Computerized densitometric measurement system (CDMS) for the quantitative analysis of diffuse retinal nerve fiber layer atrophy

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Since observable retinal nerve fiber layer (RNFL) atrophy occures several years before irreversible glaucomatous visual field damage in glaucoma patients, quantitative assessment of the diffuse RNFL atrophy has been the goal of many research efforts. The loss of RNFL reflectance is informative sign of the diffuse RNFL atrophy, and appears in monochrome photograph of RNFL. We have, therefore, made a system by which we could measure the loss of RNFL reflectance and named it Computerized Densitometric Measurement System (CDMS). In this method, density profiles concentric with optical disc are extracted from the digitalized RNFL photograph. Density integral of superior and inferior segments in the density profile is used for the assessment of RNFL atrophy. Satisfactory results were obtained about the clinical utility of this method when the CDMS measurement was compared with the indicator of loss of visual function. Intra- and inter-operator reproducibility was also excellent.

Introduction

The early diagnosis of glaucoma and the early detection of glaucomatous progression is important because glaucomatous damage is irreversible. Prevention of glaucomatous injury is an essential strategy available to ophthalmologists [1].

Since observable retinal nerve fiber layer (RNFL) loss occurs several years before measurable glaucomatous visual field (VF) damage in glaucoma patients [2–4], the examination of RNFL has been regarded as a useful means to detect and monitor this glaucomatous damage [4–9]. For instance, Sommer and co-workers [10] found that defects in the RNFL predicted the development of visual field damage, and the position of RNFL atrophy was correlated with the half of the field in which functional defects were to appear.

RNFL atrophy can be divided into two kinds—localized wedge-shaped defect and diffuse atrophy of upper or lower retina [11]. Interpretation of the RNFL and determination of the levels of severity is difficult, particularly in the grading of diffuse atrophy [12].

Therefore, objective and quantitative assessment of the diffuse RNFL atrophy has been the goal of many research efforts. Systems and instruments, such as ocular coherence tomography and scanning laser polarimetry, have been developed to measure the thickness of RNFL [1,13–17]. Even though they may provide inherently quantitative information and show good correlation with the results of conventional means of assessing glaucoma, they are not readily available for clinical use because of their high costs. Therefore, many ophthalmologists still depend on the photography of RNFL, which is a subjective and qualitative method.

Although some investigators tried to improve the objectivity in interpretation of RNFL photographs by using a 4-level grading system [11] or a reference set of photographs [18], the methods are still dependent on subjective judgment by the observers.

The aim of this study was to develop a quantitative system for the measurement of diffuse RNFL atrophy, which should not be influenced by the judgment of observers and be clinically valid. The loss of RNFL reflectance that accompanies diffuse atrophy of the RNFL is informative sign that appears in monochrome RNFL photographs. We have, therefore, made a system by which we could measure the loss of RNFL reflectance by profiling the density of retinal regions in monochrome RNFL photographs, and named it Computerized Densitometric Measurement System (CDMS) for RNFL photographs.

In this paper, we will present detail description of the CDMS, and show its clinical validity by comparing the CDMS measurements to retinal sensitivities. We will also show that the CDMS measurements are barely affected by the operation of observers.

Methods

RNFL photograph

Three RNFL photographs were taken from each eye with a Canon CF60U fundus camera, fitted with a blue filter and using TMX 100 Kodak film. We selected photographs that had well-focused vessels and a width of peripapillary atrophy no larger than one-third of the vertical diameter of the optic disc. Photographs exhibiting wedge-shaped defect or re-

flection from the internal limiting membrane were excluded.

Computerized Densitometric Measurement System (CDMS)

RNFL photographs were digitized using a scanner (ScanJet 4C/T, Hewlett-Packard Co., Palo Alto, CA, USA), and saved in monochrome GIF format. A semi-automatic image analysis program was developed using MATLAB (MathWorks Inc., Natick, USA). Procedure of analysis is as follows.

Step 1: User clicks two points on the vertical ends of the optic disc and one point on the darkest area of the fovea. The distance between the points on the vertical ends was defined as the diameter of the optic disc, and the center of the points was defined as the center of the optic disc.

Step 2: Two peripapillary circles, concentric with the optical disc and of radius 1.5 and 2.5 times that of the optical disc ('1.5 times peripapillary circle' and '2.5 times peripapillary circle') were drawn (figure 1). With 0 degrees defined as temporal, the following four segments were defined: superior (32–122°), nasal (122–238°), inferior (238–328°) and temporal (328–32°).

Step 3: Density profiles (grey level variations) along both circles were measured at one degree intervals. The density profiles were scaled from 0 to 1. The maximum (brightest) value in the optic disc was set as 1 and minimum (darkest) in the fovea as 0.

Step 4: The area under the normalized density profile in each segment was defined as the 'density integral' of that segment.

Step 5: Step 1–4 was repeated three times and the density integrals of three measurements were averaged. Density integrals of superior and inferior segments of both the 1.5 times peripapillary circle (SUP 1.5 and INF 1.5) and the 2.5 times circle (SUP 2.5 and INF 2.5) were used for the study of RNFL.

Figure 2 shows sample density profiles and density integrals defined above.

Validation study

The patient whose best corrected visual acuity under 20/20 or who had any cause of visual field defect other than glaucomatous damage was excluded, e.g. diabetic retinopathy, uveitis, cataract, and any media opacities. Thirty-nine photographs of 39 patients (mean age: 45.2, range: 19–71, M/F = 24/15) were randomly selected from the RNFL photograph file. The diagnoses were primary open-angle glaucoma (9 patients), normal-tension glaucoma (11), glaucoma suspect (18), and secondary glaucoma (1). Twenty eyes were right and 19 were left.

The clinical validity of the CDMS was evaluated by comparing the density integrals in the superior (SUP 1.5 and SUP 2.5) and those in inferior segments (INF 1.5 and INF 2.5) to the calculated mean sensitivity of corresponding visual field regions (figure 3).

Spearman's rank correlation coefficient was used for this analysis. The visual field was divided into ten sectors on the basis of the glaucoma hemifield test sector definitions [19]. The superior visual field region was

Figure 1. Two concentric peripapillary circles on the retinal nerve fiber layer photograph. With zero degrees defined as temporal, four segments were defined.

Superior	: 59.9	34.3
Nasal	: 40.2	23.0
Inferior	: 65.5	37.5
Temporal	: 9.00	5.16
Total	: 174	100 %
	Density integral	Percent

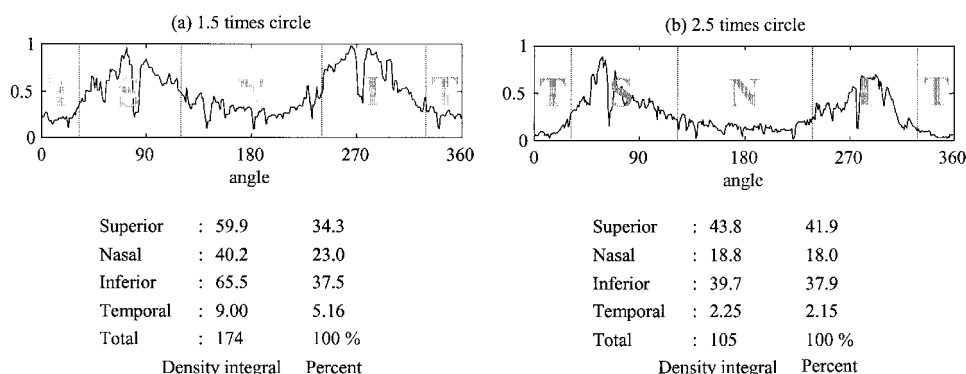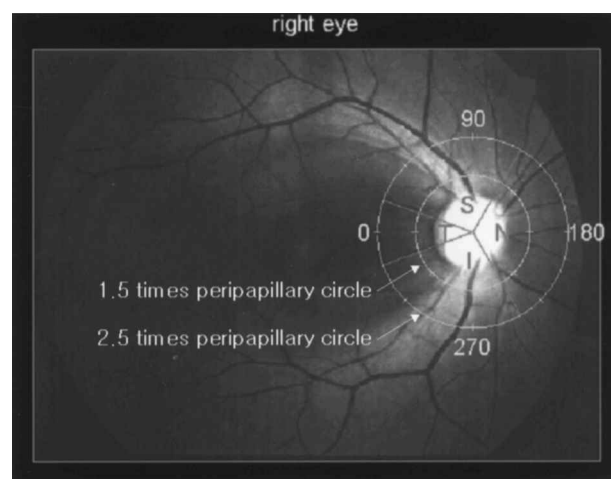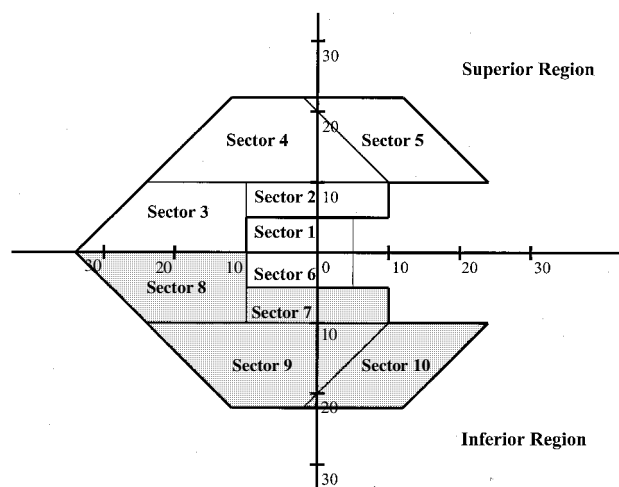| Superior | : 43.8 | 41.9 |
Nasal	: 18.8	18.0
Inferior	: 39.7	37.9
Temporal	: 2.25	2.15
Total	: 105	100 %
	Density integral	Percent

Figure 2. Density profiles (upper), and the area under the normalized density profile in each segment, which was defined as the 'density integral' of that segment (lower). (a) 1.5 times peripapillary circle, (b) 2.5 times peripapillary circle.

Figure 3. Sectors of glaucoma hemifield test. Superior field: sector 2 − 5. Inferior field: sector 7 − 10.

defined as sectors 2 through 5 and the inferior as sectors 7 through 10. SUP 1.5 and SUP 2.5 were compared to the sensitivity of inferior visual field (IVF), and INF 1.5 and INF 2.5 were compared to the sensitivity of superior visual field (SVF).

To see whether the CDMS measurements are affected by the operation of observers, two operators performed computerized densitometry independently, on the first 20 RNFL photographs on two separate occasions. Pearson's correlation coefficient was used to determine the relationship between measurements taken from the two sessions and taken by the two operators.

Results and discussion

Correlations between the CDMS measurements (SUP 1.5, SUP 2.5, INF 1.5, and INF 2.5) and mean sensitivities of visual field (IVF, and SVF) were moderate, but statistically significant (Spearman's rank correlation coefficients = 0.51 − 0.55, $p < 0.01$). Scatter plots between CDMS measurements and mean sensitivities of visual field are shown in figure 4.

The moderate relationship between CDMS and sensitivity of visual field would be satisfactory since the inherent limitation exists in the cross-sectional study in which one compare the result of structural (CDMS) measurements to that of functional (sensitivity of visual field) measurements [20]. We can consider several reasons. Visual function is often compromised only after RNFL has been damaged [4,10,12], and the amount of RNFL damage necessary for visual field loss may vary by individual. One might, therefore, expect that the strength of this association could vary depending on the glaucoma patients included in the study and the severity of their disease. Longitudinal studies of association between changes in the RNFL and changes in visual function might be more informative [20].
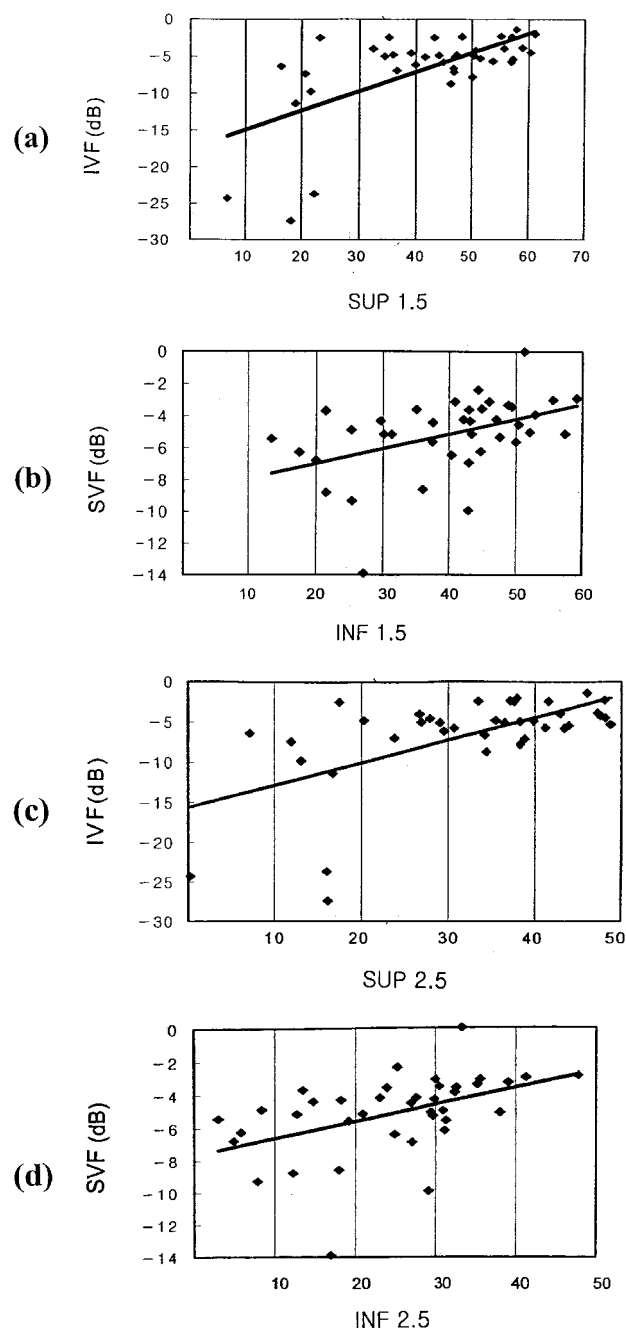

Figure 4. Scatter plots between CDMS measurement (density integral) and mean sensitivity of visual field. (a) SUP 1.5 vs. IVF (r=0.53, p <0.01), (b) INF 1.5 vs. SVF (r=0.52, p <0.01), (c) SUP 2.5 vs. IVF (r=0.51, p <0.01), (d) INF 2.5 vs. SVF (r=0.55, p <0.01).

The consistency of measurements obtained by a single operator was excellent (Pearson's correlation coefficients = 0.98 − 1.00), and the agreement between operators was also high (Pearson's correlation coefficients = 0.96 − 1.00) (tables 1 and 2). These results show that the measurements by CDMS are very robust, so that they are barely affected by the operation of the observers.

An important factor limiting the use of photography for quantitative measurements of reflectance is the dependence of film density on several poorly controlled

Table 1. Correspondences between the 1st and the 2nd measurements of density integrals in the nerve fiber layer photographs within operator.

Operator	Density integral	Pearson's correlation coefficient
1	SUP 1.5	1.00
	INF 1.5	0.99
	SUP 2.5	0.99
	INF 2.5	0.99
2	SUP 1.5	0.99
	INF 1.5	0.99
	SUP 2.5	0.99
	INF 2.5	0.98

Table 2. Correspondences between the 1st and 2nd measurements of density integrals in the nerve fiber layer photographs between operators.

Density integral	Pearson's correlation coefficient		
	1st measurement by operator 2	2nd measurement by operator 2	
SUP 1.5	1.00	0.99	1st measurement by operator 1
INF 1.5	0.98	0.97	
SUP 2.5	0.96	0.99	
INF 2.5	0.98	0.98	
SUP 1.5	1.00	0.99	2nd measurement by operator 1
INF 1.5	0.97	0.96	
SUP 2.5	0.99	0.99	
INF 2.5	0.98	0.97	

variables, e.g., development time and temperature and variability of flash intensity. In fundus photography, additional variation arises from ocular factors such as pupil size, lens opacity, fundus pigmentation and reflectivity, and so on. The method introduced in this study, normalization of photographic density to the fovea (= 0) and optic disc (= 1) under the assumption that both the fovea and disc are on the linear portion of the film's density vs. exposure curve, is new and may be useful for quantifying RNFL photographs. The relatively scaled photographic density would reduce the contrast- and brightness-related problems.

We also should consider the effect of individual variations in fundus pigmentation and reflectivity. In this study, we assumed that the reflectance of the disc and fovea is uniform among patients. Although this assumption is probably correct in our case, in which all the subjects were Asian, there is a possibility that the normalization method does not work as well with a less homogeneous population. Extensive study with different fundus pigmentation would be necessary. Another assumption was that the background reflectance (the fundus reflectance under the RNFL) does not vary among individuals: this assumption would also be affected by fundus pigmentation. Finally, the disc reflectance may vary with the size of the optic cup and the amount of disease.

Since the retinal nerve fibers are radially oriented around the optic disc, density profiles were measured along the circles concentric with the optic disc. We set two peripapillary circles with a radius of 1.5 and 2.5 times the radius of optic disc, respectively. Characteristic uniformity could disappear with large radius. On the other hand, abundant reflexes from the internal limiting membrane and the local variations in background pigmentation could impair the definition of RNFL in small radius. Correlations between density integrals and retinal sensitivities were similar between 1.5 times peripapillary circle and 2.5 times peripapillary circle.

We can find that there are large dips in the density profile shown in figure 2. This rapid decrease of intensity is due to the blood vessel in retina. This could cause an underestimation of density integral and undermine the reliability of the technique. Data preprocessing should be undertaken to compensate for this effect. We suggest a simple algorithm for this purpose although the validation of this method is not included in this work. The procedure of the algorithm is as follows (figure 5).

Step 1: Smooth each profile to remove high frequency components including abrupt changes of intensity caused by blood vessels (figure 5*b*).
Step 2: Obtain a highpass filtered profile by subtracting the smoothed profile from the original profile (figure 5*c*).
Step 3: Select global threshold level as the arithmetic mean of the maximum and minimum value of the highpass filtered profile.
Step 4: Extract vessel bearing segments using the above threshold on the highpass filtered profile. Regard the segments in which the highpass filtered profile is under the threshold (dashed line in figure 5*c*) bearing vessels.
Step 5: Compose the final compensated profile with the smoothed profile in vessel bearing segments and the original one in other areas.

Conclusions

A new method for the quantitative analysis of diffuse RNFL atrophy has been proposed, which we named CDMS. Density profiles concentric with optical disc were extracted from the digitalized RNFL photograph, and relatively scaled in order to reduce the contrast- and brightness-related problems, which was the major factor limiting the use of photography for quantitative measurements of reflectance. Density integral of superior and inferior segments in the density profile was used for the assessment of RNFL atrophy.

Satisfactory results were obtained about the clinical utility of this method when the CDMS measurement was compared with the indicator of loss of visual function (mean sensitivity of visual field). Intra- and inter-operator reproducibility was also excellent.
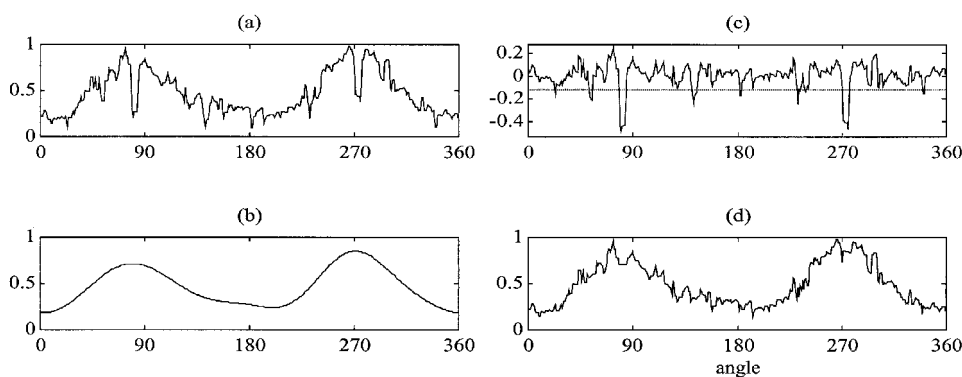
Figure 5. Processing to compensate the intensity decrease due to the vessels in retina. (a) original profile, (b) smoothed profile obtained by removing high frequency components including abrupt changes of intensity due to the vessels, (c) highpass filtered profile computed as the difference between the original and smoothed profiles (dashed line: threshold to find the vessel segments), (d) final compensated profile comprised of the smoothed profile in vessel segments and the original profile in other areas.

Also, the ubiquity of fundus cameras and the high cost of the other RNFL assessment technologies may provide a niche for this method.

Acknowledgements

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