Consistency of Structure-Function Correlation Between Spatially Scaled Visual Field Stimuli and In Vivo OCT Ganglion Cell Counts

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Submitted: December 19, 2017
Accepted: February 16, 2018

PURPOSE. To investigate the effect of stimulus size and disease status on the structure-function relationship within the central retina, we correlated the differential light sensitivity (DLS) with Goldmann stimulus size I to V (GI–V) and optical coherence tomography (OCT) derived in vivo ganglion cell count per stimulus area (GCc) within the macular area in normal subjects and patients with early glaucoma.

METHODS. Humphrey Field Analyzer 10–2 visual field data with GI through V and Spectralis OCT macular ganglion cell layer (GCL) thickness measurements were collected from normal and early glaucoma cohorts including 25 subjects each. GCc was calculated from GCL thickness data and correlated with DLS for different stimulus sizes.

RESULTS. Correlation coefficients attained with smaller stimulus size were higher compared to larger stimulus sizes in both normal (GI–GII: $R^2 = 0.41–0.43$, GIII–GV: $R^2 = 0.16–0.41$) and diseased cohorts (GI–GII: $R^2 = 0.35–0.41$, GIII–GV: $R^2 = 0.19–0.56$). Quadratic regression curves for combined GI to V data demonstrated high correlation ($R^2 = 0.82–0.90$) and differed less than 1 dB of visual sensitivity within the GC range between cohorts. The established structure-function relationship was compatible with a histologically derived model correlation spanning the range predicted by stimulus sizes GI to GIII.

CONCLUSIONS. Stimulus sizes within critical spatial summation area (GI–II) improved structure-function correlations in the central visual field. The structure-function relationship was identical in both normal and diseased cohort when GI to GV data were combined. Congruency of GI and GII structure-function correlation with those previously derived with GIII from more peripheral locations further suggests that the structure-function relationship is governed by the number of ganglion cell per stimulus area.

Keywords: ganglion cells, spatial summation, visual field, structure-function, optical coherence tomography, glaucoma

Glaucoma, one of the leading causes of irreversible blindness worldwide,1 is a progressive neurodegenerative disorder. The characteristic selective loss of retinal ganglion cells (RGCs) is thought to be the consequence of glaucomatous optic neuropathy originating at the lamina cribrosa, which results in distinct patterns of optic nerve damage along the corresponding GC axon (retinal nerve fiber) projections.2 The associated functional loss mirrors the spatial pattern of the structural loss on psychophysical assessments such as visual field testing, and the concordance between structural and functional loss is considered diagnostic of glaucoma.3,4 However, discordance between structural and functional aspects is commonly observed5,6 and may hinder the early clinical detection of glaucoma.7,8 Hence, the relationship between GC population (structure) and visual sensitivity (function) in glaucoma has been an ongoing topic of investigation.6,9–17 GC populations were initially estimated via counting GC somata from histologic sections.9–14 More recently, the development of optical coherence tomography (OCT) has allowed large-scale investigations to be conducted by utilizing thickness profile of various retinal structures such as nerve fiber layer and ganglion cell/inner plexiform layer as an in vivo surrogate measure of GC density.16–19 including models for estimating the GC counts in vivo from ganglion cell layer (GCL) thickness.20,21

The white-on-white Goldmann size III stimulus (GIII) is widely accepted as the standard stimulus size for clinical and scientific measurement of differential light sensitivity (DLS). While the choice of this stimulus size is based on historical rather than psychophysical origins,4,15,22 it has been utilized in numerous structure-function investigations.10–14,16,17 One such study by Swanson et al.13 modeled structure-function relationships between DLS measured with GIII and histologically derived GC count per stimulus area (GCc; modulated by retinal eccentricity) on a log-log plot as a two-stage linear regression with a distinct “tipping point.” The presence of the “tipping point” and two different linear regressions is likely a consequence of spatial summation characteristic altering with...
eccentricity as a result of critical summation area (Ac) enlarging with eccentricity\textsuperscript{13–25} while the stimulus size remains fixed. In the periphery, the Ac is larger than the stimulus size and a 1:1 positive correlation between structure and function is expected as a result of complete spatial summation.\textsuperscript{13} On the contrary, when the Ac is smaller than the stimulus area, a 4:1 structure-function relationship occurs as a result of incomplete summation. In other words, proportionally similar loss in function is associated with larger structural loss centrally compared to the periphery. An earlier study by Garway-Heath et al.\textsuperscript{11} also hypothesized that the nonlinearity in GCC and DLS is a result of incomplete spatial summation from using a large stimulus size and demonstrated linearity can be attained by correcting for this. In effect, the difference in the structure-function relationship between central and peripheral visual field is a result of alteration in spatial scale with eccentricity.\textsuperscript{13,14} Other models, likewise using GCC, describe a linear structure-function relationship with human autopsy data,\textsuperscript{26} primate glaucoma model data,\textsuperscript{12} or retinal layer thicknesses measured with the OCT.\textsuperscript{18} All of these, however, demonstrate an alteration in the relationship with eccentricity.

Smaller-sized targets have been suggested to be more sensitive for detecting glaucomatous defects in the past\textsuperscript{27} and an increasing number of studies suggest stimulus sizes scaled to or smaller than the Ac may be more appropriate for early diagnosis.\textsuperscript{28–32} Within the central 30\(^\circ\) of the field of vision, at which threshold visual field tests are most commonly conducted, the GIII target often exceeds spatial summation area, particularly for the central test locations.\textsuperscript{24,25} Given the importance of test size in relation to the Ac to the fidelity of visual sensitivity,\textsuperscript{15,28–30,32} we hypothesize that the GCC structure-function relationship will improve and approach 1:1 positive correlation by altering the test size within complete spatial summation. Similarly, as the summation characteristic is known to change with the disease process,\textsuperscript{30} comparisons of the structure-function relationship between normal and glaucomatous cohorts need to be conducted.

Previous attempts have been hampered by the limited range of DLS and GC density in the normal population compared to a diseased cohort.\textsuperscript{25,34} By using differently sized stimuli, it is possible to expand the range of the DLS and GC density and facilitate the investigation of the normal cohort, thus allowing improved comparison to the diseased cohort. In particular, the use of GCC as a common structural metric permits structure-function data from multiple stimulus sizes to be combined and compared directly. Therefore, we have investigated the correlation between previously developed in vivo GCC estimates\textsuperscript{37} with DLS obtained from different-sized, white-on-white visual field stimuli within the central retina in both a normal and glaucomatous cohort.

**Methods**

**Study Participants**

Participants were recruited at the Centre for Eye Health (CFEH) after receiving ethics approval from the University of New South Wales (UNSW) Australia, Human Research Ethics Advisory panel in concordance with the tenets of the Declaration of Helsinki. Written informed consent was obtained for all participants in keeping with the approved protocol.

All patients underwent an initial, single-visit glaucoma assessment, including but not limited to intraocular pressure (IOP); 24–2 SITA standard threshold visual field testing (Humphrey Field Analyzer; Carl Zeiss Meditec, Inc., Dublin, CA, USA); slit-lamp examination; gonioscopy for those with glaucoma or at risk of glaucoma; fundus examination; and OCT analysis of the macular inner retinal layer thickness and parapapillary nerve fiber layer thickness (Cirrus OCT; Carl Zeiss Meditec, Inc.). Clinical findings were first reviewed by experienced optometrists at CFEH and were subsequently reviewed by an ophthalmologist with special interest in glaucoma (MPH) if potential signs of glaucomatous damage were seen. These were defined as at least two of the following: (1) retinal changes characteristics of glaucoma were present on fundoscopy; (2) retinal changes characteristic of glaucoma were present on OCT imaging; or (3) patients exhibited visual field defect consistent with these structural findings.\textsuperscript{8,35} If deemed appropriate by the ophthalmologist, the patient was referred for further comprehensive clinical consultation with a glaucoma specialist (KM) and included in the glaucomatous group if a final diagnosis of glaucoma was established. All glaucomatous subjects in this study were initiated on topical antiglaucoma medication following the consultation and their pretreatment IOPs were recorded. Healthy volunteers recruited for the study underwent the same clinical screening procedure and were included in the normal cohort if found to be free of posterior pole disease.

Eyes with a refractive error greater than ±6 diopters (D) spherical equivalent or 3 D of astigmatism, media opacity impacting imaging of the posterior pole, visual field mean deviation (MD) of greater than –12 dB on a 24–2 SITA standard visual field testing bilaterally or presence of macular or other posterior pole disease (except for glaucoma in the disease cohort) impacting the GCC measurement at the time of data collection were excluded from the study.

One eye was included in the study for each participant. In patients with unilateral disease, the diseased eye was chosen; otherwise, the eye with lower MD was chosen for inclusion. For normal subjects, if only one eye was eligible, the eligible eye was included; otherwise, one eye was chosen using a random number generator. All scans and visual field results for the left eye were converted to right eye format for consistency.

**Ganglion Cell Count Estimate**

Posterior pole scans were obtained with the Spectralis OCT (Heidelberg Engineering, Heidelberg, Germany) consisting of 61 B-scans spaced approximately 120 μm apart and with a scan area of 30\(^\times\)25\(^\circ\) at 9 ART. OCT images were analyzed using the posterior pole analysis grid of the Heidelberg Retinal Analyzer viewing module (version 6.3.4.0). The 8 \(\times\) 8 squares grid (6880 \(\times\) 6880 μm) was centered on the fovea aligning the horizontal midline through the optic nerve head center (Figs. 1A, 1B). Following automated segmentation of the GCC by the analysis software (Fig. 1C), each frame was manually assessed and corrected for segmentation error if pronounced layer misclassification, resulting in more than 50% difference in thickness to adjacent areas, was detected by an experienced operator (NY) following previously established protocol.\textsuperscript{21} A total of 21.6% of the grids required manual correction and 4.6% individual grids were excluded from analysis as adequate segmentation was hindered due to the presence of major blood vessels; shadowing; poor quality B-scan (<15 dB signal scores); the optic nerve head encroached the grid; or more than 10% of the grid could not be analyzed because it was located outside of the scan area. Subanalysis confirmed that manual adjustments did not bias final measurements (Supplementary Fig. S1).

GCC thickness was recorded for each Spectralis measurement grid averaging a 3 \(\times\) 3 or approximately 860 \(\times\) 860 μm area, except for the central four grids corresponding to the foveal areas. The foveal area is characterized by high GC density and over 30-fold variation in the GC density within the...
investigated area. For consistent comparison between functional and structural results, GCL thickness for these four grids was modified to colocalize with the innermost 10-2 stimulus using the average of the central and four surrounding equidistant locations within an approximately 1° radius (for details, see Supplementary Fig. 1).

The number of ganglion cells per stimulus size is dependent on the areas subtended by the differently sized stimuli in square millimeters, which was defined as:

$$S_{area} = \pi \cdot \left(\frac{\Phi}{2} \cdot A_s\right)^2$$

Where $A_s$ is the linear length (millimeter) of the central retina per degree of visual angle, estimated to be 0.288 mm/degree, and $\Phi$ is the angular subtense of the visual field stimulus in degrees.

The number of ganglion cells was then inferred from the obtained GCL thickness measurements using previously established grid-wise GC density/mm$^3$ values under consideration of the above outlined visual field stimulus size as follows:

$$GCc = \left(\frac{GCL_T}{1000}\right) \cdot GC_D \cdot S_{area}$$

Where $GCc$ equals the calculated number of GC within the visual field stimulus, $GCL_T$ represents the GCL thickness in microns, and $GC_D$ represents the ganglion cell density per mm$^3$ of GCL tissue.

**Figure 1.** Measurement of GCL thickness and spatial correlation to visual field test stimuli locations. (A) The horizontal center axis of the Spectralis OCT analysis grid for GCL thickness was aligned to the line subtending the macular and optic nerve head (ONH) center (green line). (B) The analysis grid was spatially matched to the 68 stimulus locations of the 10-2 visual field test grid (white dots) and four points of the 30-2 visual field test grid at 12.7° eccentricity (pink dots), taking lateral ganglion cell displacement into account. (C) GCL thickness was measured between the retinal nerve fiber layer (turquoise line) and the boundary to the inner plexiform layer (GCL: purple line) and averaged over each grid with the exception of the central four grids, which were modified to exclude the foveal pit (for details, see Supplementary Fig. S2).
**Visual Field Data**

Visual field data were collected from the 10-2 and 30-2 test grids with the Humphrey Visual Field Analyzer (Carl Zeiss Meditec), for stimulus size I (GI) through V (GV) in full threshold mode. Given the length of the test procedure (approximately 15 minutes per field) and potential bias of results due to fatigue, data were collected in four separate sessions comprising five visual field tests each over 2 separate days. Experienced clinicians performed the procedures advising patients to take a break at least halfway through a single session or more frequently if required. To obtain functional data corresponding to structural measurements, data from all 68 stimulus locations tested by the 10-2 grid and four additional points from the 30-2 grid located at 12.7° eccentricity from fixation were collected (Fig. 1B). All sensitivity values were measured at least twice and averaged for analysis. Unreliable visual field results, defined as a false positive error rate greater than 35%, a false negative rate greater than 35% for normal, or 40% for glaucomatous subjects, and a fixation loss rate greater than 20% concordant with the gaze tracker result, were excluded from the analysis. The higher false negative rate for the glaucomatous cohort was based on the established association with glaucomatous field defect.48 For visual field results suggesting more than 20% fixation losses, the gaze tracker results were manually analyzed and visual field result excluded if the angular deviation of fixation exceeded 3° for more than 20% of the time.49

Visual field locations with a measured sensitivity of ≤0 dB were excluded from the study, as these points were outside the dynamic range of the instrument (i.e., below the “measurement floor”). Additionally, visual field sensitivity measurements below sensitivity level of 15 to 19 dB for GIII are known to be affected by high measurement variability.40,41 Initial analyses were conducted before and after exclusion of points below sensitivity level of 15 to 19 dB for GIII are known to be affected by high measurement variability.40,41 Initial analyses were conducted before and after exclusion of points below this “pseudo-measurement floor.” To minimize variability in the current study, a more stringent measurement floor of 19 dB was used for GIII as variability increases as a continuum rather than having a well-defined cutoff.42 A location-specific conversion factor was applied to determine the appropriate conversion formula subsequently applied to the pseudo-measurement floor for the GIII in dB, $F_p$, is the pseudo-measurement floor for the GIII in dB, $F_p$, is the pseudo-measurement floor equivalent to $F_p$ for the stated stimulus size, $A_C$ is the critical summation area in square millimeters expressed as dB, $n_2$ is the tangential slope of the partial summation curve, and $n_1$ is the slope for complete spatial summation (equal to 1).

Visual field points were colocated with corresponding structural data obtained from the Spectralis grid (Fig. 1B) under consideration of lateral displacement.43 Visual field sensitivity data within each Spectralis grid were averaged after conversion of the logarithmic decibel threshold values to a linear inverse Lambert (Lb) unit (Equation 1) and subsequently converted back to the original logarithmic decibel scale (Equation 2):

$$\frac{1}{L_b} = 10^{(0.1 \cdot dB)} \quad (1)$$

$$10 \cdot \log_{10} \left( \frac{1}{L_b} \right) = dB \quad (2)$$

**Age Correction**

Previous studies have indicated that GCL thickness and visual field sensitivity alter with age.21,44–46 The age difference between individual subjects and the cohorts may therefore increase the variability within the dataset and confound the comparison of structure-function relationship between the normal and glaucomatous cohort. Hence, to minimize the variability imposed by intra- and intercohort age differences, the GCL thickness and DLS of all normal and glaucomatous subjects were age-corrected to the average age of the glaucomatous cohort using previously established regression analysis models as conducted in previous studies,11,21,24–26,31,32,45,47 including the commonly used SITA-standard algorithm of the HFA.48 Specifically, for the GCL thickness, measurements from each normal and glaucomatous subjects were clustered into eight statistically separable classes and adjusted to match the average age of the glaucomatous cohort using a previously published conversion for all but the foveal area.49 For the four central grids, corresponding to the foveal area, thickness measurements were recalculated for the cohort used in the previous study (n = 201)21 as detailed in the supplementary material (Supplementary Figs. S2A–C) providing the appropriate conversion formula subsequently applied to the corresponding data in the current study (Supplementary Fig. S2D). The age correction for visual field data was established for 30-2 test pattern45; therefore, correction factors were calculated for the 10-2 test locations using a distance-wise weighted averaging method from the three or four closest 30-2 stimulus locations as described elsewhere.45

Further, subanalysis of the normal cohort was conducted to determine the efficacy of the age correction as detailed in the supplementary material. Briefly, the relationship between the GCC and DLS was compared between a glaucoma subgroup (n = 10, mean age ± SD: 59.4 ± 2.8) and an age-matched normal subgroup (n = 5, mean age ± SD: 57.5 ± 3.8) as well as an age-corrected normal subgroup comprised of the five youngest subjects (n = 5, mean age ± SD: 24.1 ± 2.6). Data confirmed that regression characteristics were similar between the age-corrected subgroup to the glaucoma subgroup and its age-matched normal subgroup: the highest discrepancy between the groups at the upper end of the GV stimulus size was contained within the measurement variability of GV at 1.7 dB (Supplementary Fig. S3).45
Statistical Analysis

Data were graphed and analyzed using statistical software (GraphPad Prism version 7.00; GraphPad Software, Inc., La Jolla, CA, USA). Correlation of GC numbers per stimulus area and averaged DLS for each grid location were analyzed fitting simple linear regression for individual stimulus size and second-order polynomial (quadratic) regression for all stimulus sizes combined. Normality of variables was assessed with the D’Agostino-Pearson normality test. Comparisons between cohorts were conducted with Welch’s t-test for normally distributed and Mann-Whitney test for non-normally distributed continuous data. Pearson’s χ² was used for categorical data.

RESULTS

Structure-function correlations were investigated for individual cohorts (Table) and stimulus sizes (Figs. 2A, 2B). In comparison to the normal cohort, the glaucomatous cohort exhibited a shift in the distribution of data toward lower DLS and GCC. As highlighted in the methods, visual function cannot be reliably assessed below a minimum sensitivity, referred to as the pseudo-measurement floor, which is determined by the stimulus size and retinal location (Figs. 2A, 2B, horizontal dotted lines). Data points containing visual field sensitivities below the pseudo-measurement floors, labeled in green, were excluded and the analysis was repeated where applicable. The effect of the exclusion of data below the pseudo-measurement floor was more prominent with smaller stimulus sizes and in patients with glaucoma, thus resulting in a more pronounced improvement in correlation coefficient in the glaucomatous cohort.

**TABLE.** Demographic Characteristics of the Normal and Glaucomatous Cohort

<table>
<thead>
<tr>
<th>Clinical Characteristics</th>
<th>Normal, n = 25</th>
<th>Glaucoma, n = 25</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, mean ± SD‡§</td>
<td>35.7 ± 12.5</td>
<td>64.5 ± 5.56</td>
</tr>
<tr>
<td>Sex, M:F†</td>
<td>10:15</td>
<td>16:9</td>
</tr>
<tr>
<td>Eye chosen, R:L†</td>
<td>12:13</td>
<td>10:15</td>
</tr>
<tr>
<td>Ethnicity, European:Asian†</td>
<td>14:11</td>
<td>16:9</td>
</tr>
<tr>
<td>Spherical error, D</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Visual acuity (LogMAR)‡§</td>
<td>$-0.10 (-0.10 to 0.00)$</td>
<td>$0.02 (0.00 to 0.09)$</td>
</tr>
<tr>
<td>IOP (mm Hg)‡§</td>
<td>15 (13 to 17.5)</td>
<td>20 (16.5 to 24)</td>
</tr>
<tr>
<td>Visual field, MD‡§</td>
<td>$-0.39 (-0.92 to 0.17)$</td>
<td>$-2.42 (-4.28 to -0.99)$</td>
</tr>
<tr>
<td>Visual field, PSD‡§</td>
<td>1.63 (1.38 to 1.89)</td>
<td>2.34 (1.99 to 4.57)</td>
</tr>
</tbody>
</table>

Interval data are presented as median and interquartile range unless indicated. IOP, intraocular pressure (pre-treatment IOP for glaucoma cohort); MD, Humphrey Visual Field 24-2 mean deviation; PSD, Humphrey Visual Field 24-2 pattern standard deviation.

* P value calculated by Welch’s t-test.
† P value calculated by Pearson’s χ².
‡ P value calculated by Mann-Whitney U test.
§ P < 0.0001.
ǁ P < 0.01.

**Figure 2.** The effect of stimulus size on structure-function correlations. Plots of differential DLS and ganglion cell per stimulus area (both in decibels) for (A) age-corrected normal and (B) glaucoma cohorts with stimulus size I to V. Simple linear regression was calculated for each plot (red line). The regression analysis was repeated after excluding data points from measurement areas that have reached the visual field measurement floor (dashed cyan lines where applicable). For comparison, individual correlations were normalized to a common origin in relation to a 1:1 line of correlation (dotted black line). The average measurement floor for each stimulus size is denoted by the horizontal dotted line. Note that some of the excluded points are above this line due to averaging of multiple visual field locations for some of the data points. R²: correlation coefficient. * R² of linear regression after excluding points below the pseudo-measurement floor (if applicable).
Disease cohorts with differing stimulus sizes and the best-fit regression curve was determined for both the normal and glaucomatous cohort (Fig. 3). The fitted second-order polynomial mirrored the steeper slope for the relationship between estimated ganglion cell numbers and light sensitivity with smaller stimulus sizes: for stimuli within Ac (=GII for central 10° of vision)24,25 larger impact on the visual sensitivity was observed with the same proportional change in GCc (Fig. 3A; regression curve left of the black dashed line versus to the right). The fitted regression curve for both the normal and glaucomatous cohorts exhibited a high correlation coefficient, weaker for the glaucomatous cohort compared to the normal cohort (R² = 0.82 and 0.90, respectively) due to the larger variability of the data (Figs. 3A, 3B). While the regression lines’ gradient was steeper for the glaucomatous cohort when each stimulus size was analyzed independently (Figs. 2A, 2B), combined analysis of all stimulus sizes resulted in an identical best fit between structure and function measurements for the normal and glaucoma cohorts (Fig. 3C). The difference plot between the two cohorts demonstrated that the disparity between the curves remained within 1 dB along the entire Gcc range (Fig. 3D). Finally, for all test sizes, the GCc values were significantly lower in the diseased cohort compared to the normal cohort (Mann-Whitney test, P < 0.0001 for all stimulus sizes).

A subset of patients from the normal cohort (n = 11), age-matched (31.1 ± 2.4 years) to a cohort previously used to establish the histologic GC population data (35.8 ± 4.0 years, P = 0.217, Welch’s t-test), was used to explore the fit of the developed model to an anatomic ground truth (Fig. 4).9,11 It should be noted that the histologic data were compared to visual field sensitivities with GII only, albeit over a larger retinal area. The change of eccentricity (and thus spatial summation characteristics)23–25 resulted in a similar effect to the change in stimulus size in the central field, thus providing data points equivalent to results obtained from GI to GII (Fig. 4).

**DISCUSSION**

**Comparison to Previous Models**

Well-established models investigating the relationship between human retinal structure and function describe the dependency of this relationship on retinal eccentricity.11,13,14 This observation was confirmed in a primate glaucoma model, demonstrating significant differences in this relationship between central and peripheral retina.12 Such a difference in the structure-function relationship can be attributed to changes in spatial summation characteristics between the central and peripheral retina, most notably when the spatial summation area exceeds stimulus size.5,15,14 Indeed, it was predicted that when spatial summation characteristics are corrected for, GCc and DLS assume a linear relationship throughout the 50-2 measurement area.11 Our results show that this apparent alteration to the structure-function relationship over different eccentricities can be replicated using differently sized stimuli within the central retinal area (Figs. 2–4). Thus, the model proposed in this paper confirms that the change in structure-function relationship at different eccentricities is a function of stimulus size under consideration of spatial summation as a result of employing fixed-sized stimuli despite spatial summation area altering with eccentricity.14,23,24

Comparison to structure-function data derived from histology9,13,15 provides direct evidence that the established model accurately describes anatomical data within a comparable range of stimulus size and eccentricity (Fig. 4, GII). Interestingly, histologic data derived with GII from peripheral locations not contained within the current dataset aligned within the predicted model area albeit in regions established with GI and GII. As the ganglion cell density is lower in the periphery compared to the center,9 the GCc is comparable in both of these cases. Furthermore, the DLS attained was found to be similar despite the difference in stimulus size and test location. This finding, therefore, suggests DLS is directly correlated to the number of GC present within the stimulus area and changes observed with eccentricity are in fact a consequence of alterations to GC density with eccentricity.14

**Practical Implications of Structure-Function Correlation With Spatially Scaled Stimulus**

Previous studies have shown that smaller stimuli lead to increased variability in the glaucomatous cohort.33,34 Our data further strongly suggests that smaller stimulus size loss rates in a better correlation between structural and functional measurements (Fig. 2). While the glaucomatous cohort revealed a gradient closer to 1:1 direct correlations for all stimulus size, even after excluding unreliable visual sensitivity values below the pseudo-measurement floor (Fig. 2),41 the best fit curve was identical between the two cohorts if all stimulus sizes were combined for analysis (Fig. 3C). The disparity between the normal and glaucomatous cohort seen in Figure 2 can be explained by a simultaneous reduction in DLS and GCc resulting in data points for the individual stimulus size shifting down along the structure-function continuum, and thus, resulting in a steeper tangential structure-function gradient (Fig. 5A). This is consistent with theories suggesting an alteration in spatial summation characteristic with disease progression14,28; the Ac increases in size with the disease process, leading to a more complete spatial summation, and thus, improves the structure-function relationship. In the current study, the use of GCc as a common structural metric enables the data from GI to GV to be combined and the structure-function relationship to be examined over a much broader range of GCc and DLS (Fig. 3). This allows greater appreciation of the relationship between structure and function, particularly for the normal cohort, whose restricted range would otherwise preclude such investigation.33,34

The observed changes in structure-function relationships with stimulus size advocate a potential advantage of using stimulus sizes smaller than Ac for assessment of the central retina. It is well acknowledged that approximately 70% loss of GC is required before a statistically significant visual sensitivity loss can be detected for the innermost 24-2/30-2 test location,33 which is equivalent to a 5-dB loss on a log scale if GII is used. Our model corroborates this assumption predicting a reduction in visual sensitivity associated with this loss of 2.4 dB, equivalent to a 2-SD reduction for this locale (Fig. 5B, GII, orange interval).48 While larger stimuli are associated with lower variability and were suggested to retain their ability to detect early visual sensitivity loss,47 loss of sensitivity (Fig. 5B, GV, red interval) falls within the measurement variability and cannot be reliably detected.49 Conversely, GI is predicted to show greater sensitivity loss (Fig. 5B, GI, green interval), but one must be judicious with the application of a stimulus that is too small, as these are associated with increased variability.19 The predicted GCc loss associated with a statistically significant (2 SD below normative data mean from Phu et al.50) visual sensitivity loss is less with GII (Fig. 5C, blue interval) compared to GII and GV (Fig. 5C, orange and red interval).
FIGURE 3. Structure-function relations across the assessed range of ganglion cell counts and visual field sensitivities. All data points were combined to obtain the best fit, depicted by a second-order polynomial curve, between estimated ganglion cell numbers and DLS in the (A) normal cohort (number of data points $N_T = 5342$) and (B) glaucomatous cohort ($N_T = 4989$) after correcting for age and pseudo-measurement floor effect. Correlation coefficient is lower in the glaucomatous cohort due to larger data variability. However, the regression curves were found to be similar between the two cohorts (C). The difference plot in DLS between the normal and glaucomatous structure-function curves demonstrates the difference to be within 1 dB (D); 95% prediction intervals of the data are represented by the dotted lines for each polynomial curve.
intervals respectively). However, due to the larger variability, a larger GCC loss is predicted with GI (Fig. 5C, green interval) compared to GII, suggesting GII to be more effective than both GI and GIII within this test condition.

Similarly, the structure-function continuum suggests glaucomatous progression can be more effectively monitored within the central retina using structural means compared to larger stimulus, such as GV. The range of GCC with GV stimulus had a range of approximately 13 dB, yet DLS had a range of approximately 3 dB suggesting a larger dynamic range with structural measurement. While the curvilinear relationship likewise suggests that smaller stimuli may be more suited for monitoring glaucoma progression than larger stimuli, this may be a disadvantage in advanced disease. Smaller stimuli are known to have limited effective dynamic range, as the variability significantly increases with decreasing visual sensitivity and become of limited clinical utility with advanced disease processes.\textsuperscript{41,50} However, our results demonstrate that it is possible to retain a sensitivity value above the measurement floor in most retinal locations in the disease cohort by increasing the stimulus size (Fig. 2B), which may be accredited to the greater number of response elements recruited with a larger stimulus size compensating for the reduced density.\textsuperscript{28} This is also consistent with the results of a previous study,\textsuperscript{51} which suggests better response characteristic can be attained in areas with sensitivity below the measurement floor with a larger stimulus size. Therefore, selection of a stimulus size within the spatial summation area but not smaller than truly necessary may maximize the signal-to-noise ratio. Previous studies have suggested the spatial summation area within the central 10° to be closest to but larger than GI,\textsuperscript{24,25,51} and therefore, consistent with our data suggesting GI is more effective than both GI and GIII for the central retina. On the other hand, spatial summation area increases with the disease process,\textsuperscript{28} and in such case, a stimulus larger than the normal spatial summation area may be more appropriate. A strategy that scales the stimulus not only for stimulus intensity but size based upon the initial measure of sensitivity and variability of the result may be of great clinical benefit.\textsuperscript{28} Such strategy would allow one to mitigate the loss of reliability posed by the use of too small a stimulus on areas with glaucomatous damage, but at the same time, allow one to capitalize on the
improved structure-function correlation to allow the detection of glaucomatous losses earlier.50,51

**Limitations of the study**

The most marked limitation of this study consists of the mismatch in age between the cohorts (Table). Additionally, the normal cohort was also more myopic and exhibited better visual acuity, although these may be attributed to the age difference.52,53 The difference in age is likely due to older age constituting a risk factor for glaucoma.54 It has been previously suggested that the spatial summation characteristic does not change significantly with age55,56 and accordingly, the difference in age should not alter the conclusion of the study. Despite the disparity in age, the actual effect on the DLS and GCL thickness/GCc is arguably small. For instance, between the age of 35 and 65 (3 decades), the average GII sensitivity within the central 12.7° is expected to alter by 1.7 dB on average (minimum: 1.65 dB; maximum: 2.1 dB)55 while the average grid GCL thickness within the equivalent measurement area may alter by 0.5 dB or 0.9 μm (minimum: 0 μm; maximum: 1.7 μm).21 The difference imposed by age between these cohorts is within the test-retest variability range of the instrument.44-50 and even before correction, there were notable overlaps between the prediction interval for the regression curves of the two cohorts (data not shown). Regardless, DLS and GCc for the normal and glaucomatous cohort were adjusted to a common age equivalent using a well-established age-correction method, frequently used in prior studies by different investigators.11,21,24-26,31,32,45,47 and regularly utilized by clinical test algorithms for the Humphrey Field Analyzer; SITA.58 A subanalysis was conducted to further confirm the validity of the age-correction technique, and good fidelity was demonstrated between the glaucomatous subgroup, and the normal age-corrected and age-matched cohorts (Supplementary Fig. S3). The age-correction models utilized for this study31,45 exhibited differences in the age-normal regression of DLS and GCL thickness (and therefore GCc); specifically, the DLS regressed at a faster rate. Such difference may be attributed to other ocular effects associated with aging that may affect the DLS, such as cataract,57 and highlights the importance of age correction for investigations of visual function.

The GC density per tissue volume was assumed to be constant and the measurement floor effect for the GCL thickness was not applied due to the unavailability of a detailed GCL floor data, which may confound the determination of GC count and density.14,59 Furthermore, retinal areas with significant glaucomatous damage are more prone to segmentation errors.59 While every effort was made to assess and correct for segmentation errors, a combination of thin residual tissue and the relatively low contrast between GCL and IPL is a potential source of error. As demonstrated in the supplementary materials, however, manual correction of such errors did not significantly alter measurement results. Lastly, “grid-wise” measurements of retinal and visual field data, as utilized in this study for GC thickness and light sensitivity, could potentially have led to uneven sampling and averaging over the study area. Future studies may apply modified measurement paradigms to better reflect the physiologic gradient in GC density and improve the spatial correlation of structural and functional data.

**Conclusions**

The findings of this study strongly suggest a better structure-function correlation in the central visual field can be achieved with stimulus sizes smaller than the local critical summation area, thus operating under complete spatial summation. Our findings are in line with a theory previously proposed by Pan and Swanson15 stating that a nonlinear structure-function relationship is likely due to the combination of fixed-size stimulus and change in the spatial summation area with eccentricity. Additionally, our data suggest that the underlying structure-function characteristics are similar between normal and glaucomatous eyes when stimulus size is accounted for and the structural metric is expressed as the number of ganglion cells per stimulus area. Therefore, physiologic structure-function correlations may be extrapolated to a diseased cohort and, as such, development of a robust structure-function model should significantly aid in diagnosis and management of glaucoma.

Acknowledgments

The authors thank Corneia Zangerl, Henrietta Wang, and Janelle Tong for assistance with data collection and analysis. Supported by Grants NHMRC 1033224 and UNSW FRGP 2014 P535406; a PhD scholarship provided by Guide Dogs NSW/ACT; and an Australian Postgraduate Award (NY, JP, AYC). SKK and MK hold a joint patent on visual field test size and disease detection. International Publication Number WO2014/094035 A1(USA) and European Patent Number 138654119.9.

Disclosure: N. Yoshioka, None; B. Zangerl, None; J. Phu, None; A.Y.J. Choi, None; S.K. Khuu, P; K. Masselos, None; M.P. Hennessy, None; M. Kalloniatis, P

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