected by gender.

### Table 2. Thickness of total retina, RNFL, GCL, and IPL (in micrometers) by the ETDRS map

| ETDRS sector | Retinal thickness, (µm) | Retinal thickness, (µm) | Retinal thickness, (µm) | Retinal thickness, (µm) |
|--------------|-------------------------|-------------------------|-------------------------|-------------------------|
|              | total retina            | RNFL                    | GCL                     | IPL                     |
|              | mean 95% CI             | mean 95% CI             | mean 95% CI             | mean 95% CI             |
| Central area |                         |                         |                         |                         |
| Inner ring   |                         |                         |                         |                         |
| Inner superior | 340.2 338.4, 342.1 | 23.2 23.0, 23.4 | 52.3 51.8, 52.7 | 41.8 41.5, 42.1 |
| Inner nasal  | 341.5 339.4, 343.6 | 20.2 20.0, 20.5 | 51.3 50.7, 51.8 | 42.6 42.2, 43.0 |
| Inner inferior | 336.5 334.5, 338.4 | 24.9 24.7, 25.2 | 51.9 51.3, 52.4 | 41.5 41.1, 41.8 |
| Inner temporal | 326.0 324.2, 327.8 | 17.2 17.1, 17.3 | 47.2 46.7, 47.7 | 39.5 39.1, 39.9 |
| Outer ring   |                         |                         |                         |                         |
| Outer superior | 300.7 298.9, 302.5 | 37.5 37.2, 37.8 | 36.3 35.9, 36.6 | 29.4 29.1, 29.7 |
| Outer nasal  | 317.0 316.0, 320.0 | 47.0 46.5, 47.5 | 39.5 39.2, 39.8 | 31.3 31.0, 31.6 |
| Outer inferior | 286.8 285.0, 288.6 | 40.8 40.4, 41.2 | 33.8 33.4, 34.2 | 31.8 31.0, 32.6 |
| Outer temporal | 283.4 281.7, 285.1 | 18.6 18.4, 18.8 | 34.8 34.4, 35.2 | 31.2 29.9, 33.3 |

ETDRS, Early Treatment Diabetic Retinopathy Study; SD, standard deviation; RNFL, retinal nerve fiber layer; GCL, ganglion cell layer; IPL, inner plexiform layer.
Nieves-Moreno et al. [27] demonstrated a greater influence of age on GCL and IPL thickness (compared to RNFL), and of gender on GCL thickness (compared to IPL and RNFL). The impact of AXL was also detected on some sectors of RNFL. Additionally, an age-related effect on macular thickness was reported by Chauhan et al. [28] as a thinning rate of 2.8% and 2.1% per decade for GCL and IPL, respectively. Consistent with previous reports, the thickness reference database in this study displayed varying effects, in terms of direction and magnitude in each specific layer and retinal area, of age, gender, and AXL on the retinal thickness. Even though the diversities

| Temporal | Total retina layer | Nasal |
|----------|-------------------|-------|
| 230.8 (229.5, 232.1) | 241.7 (240.4, 243.0) | 254.7 (253.3, 256.1) |
| 268.0 (266.5, 269.5) | 279.3 (277.8, 280.9) | 285.0 (283.3, 286.6) |
| 294.2 (292.2, 296.3) | 309.6 (307.3, 312.0) |
| 238.1 (236.8, 239.5) | 254.4 (253.5, 255.8) | 274.5 (272.9, 276.0) |
| 295.0 (293.2, 296.7) | 306.3 (304.5, 308.1) | 302.9 (301.2, 304.7) |
| 300.7 (298.8, 302.5) | 314.0 (311.5, 316.4) |
| 248.7 (247.2, 250.2) | 272.8 (271.2, 274.4) | 305.5 (303.8, 307.3) |
| 333.9 (332.0, 335.7) | 343.4 (341.4, 345.4) | 331.7 (329.7, 333.8) |
| 314.7 (312.8, 316.7) | 314.0 (311.8, 316.2) |
| 254.5 (253.0, 256.1) | 286.6 (284.8, 288.4) | 323.3 (321.5, 325.1) |
| 304.3 (302.3, 306.3) | 310.2 (308.1, 312.4) | 348.0 (345.9, 350.1) |
| 325.4 (323.2, 327.5) | 302.8 (300.7, 305.0) |
| 256.2 (254.7, 257.7) | 289.1 (287.4, 290.9) | 327.9 (326.1, 329.7) |
| 308.4 (306.4, 310.4) | 309.5 (307.4, 311.7) | 347.2 (345.1, 349.2) |
| 347.2 (345.1, 349.2) | 302.3 (300.0, 304.6) |
| 246.5 (245.0, 247.9) | 271.1 (269.4, 272.9) | 305.5 (303.6, 307.4) |
| 331.8 (329.8, 333.8) | 337.4 (335.3, 339.4) | 327.2 (325.0, 329.3) |
| 309.8 (307.7, 311.9) | 320.5 (317.7, 323.3) |
| 234.0 (232.5, 235.5) | 248.8 (247.2, 250.4) | 267.3 (265.5, 269.0) |
| 283.8 (281.8, 285.6) | 292.3 (290.2, 294.3) | 294.6 (292.5, 296.7) |
| 306.2 (303.6, 308.9) | 326.7 (324.0, 329.4) |
| 230.9 (229.4, 232.4) | 240.6 (239.1, 242.2) | 251.9 (250.2, 253.5) |
| 263.8 (262.0, 265.5) | 275.9 (273.9, 278.0) | 292.2 (289.7, 294.1) |
| 303.5 (301.0, 306.1) | 294.1 (291.1, 297.2) |

**Fig. 2.** Mean and 95% CI of the thickness (in micrometers) of total retina (a), RNFL (b), GCL (c), and IPL (d) by each cell of posterior pole thickness map analysis. The gray shaded grid cells represent 4 grid cells around the foveal center. F, foveal center.

(Color version available online)
in eligible participants may prohibit the direct comparison of results between publications, the evidence sheds light on the complexity of assessing quantitative retinal thickness values in clinical practice. Due to the inter-eye and inter-individual variations, several authors investigated a role of intraocular macular thickness asymmetry between superior and inferior retinal hemispheres, as a structural biometric for de-

| Temporal | Ganglion cell layer | Nasal |
|----------|---------------------|-------|
| 21.5     | 23.5                | 25.5  |
| 25.5     | 27.3                | 28.1  |
| 27.3     | (21.3, 21.8)        | (25.3, 25.8) |
| 25.5     | 28.7, 28.4          | 26.0  |
| 26.0     | 25.7, 26.3          | 23.5  |
| 23.5     | (21.5, 22.2)        | 21.8  |

| Temporal | Inner plexiform layer | Nasal |
|----------|-----------------------|-------|
| 17.7     | 19.4                  | 21.3  |
| 21.3     | (19.1, 19.7)          | (21.0, 21.6) |
| 21.3     | 22.9                  | 24.0  |
| 22.9     | (22.6, 22.3)          | (23.7, 24.4) |
| 24.0     | 21.7                  | 19.1  |
| 21.7     | (22.4, 22.0)          | (18.8, 19.4) |
| 19.1     | 17.6                  | 17.6  |
| 17.6     | (17.2, 18.0)          | (17.2, 18.0) |

| Temporal | 26.6 |
|----------|------|
| 26.6     | 30.9 |
| 30.9     | (26.2, 27.9) |
| 26.2     | (30.5, 31.2) |
| 30.5     | (35.3, 36.0) |
| 35.3     | (40.2, 41.1) |
| 40.2     | (39.9, 40.6) |
| 40.6     | (41.0, 41.5) |
| 41.1     | (36.0, 36.4) |
| 36.0     | (35.6, 36.4) |
| 35.6     | (28.5, 29.2) |
| 28.8     | (22.1, 22.8) |
| 22.4     | (24.9, 25.7) |

| Temporal | 30.6 |
|----------|------|
| 30.6     | 36.6 |
| 36.6     | (30.3, 30.9) |
| 30.3     | (36.3, 37.0) |
| 36.3     | (43.0, 43.3) |
| 43.0     | (42.6, 43.3) |
| 42.6     | (32.4, 32.8) |
| 32.4     | (32.0, 32.8) |
| 32.0     | (32.5, 33.4) |
| 32.5     | (33.4, 33.4) |
| 33.4     | (34.0, 34.0) |
| 34.0     | (33.5, 33.4) |
| 33.5     | (32.9, 33.4) |
| 32.9     | (35.9, 35.9) |
| 35.9     | (35.5, 35.9) |
| 35.5     | (28.6, 29.0) |
| 28.6     | (22.2, 22.6) |
| 22.2     | (24.9, 25.7) |
| 24.9     | (25.5, 26.2) |
| 25.5     | (17.4, 18.3) |
| 17.4     | (18.7, 19.3) |
| 18.7     | (21.5, 22.1) |
| 21.5     | (28.9, 29.2) |
| 28.9     | (34.9, 35.2) |
| 34.9     | (42.7, 43.1) |
| 42.7     | (43.4, 43.1) |
| 43.4     | (32.5, 33.4) |
| 32.5     | (34.0, 34.0) |
| 34.0     | (33.5, 33.4) |
| 33.5     | (30.9, 32.8) |
| 30.9     | (35.9, 36.3) |
| 35.9     | (35.5, 35.9) |
| 35.5     | (28.6, 29.0) |
| 28.6     | (22.2, 22.6) |

Fig. 3. Absolute mean difference and 95% CI of the thickness (in micrometers) for 32 intraocular corresponding pairs across posterior pole asymmetry analysis map of total retina (d), RNFL (b), GCL (c), and IPL (d). The gray shaded grid cells indicate the area with a mean difference more than 10 microns. T temporal side; N, nasal side). (For figure see next page.)

Inner Retinal Layer Thickness in Thai Healthy Adult

DOI: 10.1159/000525512

Ophthalmic Res 2022;65:668–677

673
| Vascular arcade | Total retinal layer | Retinal nerve fiber layer | Ganglion cell layer | Inner plexiform layer |
|-----------------|---------------------|---------------------------|---------------------|-----------------------|
| **T** | **N** | **T** | **N** | **T** | **N** | **T** | **N** | **T** | **N** |
| **Foveal center** | | | | |
| 7.0 (6.4, 7.7) | 7.7 (7.0, 8.4) | 8.8 (8.0, 9.6) | 9.5 (8.6, 10.4) | 10.9 (9.9, 12.0) | 13.5 (12.2, 14.8) | 16.8 (15.2, 18.3) | 23.4 (21.3, 25.5) |
| 7.0 (6.4, 7.7) | 8.1 (7.3, 8.8) | 9.4 (8.6, 10.2) | 12.5 (11.5, 13.6) | 15.0 (13.9, 16.1) | 11.1 (10.2, 12.1) | 12.0 (10.7, 13.3) | 17.7 (16.12, 19.3) |
| 6.2 (5.6, 6.7) | 6.4 (5.8, 7.0) | 6.2 (5.6, 6.8) | 6.4 (5.9, 7.0) | 7.9 (7.2, 8.6) | 7.3 (6.6, 8.0) | 8.2 (7.5, 8.9) | 11.3 (10.1, 12.4) |
| 3.7 (3.8, 4.1) | 4.1 (3.8, 4.5) | 5.1 (4.7, 5.5) | 5.9 (5.3, 6.4) | 4.9 (4.4, 5.5) | 2.8 (2.5, 3.1) | 3.9 (3.5, 4.3) | 6.5 (5.8, 7.2) |

| Foveal center | | | |
| 9.2 (8.5, 9.8) | 10.8 (10.0, 11.6) | 11.9 (11.0, 12.8) | 13.7 (12.7, 14.8) | 17.7 (16.1, 19.2) | 28.7 (26.7, 30.6) | 31.5 (29.3, 33.7) | 20.5 (18.8, 22.2) |
| 7.3 (6.8, 7.9) | 8.6 (8.1, 9.2) | 8.8 (8.2, 9.3) | 6.9 (6.4, 7.3) | 5.3 (4.8, 5.8) | 7.5 (6.6, 8.4) | 21.8 (19.9, 23.8) | 34.2 (32.0, 36.4) |
| 3.7 (3.3, 4.0) | 5.7 (5.3, 6.0) | 6.7 (6.3, 7.1) | 4.7 (4.4, 5.0) | 2.7 (2.5, 3.0) | 3.1 (2.8, 3.5) | 5.1 (4.5, 5.7) | 17.5 (16.0, 19.0) |
| 2.6 (2.3, 2.8) | 2.4 (2.2, 2.7) | 3.2 (3.0, 3.5) | 1.3 (1.2, 1.5) | 1.0 (0.9, 1.1) | 2.1 (1.9, 2.3) | 3.7 (3.4, 4.1) | 6.2 (5.6, 6.8) |

| Foveal center | | | |
| 1.5 (1.4, 1.7) | 1.8 (1.6, 1.9) | 2.2 (2.0, 2.4) | 3.1 (2.8, 3.3) | 4.0 (3.7, 4.3) | 3.4 (3.1, 3.7) | 2.7 (2.5, 3.0) | 2.6 (2.5, 2.9) |
| 1.8 (1.6, 2.0) | 2.6 (2.4, 2.8) | 3.5 (3.2, 3.8) | 3.5 (3.2, 3.7) | 3.8 (3.5, 4.1) | 2.7 (2.4, 2.9) | 2.7 (2.4, 3.0) | 2.9 (2.6, 3.2) |
| 3.2 (2.9, 3.2) | 2.4 (2.2, 2.6) | 2.3 (2.1, 2.5) | 2.3 (2.1, 2.5) | 2.0 (1.8, 2.2) | 2.1 (1.9, 2.3) | 2.1 (1.8, 2.7) | 1.9 (1.6, 2.1) |
| 4.9 (4.6, 5.2) | 4.6 (4.2, 4.9) | 3.7 (3.4, 3.9) | 3.1 (2.8, 3.4) | 2.6 (2.3, 2.9) | 1.2 (1.0, 1.3) | 1.6 (1.4, 1.8) | 1.9 (1.7, 2.1) |

| Foveal center | | | |
| 1.8 (1.6, 2.0) | 2.2 (2.0, 2.4) | 2.4 (2.1, 2.6) | 2.9 (2.7, 3.2) | 4.0 (3.7, 4.3) | 3.6 (3.3, 3.9) | 3.0 (2.7, 3.3) | 3.3 (3.0, 3.0) |
| 2.7 (2.4, 2.9) | 2.7 (2.5, 3.0) | 3.0 (2.8, 3.3) | 3.4 (3.2, 3.7) | 2.7 (2.5, 3.0) | 2.3 (2.1, 2.5) | 2.3 (2.1, 2.6) | 2.6 (2.3, 2.8) |
| 2.9 (2.7, 3.2) | 2.5 (2.3, 2.7) | 2.0 (1.9, 2.2) | 1.7 (1.5, 1.8) | 1.8 (1.6, 2.0) | 1.9 (1.7, 2.1) | 1.7 (1.6, 2.0) | 1.9 (1.8, 2.1) |
| 2.4 (2.2, 2.6) | 2.4 (2.2, 2.6) | 1.6 (1.4, 1.7) | 2.5 (2.3, 2.7) | 1.7 (1.6, 1.9) | 1.5 (1.3, 1.6) | 1.4 (1.3, 1.6) | 1.7 (1.5, 1.8) |
testing pathological conditions. Various methods of estimation including an absolute mean difference, an inferior to superior retinal thickness ratio, and asymmetric index (a logarithmic ratio of inferior/superior retinal thickness) have confirmed a discriminative ability of intraocular thickness asymmetry to define pathologies [29–38]. In a report using the Spectralis posterior pole retinal thickness scan protocol, Sullivan-Mee et al. [29] found that a global absolute mean intraocular macular thickness asymmetry was one of the robust OCT diagnostic parameters for differentiating healthy and early glaucoma subjects, with 77% sensitivity and 80% specificity using a 9-µm cut-off point. Similarly, Um et al. [31] demonstrated that when 2 pairs (out of 32 pairs) of superior versus inferior posterior pole retinal thickness met the difference criteria of >30 µm, a sensitivity of 95.2% and a specificity of 81.1% were achieved for detecting localized RNFL defects in the open-angle glaucoma patients.

However, applying one fixed cut-off retinal thickness asymmetry value to the entire macular region could not reveal small localized macular alterations. More detailed macular asymmetry topographic maps have been investigated with more reliable results. Um et al. [31] reported that when applying the glaucoma hemifield testing by dividing the Spectralis posterior pole scan into five sectors, the macular asymmetry values varied from 7.7 µm to 11.7 µm depending on the retinal topography areas. In addition, Yamashita et al. [39] demonstrated that mean absolute macular thickness differences of 32 corresponding superior and inferior posterior retinal pairs had great anatomic variations ranging from 3.1 to 23.2 µm.

Apart from total retinal thickness asymmetry, the automated retinal segmentation software has been developed to evaluate the asymmetry of other retinal layers [27, 33, 35, 36, 40]. For physiologic inner retinal thickness asymmetry in a healthy Caucasian population, Al-tan et al. [35] demonstrated that a mean intraocular asymmetry of RNFL was highest in area overlying peripheral vascular areas (5.8 µm difference in area corresponding to zone 5 of glaucoma hemifield), whereas the highest mean asymmetry of GCL thickness was shown on the temporal macular area adjacent to the horizontal hemisphere line (2.9 µm difference in area corresponding to zone 3 of glaucoma hemifield). The study also reported a nonstatistically significant impact of gender to thickness asymmetry of RNFL and GCL but demonstrated a negative correlation of age to some areas of thickness asymmetry in parts of RNFL [35]. In concordance with a previous study, this study found a greater degree of physiologic intraocular asymmetry for total retina and RNFL, particularly toward nasal-peripheral vascular arcade, compared to asymmetry of GCL and IPL. It is assumed that high variations in thickness asymmetry of total retina and RNFL may be partially explained by the presence of vascular components incorporated within these layers. Findings from this study support the less physiologic variations of OCT intraocular thickness asymmetry for GCL and IPL than total retina and RNFL proposed in previous studies. Moreover, as the intraocular thickness asymmetry for GCL and IPL was less influenced by interindividual factors, the clinical utility could be more substantial.

Limitations of this study include the cross-sectional design that does not allow the study of longitudinal age-related changes. Also, the reference database from this study was derived only from participants of Thai ethnicity. As children, subjects with high refractive errors, and the patients with macular abnormalities were excluded, the findings cannot directly apply to those specific populations. Moreover, associations between retinal structures and visual function in diseased subjects were not evaluated; therefore, the optimal sensitivity and specificity cut points were not determined for pathological conditions. However, this study defined healthy subjects based on ophthalmic examinations and OCT findings which could not totally exclude very early stages of disease and thus may overestimate a retinal thickness asymmetry pattern of the healthy population. Despite these limitations, this study provides a reference database of inner retinal thickness and their asymmetry distribution that would be useful for clinical applications.

**Conclusion**

This study describes a quantitative reference database of retinal thickness and physiologic asymmetry of total retina and individualized inner retinal layers in healthy eyes measured by an automated Spectralis posterior pole analysis protocol. The findings of OCT parameters would be useful for assessing and monitoring several pathological conditions, particularly in the Thai population.

**Acknowledgment**

We would like to thank Barbara Metzler, a director of the Chiang Mai University English Language Team, for helping with manuscript editing.
Statement of Ethics

This cross-sectional study was performed in accordance with the Declaration of Helsinki. The study protocol was reviewed and approved by the Chiang Mai University Ethics Committee (approval number 278/2563) and registered in the Thai clinical trial registration (TCTR ID Number: 20190919002). All research participants provided a written informed consent.

Conflict of Interest Statement

The authors declare that they had no conflicts of interests.

Funding Sources

This study received funding support from the Faculty of Medicine Endowment Fund, Chiang Mai University, Thailand, under Grant No. (1) 068/2563. The authors have no commercial interest in any materials discussed in this article.

References

1. Lu S, Cheung CY, Liu J, Lim JH, Leung CK, Wong TY. Automated layer segmentation of optical coherence tomography images. IEEE Trans Biomed Eng. 2010 Oct;57(10):2605–8.
2. Raza AS, Cho J, de Moraes CG, Wang M, Zhang X, Kardon RH, et al. Retinal ganglion cell layer thickness and local visual field sensitivity in glaucoma. Arch Ophthalmol. 2011 Dec;129(12):1529–36.
3. Lang A, Carass A, Sotirchos E, Calabresi P, Prince JL. Segmentation of retinal OCT images using a random forest classifier. Proc SPIE Int Soc Opt Eng. 2013 Mar;8669:1667/494.
4. Niu S, Chen Q, de Sisternes L, Rubín DL, Zhang W, Liu Q. Automated retinal layers segmentation in SD-OCT images using dual-gradient and spatial correlation smoothness constraint. Comput Biol Med. 2014 Nov;54:116–28.
5. Resch H, Mitsch C, Pereira I, Schwarzhans F, Wasserman L, Hommer A, et al. Optic nerve head morphology in primary open-angle glaucoma and nonarteritic anterior ischaemic optic neuropathy measured with spectral domain optical coherence tomography. Acta Ophthalmol. 2018 Dec;96(8):e1018–24.
6. Polo V, Garcia-Martín E, Bambo MP, Pinilla J, Larrosa JM, Satue M, et al. Reliability and validity of Cirrus and Spectralis optical coherence tomography for detecting retinal atrophy in Alzheimer’s disease. Eye. 2014 Jun;28(6):680–90.
7. Dave P, Jethani J, Shah J. Asymmetry of retinal nerve fiber layer and posterior pole asymmetry analysis parameters of spectral domain optical coherence tomography in children. Semin Ophthalmol. 2017;32(4):443–8.
8. Arcinue CA, Bartsch DU, El-Emamy SY, Ma F, Doede A, Sharpsten L, et al. Retinal thinning and photoreceptor loss in HIV eyes without retinitis. PLoS One. 2015;10(8):e0132996.
9. Asrani S, Rosdahl JA, Allingham RR. Novel software strategy for glaucoma diagnosis: asymmetry analysis of retinal thickness. Arch Ophthalmol. 2011 Sep;129(9):1205–11.
10. Nguyen J, Rothman A, Gonzalez N, Avornu A, Ogbuo-kiri E, Balcer LJ, et al. Macular ganglion cell and inner plexiform layer thickness is more strongly associated with visual function in multiple sclerosis than bruch membrane opening-minimum rim width or peripapillary retinal nerve fiber layer thicknesses. J Neuroophthalmol. 2019;12;39(4):444–50.
11. Leung CK, Cheung CY, Weinreb RN, Qiu Q, Liu S, Li H, et al. Retinal nerve fiber layer imaging with spectral-domain optical coherence tomography: a variability and diagnostic performance study. Ophthalmology. 2009 Jul;116(763):1257e1–632.
12. Hammel N, Belghith A, Weinreb RN, Meireiras FA, Mendoza N, Zangwill LM. Comparing the rates of retinal nerve fiber layer and ganglion cell-inner plexiform layer loss in healthy eyes and in glaucoma eyes. Am J Ophthal-mol. 2017 Jun;178:38–50.
13. Pazos M, Dyrdal AA, Biarnés M, Gómez A, Martín C, Mora C, et al. Diagnostic accuracy of spectralis sd-oct automated macular layers segmentation to discriminate normal from early glaucomatous eyes. Ophthalmology. 2017;124(8):1218–28.
14. Langenegger SJ, Funk J, Töteberg-Harms M. Reproducibility of retinal nerve fiber layer thickness measurements using the eye tracker and the retest function of Spectralis SD-OCT in glaucomatous and healthy control eyes. Invest Ophthalmol Vis Sci. 2011 May;52(6):3338–44.
15. 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(9):e0137316.
16. Fiore T, Lupidi M, Androudi S, Gianfanti F, Fruttini D, Cagini C. repeatability of retinal macular thickness measurements in healthy subjects and diabetic patients with clinically significant macular edema: evaluation of the follow-up system of Spectralis optical coherence tomography. Ophthalmologica. 2015;233(3–4):186–91.
17. Çetinkaya E, Duman R, Duman MC, Sabaner MC. Repeatability and reproducibility of automatic segmentation of retinal layers in healthy subjects using Spectralis optical coherence tomography. Arq Bras Oftalmol. 201; 80(6):378–81.
18. Zangwill LM, Weinreb RN, Berry CC, Smith AR, Dirkes KA, Coleman AL, et al. Racial differences in optic disc topography: baseline results from the confocal scanning laser ophthalmoscopy ancillary study to the ocular hypertension treatment study. Arch Ophthalmol. 2004 Jan;122(1):22–8.
19. Song WK, Lee SC, Lee ES, Kim CY, Kim SS. Macular thickness variations with sex, age, and axial length in healthy subjects: a spectral domain-optical coherence tomography study. Invest Ophthalmol Vis Sci. 2010 Aug;51(8):3913–8.
20 Ooto S, Hangai M, Tomidokoro A, Saito H, Araie M, Otani T, et al. Effects of age, sex, and axial length on the three-dimensional profile of normal macular layer structures. Invest Ophthalmol Vis Sci. 2011 Nov 15;52(12): 8769–79.
21 Knight OF, Girkin CA, Budenz DL, Durbin MK, Feuer WJ, Group CONDS. Effect of race, age, and axial length on optic nerve head parameters and retinal nerve fiber layer thickness measured by Cirrus HD-OCT. Arch Ophthalmol. 2012 Mar;130(3):312–8.
22 Yamashita T, Tanaka M, Kii Y, Nakao K, Sakamoto T. Association between retinal thickness of 64 sectors in posterior pole determined by optical coherence tomography and axial length and body height. Invest Ophthalmol Vis Sci. 2013 Nov 13;54(12):7478–82.
23 Higashide T, Ohkubo S, Hangai M, Ito Y, Shimada N, Ohno-Matsui K, et al. Influence of clinical factors and magnification correction on normal thickness profiles of macular retinal layers using optical coherence tomography. PLoS One. 2016;11(1):e0147782.
24 Kim KY, Kwak HW, Kim M, Kim YG, Yu SY. New profiles of posterior pole retinal thickness map in healthy Korean eyes measured by spectral-domain optical coherence tomography. Retina. 2013;33(10):2139–48.
25 Invernizzi A, Pellegrini M, Acquistapace A, Benatti E, Erba S, Cozzi M, et al. normative data for retinal-layer thickness maps generated by spectral-domain OCT in a white population. Ophthalmol Retina. 2018;2(8):808–e1.
26 Mauschitz MM, Holz FG, Finger RP, Breteler MM. determinants of macular layers and optic disc characteristics on SD-OCT: the Rhineland Study. Transl Vis Sci Technol. 2019 May;8(3):34.
27 Nieves-Moreno M, Martínez-de-la-Casa JM, Cifuentes-Canorea P, Sastre-Ibáñez M, Santos-Bueso E, Sáenz-Francés F, et al. Normative database for separate inner retinal layers thickness using spectral domain optical coherence tomography in Caucasian population. PLoS One. 2017;12(7):e0180450.
28 Chauhan BC, Vianna JR, Sharpe GP, Demirel S, Girkin CA, Mardin CY, et al. differential effects of aging in the macular retinal layers, neuroretinal rim, and peripapillary retinal nerve fiber layer. Ophthalmology. 2020 02; 127(2):177–85.
29 Sullivan-Mee M, Ruegg CC, Pensyl D, Halverson K, Qualls C. Diagnostic precision of retinal nerve fiber layer and macular thickness asymmetry parameters for identifying early primary open-angle glaucoma. Am J Ophthalmol. 2013 Sep;156(3):567–77.e1.
30 Seo JH, Kim TW, Weinreb RN, Park KH, Kim SH, Kim DM. Detection of localized retinal nerve fiber layer defects with posterior pole asymmetry analysis of spectral domain optical coherence tomography. Invest Ophthalmol Vis Sci. 2012 Jul 1;53(8):4347–53.
31 Um TW, Sung KR, Wollstein G, Yun SC, Na JH, Schuman JS. Asymmetry in hemifield macular thickness as an early indicator of glaucomatous change. Invest Ophthalmol Vis Sci. 2012 Mar 2;53(3):1139–44.
32 Hwang YH, Ahn SI, Ko SJ. Diagnostic ability of macular ganglion cell asymmetry for glaucoma. Clin Exp Ophthalmol. 2015 Nov;43(8):720–6.
33 Yamada H, Hangai M, Nakano N, Takayama K, Kimura Y, Miyake M, et al. Asymmetry analysis of macular inner retinal layers for glaucoma diagnosis. Am J Ophthalmol. 2014 Dec;158(6):1318–e3.
34 Kawaguchi C, Nakatani Y, Ohkubo S, Higashide T, Kawaguchi I, Sugiyama K. Structural and functional assessment by hemispheric asymmetry testing of the macular region in preperimetric glaucoma. Jpn J Ophthalmol. 2014 Mar;58(2):197–204.
35 Altan C, Arman BH, Arici M, Urdem U, Solmaz B, Pasaoğlu I, et al. Normative posterior pole asymmetry analysis data in healthy Caucasian population. Eur J Ophthalmol. 2019 Jul;29(4):386–93.
36 Casado A, Cerveró A, López-de-Eguieta A, Fernández R, Fonseca S, González JC, et al. Topographic correlation and asymmetry analysis of ganglion cell layer thinning and the retinal nerve fiber layer with localized visual field defects. PLoS One. 2019;14(9):e0222347.
37 Chen MJ, Yang HY, Chang YF, Hsu CC, Ko YC, Liu CJ. Diagnostic ability of macular ganglion cell asymmetry in preperimetric glaucoma. BMC Ophthalmol. 2019 Jan 8;19(1):12.
38 Takemoto D, Higashide T, Ohkubo S, Udagawa S, Sugiyama K. Ability of macular inner retinal layer thickness asymmetry evaluated by optical coherence tomography to detect preperimetric glaucoma. Transl Vis Sci Technol. 2020;9(5):8.
39 Yamashita T, Sakamoto T, Kakiuchi N, Tanaka M, Kii Y, Nakao K. Posterior pole asymmetry analyses of retinal thickness of upper and lower sectors and their association with peak retinal nerve fiber layer thickness in healthy young eyes. Invest Ophthalmol Vis Sci. 2014 Aug 12;55(9):5673–8.
40 Lee KM, Lee EJ, Kim TW, Kim H. Comparison of the abilities of SD-OCT and SS-OCT in evaluating the thickness of the macular inner retinal layer for glaucoma diagnosis. PLoS One. 2016;11(1):e0147964.