

1 Longitudinal analysis of the peripapillary retinal nerve fiber layer thinning in

2 patients with retinitis pigmentosa

3 Running head: longitudinal analysis of RNFL in RP

4 Akio Oishi, Ken Ogino, Satoko Nakagawa, Yukiko Makiyama, Masafumi

5 Kurimoto, Atsushi Otani, Nagahisa Yoshimura

6

7 Department of Ophthalmology and Visual Sciences, Kyoto University Graduate

8 School of Medicine, Kyoto, Japan

9

10 Corresponding author: Akio Oishi

11 54, Shougoin Kawahara-cho, Sakyo-ku, Kyoto 606-8507, Japan

12 Telephone number: (81)-75-751-3248

13 Fax number: (81)-75-752-0933

14 E-mail address: aquio@kuhp.kyoto-u.ac.jp

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18 tomography

19 **Abstract**

20 Purpose: To investigate longitudinal changes in peripapillary retinal nerve fiber
21 layer (RNFL) thickness in patients with retinitis pigmentosa (RP).

22 Methods: We re-examined 103 RP patients whose RNFL thickness was
23 previously examined and reported. RNFL thickness was measured using Stratus
24 optical coherence tomography and was compared with the previous
25 measurements. The results were also compared with that of previously reported
26 normal subjects. Association between the decrease rate and visual acuity and
27 visual field was also investigated.

28 Results: The mean follow-up period was 56.9 months. After excluding patients in
29 whom RNFL images were of poor quality, 88 patients were eventually analyzed.
30 The average RNFL thickness decreased from 105.8 to 98.2 μm during the period,
31 with the average rate of decrease being 1.6 $\mu\text{m}/\text{year}$. The decrease in RNFL was
32 more evident in superior and inferior sectors. Cross sectional linear regression
33 analysis also revealed an age-dependent decrease in RNFL, with the slower
34 rate of decrease being 0.94 $\mu\text{m}/\text{year}$. The decrease in RNFL thickness was
35 significantly faster than that reported in normal subjects. The decrease rate was
36 not associated with visual functions.

37 Conclusion: Age-dependent RNFL thinning occurs at a faster rate in RP patients
38 as compared to that in normal subjects. The result supports the notion that
39 pathologic changes involve inner retina as well as outer retina in eyes with RP.
40 Considering the discrepancy in the rate of RNFL thinning estimated from trend
41 analysis and longitudinal measurement, care should be taken when interpreting
42 the result of cross sectional analysis.

43

44 **Introduction**

45 Retinitis pigmentosa (RP) is a hereditary heterogenous disease, which primarily
46 affects rod photoreceptors. As a consequence of rod photoreceptor death,
47 patients experience night blindness and peripheral visual field loss in the early
48 stages of the disease.¹ Several therapeutic strategies for RP, including gene
49 therapy, cell transplantation therapy, and retinal prosthesis, have been
50 intensively investigated in recent times.² Each of these has demonstrated
51 promising effects, and some are currently under clinical trials.^{3, 4}

52 However, the effect of RP on the preservation of second or third neurons—which
53 are necessary for conveying visual information to the lateral geniculate bodies or
54 visual cortex remains unclear.⁵ If the status of inner retina varies in each patient,
55 selection of patients based on the preservation of inner retinal structures and
56 functions would be helpful to achieve maximal effect from the treatments.⁶

57 Histology studies concerning the status of the inner retina in RP are limited.⁷⁻¹⁰

58 These histologic reports showed that up to 75% of ganglion cell layer cells are
59 retained in the macular area in patients with RP^{7, 8}; however, the percentage
60 decreases to 20%-30% in extra-macular region⁹ and 70–90% of total ganglion
61 cells or theirs axons are lost eventually¹⁰, suggesting that inner retinal cells are

62 partially preserved in RP but degenerate as the disease progresses. These
63 reports provide important information; however, the method of postmortem
64 analyses cannot be used for the evaluation of patient suitability for the future
65 treatment.

66 Several groups, including ours, have attempted to evaluate the inner retinal
67 status *in vivo* using optical coherence tomography (OCT) (Table 1). For example,
68 Walia et al. reported abnormal thinning and thickening of the retinal nerve fiber
69 layer (RNFL).^{11, 12} The abnormal thickening of the RNFL was also observed by
70 Hood et al.⁵ Consistently, a recent study dealing young subjects also showed
71 relatively thick RNFL.¹³ We have previously reported wide variations in RNFL
72 thickness in RP patients albeit with the average thickness being similar to that in
73 normal eyes. In addition, RNFL thickness in RP patients appears to decrease
74 faster than that observed in normal eyes.¹⁴ Anastasakis et al. used a more
75 recent model of OCT and confirmed the abnormal thickening and thinning of
76 RNFL. In addition, they reported a similar rate of age-dependent decrease in
77 RNFL thickness.¹⁵ The effect of using different models of OCT can be estimated
78 by the result of another recent report.¹⁶ However, all these reports are cross
79 sectional studies and information regarding the changes in RNFL thickness over

80 time remains limited. The rate of age-dependent change estimated from trend
81 analysis does not necessarily coincide with longitudinal measurement. In fact, a
82 recent study concerning the RNFL thickness in normal subjects revealed
83 discrepancies in the longitudinal and cross-sectional data;¹⁷ therefore,
84 longitudinal measurement is necessary to evaluate time-dependent changes in
85 RP patients in clinical practice.

86 In the present study, in order to evaluate longitudinal changes in RNFL thickness
87 in RP patients, we re-examined patients in whom the RNFL thickness was
88 previously assessed and reported approximately 5 years ago. Further, we
89 statistically analyzed these changes in RNFL thickness for age dependence and
90 disease progress.

91 **Methods**

92 **Subjects**

93 We re-recruited RP patients whose RNFL thickness results we previously
94 reported in 2008.¹⁴ The study sample consisted of 137 eyes from 137 RP
95 patients (including 2 patients with Usher syndrome) who were first examined
96 between January 2006 and April 2007. The exclusion criteria were as follows:
97 best-corrected visual acuity worse than 0.1 (20/200), presence of optic nerve

98 diseases or retinal vascular diseases, refractive errors greater than $-6D$, OCT
99 signal strength < 6 , or OCT image showed evident artifact. These patients were
100 re-examined between May 2011 and April 2012 during a follow-up visit to our
101 institution.

102 All procedures conformed to the tenets of the Declaration of Helsinki, and the
103 study design was approved by the institutional review board and the ethics
104 committee of the Kyoto University Graduate School of Medicine. The aim of the
105 study and the measurement procedures were explained to the study participants.
106 The review board waived the need for written informed consent. We certify that
107 all applicable institutional and governmental regulations concerning the ethical
108 use of human volunteers were followed during this research.

109 Patients were examined using the Stratus OCT (Carl Zeiss Meditec, Inc., Dublin,
110 CA), which is not a recent OCT; however, this was the model used in the
111 previous study and was re-used to avoid interdevice variations. Peripapillary
112 RNFL was measured with the Fast RNFL scan option, comprising 3 circular
113 scans of a diameter of 3.4 mm around the optic disc. As in the previous study, we
114 excluded the patients in whom signal strength of the image did not reach 7 or in
115 whom segmentation of the image exhibited artifacts.

116 Among the measurement parameters, the RNFL thickness in the 12 divided
117 sectors, quadrant sectors, and the average RNFL thickness were used for
118 statistical analyses. In addition, according to the study of Wallia et al.,¹¹ we
119 counted the number of sectors showing abnormal thinning or thickening in 12
120 divided sectors. When the color map image showed yellow or red, the sector
121 was judged as “thinning” and white was judged as “thickening”.¹¹
122 We assigned a visual field score for each case based on the previously reported
123 system¹⁴ with some modifications. Based on a previous histological report,
124 which indicated that the central 10- and 30-degree of retina contains up to 34%
125 and 69% of the total number of retinal ganglion cells, respectively,¹⁸ we divided
126 the visual field into concentric circles of central 0–10 degrees, central 10–30
127 degrees, and >30 degrees. Each concentric circle was further divided into
128 quadrant sectors. Theoretically, each sector contains a similar number of
129 ganglion cells (central 0–10 degrees: 8.5%, 10–30 degrees: 8.5%, and >30
130 degrees: 7.8%). We assigned a score for remaining visual field measured with
131 the V/4e isopter of Goldmann kinetic perimetry (GP). A score of 1 was assigned
132 for the remaining visual field in each sector. When the remaining visual field
133 occupied more than half but not the total extent of the sector, we gave a score of

134 0.5 (Figure 1).

135

136 Statistical analysis

137 The statistical program SPSS version 19 (IBM Japan, Tokyo, Japan) was used

138 for the analysis. Excel 2010 version 14.0.6112.5000 (Microsoft Japan, Tokyo,

139 Japan) was also used to compare the present data with previously reported

140 values. Descriptive analyses are reported as means $\pm$ SD unless otherwise

141 specified. The average RNFL thickness, RNFL thickness of each sector, and

142 signal strength of the OCT image in the present study were compared with the

143 previous measurements using the paired t-test. To estimate the age-dependent

144 decrease in RNFL thickness, a linear regression model was used with the

145 average RNFL as the dependent variable and age, refraction error, visual acuity,

146 and the visual field score as independent variables. To investigate the effect of

147 the changes in each parameter for changes in RNFL thickness, another linear

148 regression analysis was performed with the change of average RNFL thickness

149 as the dependent variable and observation period, change of OCT signal,

150 change of visual acuity, and change of visual field score as independent

151 variables. Correlations between each parameter were further analyzed with

152 Spearman's rank correlation test. P values less than 0.05 was regarded as
153 significant. We searched for previous reports on PubMed concerning Stratus
154 OCT-measured RNFL of normal subjects. Among them, articles that included
155 linear regression coefficients with 95% confidential intervals were selected for
156 analysis.¹⁹⁻²² We calculated the standard error from the 95% confidential
157 intervals and compared these values with the measurements in the present
158 study using the *t*-test.

159 **Results**

160 We successfully reevaluated 103 (75.2%) patients out of the original population
161 of 137 patients. Generally, the examination was performed smoothly; however,
162 15 patients were excluded due to poor OCT image quality (7 or worse; 9
163 patients) and evident artifacts in RNFL segmentation (6 patients). Poor image
164 quality was due to progression of cataract, vitreous opacity, or fixation loss. A
165 decrease in signal strength, which affects thickness measurement,^{23, 24} was
166 observed even in the included subjects (9.0 ± 1.1 to 8.5 ± 1.1 , $P = 0.002$). Thus,
167 the final study population consisted of 88 patients (39 men and 49 women). At
168 the initial examination, the mean age was 50.4 ± 13.8 years (range, 20–77
169 years); the refractive error, -1.5 ± 2.4 D (range, +3.75 to -5.875); logMAR, $0.18 \pm$

170 0.33 units (range, -0.18 to 1.0); and GP score, 7.6 ± 2.8 (range, 2–12). The
171 average duration between the previous and the present examination was $56.9 \pm$
172 4.4 months (range, 50.8–68.7 months).

173 The average RNFL thickness decreased from $105.8 \pm 22.7 \mu\text{m}$ to $98.3 \pm 23.2 \mu\text{m}$
174 in the 5-year period (Figure 2A). Linear regression model showed that the
175 change of OCT signal is partly responsible for the decrease of measurement.
176 ($2.1 \mu\text{m}$ of decrease/1 unit of change, $P=0.007$) The mean rate of decrease was
177 calculated as $1.63 \pm 2.0 \mu\text{m/year}$ (range, -8.3–+3.4 $\mu\text{m/year}$). The decrease was
178 more evident in the superior and inferior sectors (Figure 2B, exact values are
179 presented in online only table). OCT images from a representative case are
180 shown in Figure 3.

181 A negative correlation was noted between the average RNFL and age; using
182 cross-sectional multivariate linear regression analysis, the RNFL thickness was
183 noted to decrease by $0.94 \mu\text{m/year}$ ($P<0.001$, 95% CI: 0.64 to 1.24, Figure 2 C).

184 Baseline age was also associated with change of RNFL in the observation
185 period ($\rho = -0.2$, $P=0.05$). Refractive errors, visual acuity, and visual field had no
186 significant effects on RNFL thickness in multivariate linear regression analysis.

187 The result was consistent with the previous result that RNFL thinning is not

188 necessarily associated with the degree of visual impairment.¹⁴ However,
189 refractive error and the rate of decrease in RNFL thickness showed a weak
190 association ($\rho = -0.30$, $P = 0.004$; hyperopic eyes showed a faster rate of
191 decrease). GP score did not show significant effect on average RNFL thickness
192 but was associated with decrease of RNFL in the inferior sector ($\rho = 0.21$,
193 $P=0.048$; better GP score showed a smaller decrease of the inferior sector
194 RNFL).

195 Figure 2D shows the longitudinal measurements for the decrease in RNFL
196 thickness in each patient based on the baseline age. Most patients showed a
197 decrease in the RNFL thickness at a rate of 0 to $-4 \mu\text{m}/\text{year}$; however, 13
198 patients (14.8%) showed an increase in RNFL thickness. We compared these 13
199 patients and the rest of the subjects in age, sex, refractive error, visual acuity,
200 and visual field score but there was no significant differences ($P=0.38$, 0.20 , 0.07 ,
201 0.44 , 0.14 , respectively).

202 Since we did not have healthy controls who were followed up for 5 years, we
203 compared the previously reported rates of decrease in RNFL thickness in normal
204 eyes. We found 4 studies that examined normal subjects using Stratus OCT and
205 reported the rate of decrease in RNFL thickness along with 95% confidence

206 intervals (Table 2). The reported rate of decrease in RNFL thickness ranged
207 between -0.16 and -0.26 $\mu\text{m}/\text{year}$.¹⁹⁻²² We calculated standard deviations and
208 standard errors from the reported means and 95% CIs and we then compared
209 the value with the present result. The regression coefficient in the present study
210 indicated a significantly faster rate of decrease in RNFL thickness than that in
211 these 4 reports ($P < 0.001$ for all 4 comparisons).
212 We also investigated the abnormal thinning and thickening noted in certain
213 patients. At the baseline examination, abnormal thinning was noted in 1.1 ± 1.9
214 sectors, while thickening was noted in 2.7 ± 2.6 sectors. In the present
215 assessment, the number of sectors with abnormal thinning increased to 1.8 ± 2.2
216 ($P < 0.001$) and with thickening decreased to 2.4 ± 2.3 with non-significant
217 P-value ($P = 0.057$).

218

219 **Discussion**

220 The present study investigated time-dependent changes in RNFL thickness in
221 RP patients. The average RNFL thickness decreased by 7.1% in approximately
222 5 years in these patients, and the rate of RNFL thinning was higher than that
223 previously reported in healthy subjects.

224 The linear regression model showed a decrease in RNFL thickness of 0.94

225 $\mu\text{m}/\text{year}$ in the RP patients, which was similar to that previously reported (-0.65
226 μm per year, within the 95% CI in the present study) using a different OCT
227 model.¹⁵ However, longitudinal RNFL thickness measurements demonstrated
228 that RNFL thickness in RP patients decreased at a rate of $1.63 \mu\text{m}/\text{year}$. A similar
229 discrepancy in longitudinal measurements and linear regression coefficient
230 measurements for RNFL thickness was recently reported in normal subjects,¹⁷
231 suggesting that applying the linear regression model may not always be valid for
232 evaluating RNFL thinning in RP patients. Leung et al. clarified that age-related
233 changes in individuals should be determined from longitudinal data and not
234 based on the extrapolation of cross-sectional data;¹⁷ this can also be applied in
235 the present case.

236 The RNFL thinning did not progress equally in each quadrant. The sectoral
237 difference was already reported in normal subjects but it is not consistent as to
238 which quadrant significantly decreases with age.^{17, 19, 21} In the present study,
239 inferior and superior sector showed significant thinning but nasal and temporal
240 sector did not. In addition, association between visual field score and RNFL
241 decrease rate was found only in inferior quadrant implying the correlation
242 between disease stage and progression pattern of RNFL thinning. Sectoral

243 difference can be an important issue when considering the site of visual
244 prosthesis implantation or cell transplantation. It should be further investigated.
245 Abnormal thickening as well as thinning of the RNFL has been previously
246 reported in RP patients.^{5, 11, 12} In fact, some patients showed thick RNFL at
247 baseline and after the follow-up. However, the number of sectors with thinning or
248 thickening also showed the trend of thinning; the number of sectors with RNFL
249 thinning increased, while that with RNFL thickening tended to decrease. These
250 findings suggest that abnormal RNFL thickening certainly occurs in RP patients;
251 however, the overall RNFL thickness continues to decrease over time. If patients
252 are examined over longer follow-up periods or only patients in advanced stages
253 are examined, a predominance of abnormal thinning may be noted with no
254 findings of RNFL thickening. Although the patients whose RNFL thickness
255 increased during the follow up period did not show specific characteristics in the
256 present study and the change can be a variability of the measurement,
257 investigating which patients and when these patients show thickening of RNFL
258 would be of interest since it would highlight the pathological process of RP in the
259 retinal cells other than photoreceptors.
260 RNFL thickness is reported to be affected by signal strength, with low signal

261 strength being associated with RNFL thinning.^{23, 24} Generally, obtaining
262 good-quality OCT images in RP patients is more difficult than that in normal
263 subjects due to cataract, vitreous opacity, unstable fixation, etc. Increasing
264 patient age and disease progression render obtaining OCT images even more
265 difficult. In the present study, 15 patients were excluded due to signal strength <
266 6 or the presence of evident artifacts. Moreover, the average signal strength of
267 the included patients was also observed to have decreased in comparison to the
268 baseline. According to the linear regression analysis, around 1 μ m of the
269 decrease observed in the present study could be explained by the change of
270 signal strength.

271 In the present study, we used the Stratus OCT instead of a later model. Although
272 the latest models of spectral domain OCT provide better resolution and higher
273 reproducibility,¹⁶ previous reports comparing measurements from these models
274 showed that the results from differing models, while highly correlated, are not
275 interchangeable.²⁵⁻²⁸ Since the Stratus OCT was used in our previous study of
276 the same study population, we used the same model for the present study. The
277 Stratus OCT does not have an eye-tracking system and automated registration.
278 In addition, acquisition of fundus image is done after the OCT measurement is

279 finished thus the placement of scan circle is not completely precise. These
280 limitations in variation of measurement should be noted. We now examine
281 patients with spectral domain OCT, and longitudinal analyses in the future will be
282 based on the result from the latest OCT models.

283 The present study was limited by its non-prospective design and lack of normal
284 control subjects since we did not have access to healthy subjects who were
285 followed up for 5 years; this is probably the case for most institutions. Age-,
286 gender-, and ethnicity- matched control would provide more robust conclusion. A
287 prospective study that strictly compares the rates of change in RNFL thickness
288 between normal controls and RP patients is required in the future.

289 The results of the present study demonstrated progressive age-related loss of
290 RNFL thickness in RP patients based on a longitudinal analysis of OCT images.

291 Our results indicate that the integrity of the inner retina should be carefully
292 evaluated in each RP patient before determining the therapeutic strategy.

293
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295

296 **References**

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431

432 **Figure legends**

433

434 Figure 1. Method and an example of visual field scoring in the present study. A:

435 We divided the visual field into concentric areas of 0–10 degrees, 10–30

436 degrees, and >30 degrees. Each area was further divided into quadrant sectors.

437 When the remaining visual field occupied a sector, one point was assigned.

438 When the visual field occupied more than half but not all of each sector, a score

439 of 0.5 was assigned. B: In the presented case, 1 point × 4 quadrant sectors of

440 central 10 degree and 0.5 point × 2 quadrants to lower > 30 degree were

441 assigned. Visual field in 10–30 degree sectors and in upper sectors of >30

442 degree were judged as less than half of the sector and points were not assigned;

443 i.e. visual field score for the case was 5 points.

444

445 Figure 2. Changes in retinal nerve fiber layer (RNFL) thickness in patients with

446 retinitis pigmentosa. RNFL thickness was measured after approximately 5 years.

447 The scatter plot demonstrates that most patients experienced a decrease in

448 RNFL thickness (A). The line chart shows the changes in the RNFL thickness in

449 each sector (B). The upper and lower lines represent the baseline and the

450 follow-up (after 5 years) measurements of RNFL thickness, respectively.
451 Decrease in RNFL thickness was statistically significant in the inferior and
452 superior sectors. Figure 2 C and D shows correlation between age and RNFL
453 thickness. The regression line indicates a slope of -0.938 for the multiple linear
454 regression model (C). Baseline age and the rate of RNFL changes calculated
455 from longitudinal measurements did not show evident trend (D). T: temporal, S:
456 superior, N: nasal, I: inferior, *:P<0.05, **:P<0.01.

457

458 Figure 3. A representative case with retinitis pigmentosa showing marked
459 thinning of the retinal nerve fiber layer (RNFL). RNFL thickness was measured in
460 this 51-year-old woman after a 5-year interval. Fundus photographs show the
461 progression of the disease as indicated by pigmentation and retinal pigment
462 epithelium atrophy.(A, E) Scan alignment, image quality, and segmentation of
463 RNFL were confirmed for each measurement.(B, C, F, G) The average RNFL
464 thickness decreased from $102.88\text{ }\mu\text{m}$ (D) to $75.82\text{ }\mu\text{m}$ (H) at the end of 5 years
465 follow up in this patient.

466

467 Table 1 Previous reports on RNFL thickness in RP patients

Report	Numbers of eyes/patients	OCT model	Age (years, range)	RNFL thickness (μm)	Rate of decrease in RNFL thickness ($\mu\text{m}/\text{year}$)
Walia ¹¹	25/25	Stratus	48.6 (23 to 73)	97.0 $\pm$ 19.7	NA
Walia ¹²	97/52	Optovue	39.7 (12 to 78)	NA, abnormal thinning in 38.1%, thickening in 21.7%	NA
Oishi ¹⁴	137/137	Stratus	50.0 $\pm$ 14.1 (15 to 78)	104.1 $\pm$ 21.7	−0.83 (95% CI, −0.60 to −1.07)
Hood ⁵	30/30	Spectralis	33.1 $\pm$ 15.9 (11 to 65)	128.2 $\pm$ 16.7	NA

Tamaki ²⁹	86/45	Cirrus	58.7 (13 to 79)	Right: 93.2 ± 14.6 Left: 84.6 ± 17.4	NA
Anastasakis ¹⁵	50/30	OPKO SD-OCT	45.8 ± 16.3 (15 to 73)	100.1 ± 18.8	−0.65
Sliesoraityte ³⁰	24/12	Spectralis	44 ± 14	NA	NA
Garcia-Martin ¹⁶	42/42	Stratus Cirrus Spectralis	40.0 ± 8.6 (35 to 69)	78.1 ± 14.5 76.4 ± 9.3 82.9 ± 10.4	NA
Hwang ¹³	36/36	Cirrus	23.1 ± 3.6 (20 to 30)	112.8 ± 17.0	NA

468 NA: Not available

469

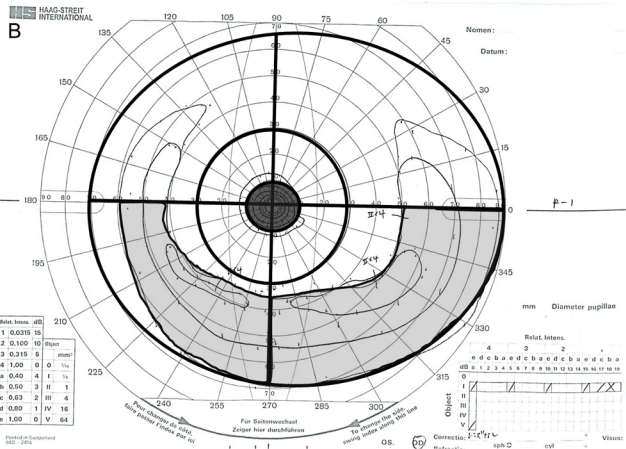
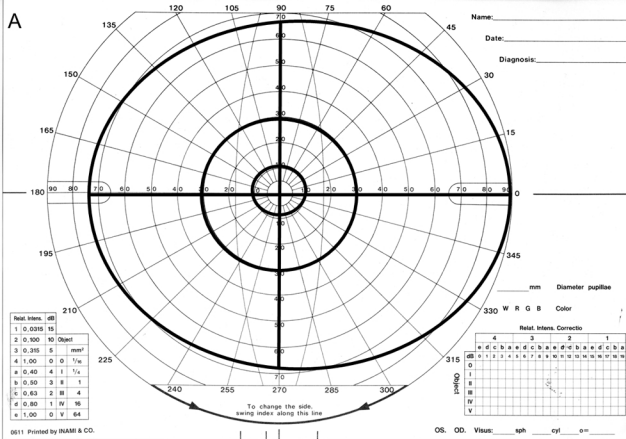
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471 Table 2 Previous reports on RNFL thickness of healthy subjects measured with

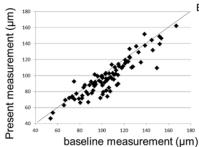
472 Stratus OCT

Report	Numbers of eyes/subjects	Age (years, range)	RNFL thickness (μm)	Rate of decrease in RNFL thickness ($\mu\text{m}/\text{year}$) (95% CI)
Parikh ¹⁹	187/187	33.0 ± 19.7 (5 to 75)	97.3 ± 11.3	-0.16 (-0.1 to -0.24)
Budenz ²⁰	328/328	47.4 ± 15.8 (18 to 85)	100.1 ± 11.6	-0.199 (-0.279 to -0.119)
Sung ²¹	226/124	47.5 ± 15.9 (18 to 85)	100.8 ± 10.5	-0.255 (-0.439 to -0.071)
Feuer ²²	425/425	46 ± 16 (18 to 85)	104.7 ± 10.8	-0.24 (-0.31 to -0.18)

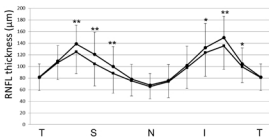
473



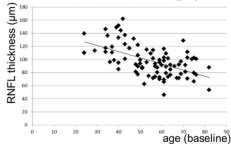
A



B



C



D

