Evaluation of the Structure–Function Relationship in Glaucoma Using a Novel Method for Estimating the Number of Retinal Ganglion Cells in the Human Retina

Ali S. Raza^{1,2} and Donald C. Hood^{1,3}

¹Department of Psychology, Columbia University, New York, New York, United States

²Department of Neurobiology and Behavior, Columbia University, New York, New York, United States

³Department of Ophthalmology, Columbia University, New York, New York, United States

Correspondence: Donald C. Hood, Department of Psychology, 406 Schermerhorn Hall, 1190 Amsterdam Avenue, MC 5501, Columbia University, New York, NY 10027, USA; dch3@columbia.edu.

Submitted: December 31, 2014

Accepted: July 16, 2015

Citation: Raza AS, Hood DC. Evaluation of the structure–function relationship in glaucoma using a novel method for estimating the number of retinal ganglion cells in the human retina. *Invest Ophthalmol Vis Sci*. 2015;56:5548–5556. DOI:10.1167/iov.14-16366

PURPOSE. We developed a simple method for estimating the number of retinal ganglion cells (RGCs) in the human retina using optical coherence tomography (OCT), compared it to a previous approach, and demonstrated its potential for furthering our understanding of the structure–function relationship in glaucoma.

METHODS. Swept-source (ss) OCT data and 10-2 visual fields (VFs) were obtained from 43 eyes of 36 healthy controls, and 50 eyes of 50 glaucoma patients and suspects. Using estimates of RGC density from the literature and relatively few assumptions, estimates of the number of RGCs in the macula were obtained based on ssOCT-derived RGC layer thickness measurements.

RESULTS. The RGC estimates were in general agreement with previously published values derived from histology, whereas a prior method based on VF sensitivity did not agree as well with histological data and had significantly higher ($P = 0.001$) and more variable ($P < 0.001$) RGC estimates than the new method based on ssOCT. However, the RGC estimates of the new approach were not zero for extreme VF losses, suggesting that a residual, non-RGC contribution needs to be added. Finally, the new ssOCT-derived RGC estimates were significantly ($P < 0.001$ to $P = 0.018$) related to VF sensitivity (Spearman's $\rho = 0.26$ – 0.47), and, in contrast to claims made in prior studies, statistically significant RGC loss did not occur more often than statistically significant visual loss.

CONCLUSIONS. The novel method for estimating RGCs yields values that are closer to histological estimates than prior methods, while relying on considerably fewer assumptions. Although the value added for clinical applications is yet to be determined, this approach is useful for assessing the structure–function relationship in glaucoma.

Keywords: glaucoma, retinal ganglion cells, optical coherence tomography, visual fields, structure versus function, macula

Given that glaucoma is a disease of the retinal ganglion cells (RGCs), it is not surprising that there is interest in relating visual sensitivity to estimates of the number of RGCs. Harwerth et al.^{1–3} previously developed a model for estimating the number of RGC bodies based on visual sensitivity as well as the number of RGC axons based on optical coherence tomography (OCT) scans of the optic nerve. However, because this model uses information at the optic disc, it must make assumptions about the relationship between the RGC axons and the location of the corresponding RGC bodies in the form of a topographic map. In fairness, with a few notable exceptions,^{4,5} the vast majority of work using the older time-domain OCT technology to study RGCs has been based on imaging of the optic disc as a proxy for RGC axons. However, newer frequency-domain OCT (fdOCT) technology allows for higher-resolution imaging of the RGC layer within the macula.⁶ In particular, the combined RGC layer and inner plexiform layer (IPL) thickness can be obtained from volume scans covering approximately the central 10° of visual angle.

We have used this newer fdOCT technology to measure the combined RGC and IPL thickness, and also to relate this structural measurement to local sensitivity loss in a group of glaucomatous eyes.^{7,8} This work illustrated the importance of correcting for the centripetal displacement of macular RGC bodies away from the fovea,⁸ and we accounted for this by adjusting our data based on the histological estimates of displacement by Drasdo et al.⁹ As we were comparing local sensitivity at many regions within the central 10° (i.e., the 10-2 protocol), we used volume scans instead of the higher-quality averaged line scans. However, given the quality of the fdOCT volume scans at the time, the use of volume scans prevented us from confidently separating the RGC layer from the IPL. Therefore, we took a conservative approach and simply combined the two. We later found that, in regions corresponding to severe glaucomatous visual field (VF) loss, the thickness of the IPL decreased by a relatively smaller amount than did the thickness of the RGC layer.¹⁰ Thus, the ability to separate the two layers is important from the standpoint of simplifying

TABLE. An Overview of Several Structure-Function Models Described in the Literature

Model	Central Retina	Peripheral Retina	Structural Measure
Garway-Heath et al., ¹⁴ 2000*	Power law	Linear	RGC RF density
Kerrigan-Baumrind et al., ²⁵ 2000†	Inverse power law	Inverse power law	RGC count
Swanson et al., ¹⁹ 2004; Pan et al., ²⁰ 2006*	Nonlinear	Linear	RGC RF density
Harwerth et al., ¹ 2004†	Inverse power law	Inverse power law	RGC density
Harwerth et al., ^{2,3} 2007, 2010*	Inverse power law	Inverse power law	RNFL thickness
Hood and Kardon ¹⁸ , 2007*	Uncertain	Linear	RNFL thickness
Drasdo et al., ²¹ 2008*	Fourth-order polynomial	Linear	RGC RF density
Wollstein et al., ²⁴ 2012†	Segmented linear	Segmented linear	RNFL thickness

RF, receptive field.

* Denotes a theoretical model (including those with empirically-derived parameters from a separate dataset).

† Denotes an empirical best-fit model (without a theoretical basis).

future evaluations of structure-function models as well as for advancing our general understanding of glaucoma.

Recently, swept-source OCT (ssOCT) technology,^{11,12} which has a faster acquisition rate than fdOCT, has become available. With ssOCT, it now is easier to separate the RGC layer from the IPL in volume scans, and, thus, to determine RGC layer thickness in the macula. This creates the possibility of estimating the number of RGCs directly from structural measures, and in this study, we demonstrated a simple yet novel method for using ssOCT data to estimate the number of RGCs in the macula for a group of healthy and glaucomatous eyes. Previously, Curcio and Allen¹³ published densely sampled estimates of RGC density along the horizontal and vertical meridians of the human retina, and Garway-Heath et al.¹⁴ subsequently interpolated the data of Curcio and Allen¹³ into a 2-dimensional estimate of RGC density. To estimate the number of RGCs for a particular eye, a method comparable to that of Garway-Heath et al.¹⁴ was needed to apply the Curcio and Allen¹³ RGC density estimates to 2-dimensional thickness measurements obtained from ssOCT. A similar approach has been presented previously by Turpin et al.,¹⁵ although it was used in a different context and was based on the combined thickness of the retinal nerve fiber layer (RNFL), RGC layer, and IPL. In this study, we isolated the thickness of the RGC layer by leveraging our previously developed segmentation techniques.^{8,16,17} The resulting RGC estimates are compared to the previous method by Harwerth et al.³ as well as to RGC estimates from independent histological studies. Finally, our approach for estimating the number of RGCs allows for evaluation of models of structure-function (see Table) that relate visual sensitivity with the number, density, or receptive field density of RGCs.^{1-3,18-25} This is illustrated by assessing the conclusions of a well-cited study by Kerrigan-Baumrind et al.²⁵ in the context of our results.

METHODS

Subjects

Data were collected from human participants divided into two groups: 43 eyes of 36 healthy controls (age = 53.8 ± 9.1 years [mean $\pm$ SD]) with normal vision and 50 eyes of 50 glaucoma patients and suspects (age = 54.1 ± 14.2 years [mean $\pm$ SD]) with mostly mild to moderate vision loss based on the Hodapp-Anderson-Parrish classification system.²⁶ The University of California, San Diego (San Diego, CA, USA) contributed data for 21 of the 43 control eyes. Data for the remaining control eyes, as well as all of the glaucomatous eyes, were from Columbia University (New York, NY, USA).

Control eyes were included based on the following criteria: a normal clinical examination (including a normal-appearing

optic disc) and normal VFs. A subset of controls (seven eyes) did not have visual sensitivity data and were used as a separate group (as explained in Results). Subjects were excluded if they had a history of ocular disease or a family history of glaucoma. Controls were enrolled prospectively. The glaucomatous group consisted of patients in which at least one eye exhibited glaucomatous optic neuropathy, defined based on stereophotography evaluation by glaucoma specialists. All eyes had open angles as viewed during gonioscopic examination. Consecutive patients were enrolled prospectively. Patients with cataracts, a history of ocular surgery, or a history of any other ocular or neurological diseases that could affect structural or functional measures were excluded. For both groups, structural and functional measures were required to be within 1 year of each other.

Written, informed consent was obtained from all of the participants. Procedures followed the tenets of the Declaration of Helsinki, and the protocol was approved by the institutional review boards of Columbia University; the University of California, San Diego; and the New York Eye and Ear Infirmary.

Function: VF Sensitivity

Participants were tested with standard automated perimetry (10-2 and 24-2; SITA Standard²⁷ protocol, Humphrey 750i Visual Field Analyzer; Carl Zeiss Meditec, Inc., Dublin, CA, USA). Visual fields were required to have fixation losses $\leq 33\%$, false negatives $\leq 33\%$, and false positives $\leq 15\%$. The 24-2 VF test was used only for inclusion criteria for the glaucomatous group (mean deviation [MD] ≥ -6 dB). From the larger prospective dataset of glaucoma patients and suspects with 10-2 VF tests, a subset was selected based on an inclusion criterion of at least one total deviation sensitivity value ≤ -3 dB within the central $\pm 8^\circ$. The 10-2 VF MD (mean $\pm$ SD) for the control group was -0.5 ± 1.1 dB and for the glaucomatous group was -3.3 ± 1.9 dB.

Structure: ssOCT

All participants also were tested using ssOCT (Fig. 1; DRI-OCT; Topcon Medical Systems, Inc., Oakland, NJ, USA) with the volume (cube) "widefield" scan protocol (9×12 -mm, 256 horizontal B-scans with 512 A-scans each) with an internal fixation target between the fovea and optic disc. Scans with poor quality (e.g., poor fixation or blink artifacts) were rejected. The thickness of retinal layers was determined using a previously validated segmentation algorithm,¹⁶ which was manually corrected as necessary^{8,17} based on the performance of the automated algorithm. The manual correction was done by individuals masked to the classification of each eye (i.e., healthy or glaucomatous) as well as to the purpose of this study. It was possible to determine the border between the RGC and IPL in all of the eyes in this study and most eyes

FIGURE 1. (A) The RGC layer as obtained by ssOCT. (B) The same as in (A), but without segmentation lines. (C) The image in (B) modified by smoothing (Gaussian blur). (D) The image in (B) modified with higher contrast. Together, (C) and (D) better illustrate the separation between the RGC layer and IPL for those less familiar with OCT imaging.

required little to no manual correction, though recall that poor quality scans were rejected and that the eyes in this study ranged only from controls to mild glaucoma. The center of the fovea was manually determined according to previously established guidelines¹⁷ by taking into account features, such as the location of the peak of the inner-segment ellipsoids and the minimum thickness of the retina; because the dataset consisted of volume scans, the center was determined by using horizontal and vertical cross-sections of the retina (i.e., a B-scan and an extracted vertical “B-scan” equivalent). Scans then were centered by shifting the data based on the coordinates of the manually marked location of the foveal center.

A Novel Method for Estimating RGCs

To convert RGC layer thickness to an estimated number of RGCs, we first obtained measurements of the thickness of the RGC layer independent of the IPL. The segmentation of the RGC layer can be seen in Figure 1A alongside the unsegmented image in Figure 1B. While the boundary between the RGC and IPL layer in Figure 1B probably is obvious to those familiar with interpreting OCT images, the altered images in Figure 1C (smoothing) and Figure 1D (increased contrast) illustrate the boundary more clearly. An automated algorithm that we previously validated¹⁶ uses a strategy employing a combination of image modifications similar to those seen in Figures 1C and 1D. Moreover, for manual corrections of the automated algorithm, it is possible, at the discretion of the user, to apply smoothing (spatial averaging across neighboring cross-section-

al B-scans) or a change in contrast. Thus, a combination of the increased acquisition rate of the ssOCT, our use of a previously validated high-performing automated algorithm,¹⁶ and our use of a previously validated¹⁷ technique for manual segmentation allowed us to measure the RGC layer thickness.

Given a measure of RGC layer thickness, it is possible to directly estimate the number of RGCs if the density of RGCs per unit volume can be estimated. The previously published data of Curcio and Allen¹³ provide the number of RGCs per unit area (RGCs/mm²) rather than per unit volume (RGCs/mm³). To obtain an estimate of RGCs/mm³, first, the raw data for the number of RGCs/mm² were obtained from Curcio and Allen¹³ ($n = 6$ eyes, 992 histological samples) for the superior, inferior, temporal, and nasal retina. Next, from these values, a 2-dimensional approximation of RGCs/mm² was generated by linear interpolation in polar coordinates (Fig. 2A) in a manner similar to that of Garway-Heath et al.¹⁴ The data of Curcio and Allen¹³ were aligned such that the fovea and optic disc were both on the horizontal meridian; therefore, the interpolated 2-dimensional approximation of RGCs/mm² then was rotated based on independent estimates of the average location of the optic disc, corresponding to an angle of elevation from the horizontal meridian of approximately 6.3°.²⁸ Next, an estimate of the RGC layer thickness for controls (Fig. 2B) was obtained by averaging the OCT-derived values for all controls ($n = 43$ eyes). In addition, when estimating the number of RGCs for each individual control eye, a “leave-one-out” approach was implemented such that the average was calculated using all other controls. Because the position of the ssOCT scans could

FIGURE 2. (A) The 2-dimensional RGC density per unit area extrapolated from the histological data of Curcio and Allen¹³ (from their fig. 6), (B) RGC layer thickness derived from ssOCT from the healthy controls in this study, and (C) RGC density per unit volume (see Methods) based on (A) and (B).

vary somewhat from eye to eye, the data for the RGC layer thickness (and consequently all other data shown here) were truncated beyond a radius of 2.8 mm from the foveal center to ensure that equivalent data were available for each eye after centering the scans. The RGCs/mm² and RGC layer thickness (μm) were interpolated in 2-dimensions, such that each pixel represented 0.0001 mm² of area on the retina. Next, the RGCs/mm² was divided by RGC layer thickness in mm to yield an estimate of RGCs/mm³ (Fig. 2C). To obtain a direct estimate of the number of RGCs at a particular location on the retina for a given individual, this “volumetric density map” in RGCs/mm³ can then be convolved with an individual’s RGC layer volume, divided into columns with a base of an arbitrary area (a “pixel”) and a height based on that individual’s RGC layer thickness measurement at that particular pixel. Finally, a direct estimate of the total number of RGCs for a given area of the retina can be obtained by summation of the number of RGCs per unit area of all pixels within that region.

Evaluation of RGC Estimates

The number of RGCs estimated from the method described here were compared to RGC estimates derived from the method of Harwerth et al.,³ the details of which have been described previously.^{3,29,30} In brief, the VF total deviation sensitivity values, which are adjusted based on age-matched controls, were converted to absolute sensitivity values using formulas³¹ provided by the manufacturer (Carl Zeiss Meditec, Inc.). Then, using the most recently published parameter values, the relevant Harwerth et al.^{3,29} equations were used to

estimate the number of RGCs corresponding to these VF absolute sensitivity values.

Both methods of estimating RGCs also were compared to independent estimates of human RGC counts derived from postmortem histology in human subjects. Raw data were digitized from four studies^{25,32–34} and obtained directly from data tables provided in two others.^{35,36} When a study included information from two eyes, the data for the two eyes were averaged. This previously described³⁰ meta-analysis yielded a sample size of 129 eyes (age = 54.3 ± 22.6 years [mean $\pm$ SD]) without optic nerve disease. Additionally, recall that the ssOCT data were truncated beyond a radius of approximately 2.8 mm, which is roughly equivalent to the central 10° tested by the 10-2 test protocol. According to Curcio and Allen,¹³ approximately 50% of the total number of RGCs in the human retina are located within 16° of the fovea; although this is a crude estimate, Dawson and Maida,³⁷ using the considerably older RGC density estimates from Van Buren,³⁸ offer a similar estimate of 50% of the total RGCs within approximately 13° of the foveola. Thus, after accounting for the differences in retinal area and RGC density, the expected number of RGCs within 2.8 mm of the fovea, corresponding to the region used by our approach and that of Harwerth et al.,³ is approximately 32% of the total number of RGCs. Therefore, the estimated number of RGC counts in the macula as determined by our method and the Harwerth et al.³ method were compared to the mean and standard deviation of 32% of the RGC counts obtained from other studies in which the total number of RGCs was reported.

For plots of the structure-function relationship, the VF absolute sensitivity data were converted from dB to linear (1/

FIGURE 3. (A) An example of estimated RGC counts for a healthy control eye. (B) Another example for a glaucomatous eye with focal damage in the inferior retina.

Lamberts or 1/L) units. For example, an absolute sensitivity value of 30 dB would be divided by 10 to convert from dB to log units, and then 10 to the power of 3 (i.e., the antilog) would yield a sensitivity of 1000 1/L in linear units. That is, the stimulus presentation at threshold was attenuated to 1000th of the maximum luminance (1 L or 3183 cd/m²) that the perimeter is capable of producing.

RESULTS

An example of estimated RGC counts for a control can be seen in Figure 3A. Note that the number of estimated RGCs per pixel is based on the density of the sampling (each pixel is 0.01 mm² in Fig. 3). Another example of the estimated RGC counts, this time for a glaucoma patient with clear focal loss in the inferior retina, is shown in Figure 3B.

Although the ideal validation of this novel method of estimating RGCs from ssOCT images will require comparison to histology in the same individual, the estimates in the group of healthy controls, as well as the variability of these estimates, can be compared to published histological data. The mean and standard deviation of the RGC counts from Curcio and Allen (their fig. 8)¹³ as well as a meta-analysis of the literature ($n = 129$ eyes),^{25,32–36} are compared (see Methods) in Figure 4 to the estimated RGC counts based on our ssOCT-derived approach. The estimated RGC counts from our method were similar to the literature values, offering some face validity to our approach.

We also compared our estimates to those based upon the Harwerth et al.³ method for estimating RGCs based on visual sensitivity, as shown in Figure 4. Qualitatively, their method differed from ours and the published histology. Notably, even though both methods for estimating RGCs used the exact same dataset, consisting of the control eyes in this study with available VF data ($n = 36$), there was a statistically significant difference between our method and the Harwerth et al.³ method in the mean (381,000 and 442,000 RGCs, respectively; $P = 0.001$, paired-sample t -test) and variance (standard deviation of 39,000 and 84,000 RGCs, respectively; $P < 0.001$, F -test) of the estimated RGCs, both of which were higher for the Harwerth et al.³ method.

FIGURE 4. The mean (dot) and standard deviation (bars) of the number of RGCs in the macula for healthy control eyes as obtained from Curcio and Allen¹³ (derived from their fig. 8), a meta-analysis of the histology literature^{25,32–36} (see Methods), and two different approaches using the data from the current study (see legend).

FIGURE 5. The average estimated number of RGCs and the average VF sensitivity (see Methods) of the corresponding region for control eyes (open circles) and glaucomatous eyes (filled circles) for the (A) entire macula, (B) superior hemifield (inferior macula), and (C) inferior hemifield (superior macula). The center of each plot is based on the mean of the control eyes on each axis.

The Relationship Between Sensitivity and Estimated Number of RGCs

The estimated number of RGCs was determined for each eye of the controls and patients and compared to the averaged absolute sensitivity in linear (1/L) units for the corresponding region. Figure 5 shows these data for the controls (open circles) and patients (filled). Despite the considerable variability on both axes, the data were fairly orderly for the overall average (Spearman's $\rho = 0.35$, $P < 0.001$, Fig. 5A) and for the superior hemifield ($\rho = 0.47$, $P < 0.001$, Fig. 5B), although less so for the inferior hemifield ($\rho = 0.26$, $P = 0.018$, Fig. 5C). Notice that there appears to be a “residual” number of RGCs associated even with a relatively large degree of sensitivity loss (see Discussion for details).

DISCUSSION

Here, a straightforward method for estimating the number of RGCs directly from OCT-derived RGC layer thickness measurements was demonstrated. The mean and standard deviation of the RGC estimates obtained showed reasonably good agreement with the histological data (Fig. 4). In addition, these RGC estimates were in general agreement with estimates of visual sensitivity (Fig. 5).

Two major lines of research have related RGC counts to loss of visual sensitivity in human eyes. The first, by Kerrigan-Baumrind et al.²⁵ and Quigley et al.,³⁹ compared loss of visual sensitivity to RGC counts based upon postmortem histology. While these data have been generally interpreted as showing that structural damage (e.g., loss of RGCs) precedes functional damage (i.e., VF sensitivity loss), the results do not support this claim. First, as Hood and Kardon¹⁸ point out, this claim is open to two interpretations. On one hand, it implies that the underlying relationship is nonlinear, that is, RGCs are lost before there is any sensitivity loss. The evidence does not support this interpretation (see prior reports^{18,23} for relevant reviews). On the other hand, this claim can be taken to mean that although structure and function may be linearly related (i.e., they decrease together), the structural loss is detected first due to lower variability in the structural measure as compared to the functional measure. For example, Kerrigan-Baumrind et al.²⁵ concluded, “At least 25% to 35% RGC loss is associated with statistical abnormalities in automated visual field testing,” which implies that substantial and detectable RGC loss occurred before sensitivity loss became statistically detectable. However, Malik et al.²³ pointed out that the data reported by Kerrigan-Baumrind et al.²⁵ do not support their conclusion. In particular, many eyes with abnormal VF values had RGC counts within normal limits. In agreement with this interpretation, our data did not support the conclusion that the detection of a loss of RGCs usually precedes detection of visual loss. For the overall average, 19 of the 50 glaucomatous eyes were outside the normal limits (i.e., $P \leq 0.05$) of the healthy controls based on the RGC count and a similar number, 25, were outside normal limits based on the VF sensitivity; thus, in our data, there was no significant difference ($P = 0.210$, McNemar's test) in detectability between structure and function. In general, whether glaucomatous damage will be detected first by structural or functional tests depends upon the nature of the test, retinal location, and individual tested.¹⁸

While Kerrigan-Baumrind et al.²⁵ and Quigley et al.³⁹ counted RGCs as seen with histology, Harwerth et al.³ converted local VF sensitivity to RGC counts in human eyes using a model based upon behavioral and histological data from monkeys. However, estimates of the number of RGCs as a function of eccentricity derived from their model designed for healthy humans, which includes an adjustment for the difference in axial length between a monkey and human eye, are substantially greater than the anatomical data of Curcio and Allen.^{13,30} In a similar manner, estimates of the total number of RGCs derived from the Harwerth et al.³ model, applied to the data in the present study, also were generally greater than the published histology data (Fig. 4), while the RGC estimates obtained from our method were in general agreement with histology. Additional concerns about the assumptions underlying the Harwerth et al.³ model have been discussed recently in detail.^{30,40,41}

However, as is obvious from Figure 5, any model evaluation is subject to the large degree of variability inherent in the data. Note that there is variability on both axes, and the variability for the controls relative to the glaucomatous eyes is larger for visual sensitivity than it is for the estimated RGC counts. That said, the standard deviation of the RGC counts for the controls

is considerable (39,000 RGCs), although when using the method of Harwerth et al.³ for estimating the number of RGCs for the same eyes in this study, the standard deviation was significantly higher (84,000 RGCs) than our method. The increased variability of the method of Harwerth et al.³ probably stems from the use of VF sensitivity data to estimate RGCs.

Previously, we focused on the extent to which the Hood and Kardon¹⁸ model explains observed data after accounting for the variability in both measures,⁴² and future work should consider structure-function models in the context of the inherent variability in the data. Notably, Drasdo et al.²¹ specifically mention the limitations of their model when applied to individual data due to variability. Given that the measurement error of OCT-derived structural data is considerably less than that of sensitivity data,⁴² theoretically it is likely that any model that predicts RGC counts from visual sensitivity for a given individual will usually perform worse than an estimate of RGC based on structure, such as that shown here, though note the caveats mentioned below. This issue of VF variability will be exacerbated further when using local VF sensitivities corresponding to individual test locations as opposed to averaging sensitivity values over larger areas. In fairness, Harwerth et al.³ also described a method for estimating RGC axons from OCT-derived RNFL thickness around the optic disc. However, by directly estimating the RGCs based on local measurements of RGC layer thickness in the macula, our method avoids assumptions about the topographic relationship between RGC axons at the disc and RGC bodies in the macula, a source of considerable variability.^{42–46}

Caveats and Assumptions

We made several simplifying assumptions to estimate the number of RGCs. First, we assumed that the RGC layer is comprised largely of RGCs. Given that we restricted our measurements to the central ± 2.8 mm (approximately $\pm 10^\circ$), this is a reasonably good assumption. The cell bodies, tightly packed in this region, are mostly RGC bodies; the displaced amacrine cells comprise a relatively smaller proportion of the cell bodies in this region than they do in the more peripheral retina.¹³ Second, we assumed that the RGC density at a given eccentricity from the fovea is relatively uniform in all three dimensions. Third, we assumed that the change in the thickness of the RGC layer due to glaucoma results in a proportional change in the number of RGCs, that is, the relationship between RGC layer thickness and RGC number remains linear during disease progression. Fourth, we assumed that there is no residual component of RGC layer thickness associated with a high degree of visual sensitivity loss; this is undoubtedly an oversimplification. In reality, the “residual RGCs” (i.e., a “floor effect”) observed in the data probably correspond to residual RGC layer thickness¹⁰ comprised of displaced amacrine cells,^{13,47} glial cells, such as Müller cells^{48–50} and microglia,^{50–53} and perhaps other nonneuronal components, such as blood vessels⁵⁴ (Kay KY, et al. *IOVS* 2008;49:ARVO E-Abstract 1184) and extracellular matrix proteins.⁵⁵ Thus, in regions with considerable sensitivity loss, we expect that the current approach overestimates the number of remaining RGCs. In the future, the estimate of RGC density per unit volume (RGCs/mm³) could be adjusted to take into account this residual.

Finally, we only studied the region within approximately 2.8 mm of the fovea, that is, the region corresponding approximately to the macula or “central area” with the highest density of RGCs.^{13,28,56} Because the RGC layer becomes very thin outside of the macula, with the maximum thickness ultimately becoming approximately the same as a single RGC, it is likely

that the loss of RGCs results in a change in RGC density without a measureable (with current technology) corresponding change in RGC layer thickness (i.e., a very limited “dynamic range”), thereby preventing an accurate estimate of RGCs in the periphery. Thus, our method is not likely to be useful outside the central $\pm 10^\circ$. That said, a large proportion of the RGCs can be found within this central region, and it is this region that is needed for critical functions, such as reading, driving, and object recognition.^{57,58}

CONCLUSIONS

We described a novel method for estimating the number of RGCs based on the thickness of the RGC layer as measured by ssOCT, with a smaller set of assumptions than previously published techniques. Whereas the value added for clinical applications is yet to be determined, estimating RGC number in this manner should improve our ability to evaluate models of the structure-function relationship in glaucoma.

Acknowledgments

The authors thank the investigators of the National Eye Institute (NEI; Bethesda, MD, USA)-sponsored Diagnostic Innovations in Glaucoma Study (NEI R01-EY011008, R01-EY021818, P30-EY-022589) conducted at the University of California, San Diego for contributing control eyes; Paula Alhadeff, MD, for assisting with data acquisition for the control eyes from Columbia University; Sarah Adams, Maria Mavrommatis, Jacoby Shelton, and Alyssa Ehrlich for helping with the manual correction of the retinal layer segmentation from the OCT images; and Linda Zangwill, PhD, and Alyssa Ehrlich for providing critical revisions to the text.

Supported by National Institutes of Health (Bethesda, MD, USA) Grants R01-EY02115 (DCH), R01-EY011008, R01-EY021818, P30-EY022589, and National Science Foundation Graduate Research Fellowship Grant DGE-11-44155 (ASR).

Disclosure: **A.S. Raza**, None; **D.C. Hood**, Topcon (F)

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