

Erratum in: “Relationship Between Orbital Optic Nerve Axon Counts and Retinal Nerve Fiber Layer Thickness Measured by Spectral Domain Optical Coherence Tomography” by Grant A. Cull, Juan Reynaud, Lin Wang, George A. Cioffi, Claude F. Burgoyne, and Brad Fortune (*Invest Ophthalmol Vis Sci.* 2012;53:7766–7773) doi:10.1167/iovs.12-10752

We have uncovered an error in the absolute values of optic nerve axon counts listed in Table 2 of our recent publication.¹ Our previous method² for counting axons in 100% of the cross-sectional area of orbital optic nerve sections relied on Bioquant software (BIOQUANT Image Analysis Corporation, Nashville, TN, USA) to control the microscope’s X-Y-Z stage as well as the image acquisition by the digital camera (Model# 01-RET-OEM-F-CLR-12; QImaging, Surrey, BC, Canada). Due to limitations of that system and outdated firmware, we have since created custom software to control the stage and image acquisition directly. Specific improvements for our new system include: (1) increased usage of the total available field of view, and also a reduction in the total number of images required to cover an entire histologic sample (image acquisition with the Bioquant software was limited to a central region of 640 × 480 pixels whereas the charge-coupled device chip is 1392 × 1040 pixels); (2) an enhanced depth of field (EDF) focus routine that guarantees focused images at the expense of longer capture times. This feature enables the capture of clear images at all locations even when the sample is not mounted perfectly flat; and (3) reduction of the total acquisition time as the current system automatically avoids capturing images that have no content (e.g., “empty” areas around the outer edge of the section).

Using our new system, we have found that the total axon counts of the same sample sections are larger by an average (\pm SD) of 11.3% \pm 1.6%. We have learned that this is due to an error in the original Bioquant software, whereby the overlap between adjacent acquired images was only approximately 10%, rather than the 15% we had set the software to use (the software was using its default setting regardless of the user input). Thus, our previous compilation routine was discounting areas assumed to be part of the 15% overlap, which were not, in fact, within any overlap region. Our new stage control, acquisition, and compilation software handles the

TABLE. Corrected Optic Nerve Axon Count for Each Eye

Animal ID	Optic Nerve Axon Count		Relative Optic Nerve Axon Count (EG:Control)
	Control	EG	
AM89	908,858	920,672	1.01
26161	1,035,189	984,967	0.95
22159	1,252,305	1,162,086	0.93
AM76	1,385,180	1,213,970	0.88
137	1,138,170	992,322	0.87
AO23	1,244,726	1,076,928	0.87
18664	1,231,868	1,098,354	0.89
26072	1,151,124	985,505	0.86
23499	950,135	804,033	0.85
25564	1,172,739	953,564	0.81
AP02	1,147,344	877,844	0.77
25357	1,080,552	770,922	0.71
25356	1,420,527	709,949	0.50
136	1,439,660	695,471	0.48
21676	1,207,766	533,825	0.44
140	964,156	424,791	0.44
23538	1,076,141	433,652	0.40
139	1,066,227	435,835	0.41
15527	1,355,174	418,615	0.31
25904	1,162,313	332,898	0.29
135	1,001,809	262,888	0.26
20377	1,107,749	285,203	0.26
Average	1,159,078	744,286	0.64
SD	149,576	305,911	0.26

EG, experimental glaucoma.

overlap areas with high-precision accounting, such that no areas within the montage are missed (or counted twice).

We regret that we had not discovered this error earlier and wish to correct the total optic nerve axon counts listed in the original Table 2,¹ which should be replaced with the corrected values listed here in the Table.

None of the other results reported in our paper are materially affected since the corrected total count of all eyes increased by an equal proportion ($11.3\% \pm 1.6\%$); thus, the conclusions also remain the same. The values provided in the two tables of the original manuscript represent the complete dataset, which would enable the reader to confirm the robustness of all reported results and conclusions after correction of the total axon counts.

References

1. Cull GA, Reynaud J, Wang L, Cioffi GA, Burgoyne CF, Fortune B. Relationship between orbital optic nerve axon counts and retinal nerve fiber layer thickness measured by spectral domain optical coherence tomography. *Invest Ophthalmol Vis Sci.* 2012;53:7766-7673.
2. Reynaud J, Cull G, Wang L, et al. Automated quantification of optic nerve axons in primate glaucomatous and normal eyes—method and comparison to semi-automated manual quantification. *Invest Ophthalmol Vis Sci.* 2012;53:2951-2959.

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