**Using Bioquant® as a Viable Method for Counting Axons**

**Pouya Jouharian1,2, Assraa Jassim-Jaboori1, and Denise M. Inman1**

1Department of Pharmaceutical Sciences, Northeast Ohio Medical University, Rootstown, OH;

2School of Biomedical Sciences, Kent State University, Kent, OH

[pjouharian@neomed.edu](mailto:pjouharian@neomed.edu)

**Objective:**

The primary objective of this study was to determine if Bioquant***®*** is a practical program to use for counting axons. Axon degeneration is the earliest pathology in glaucoma, therefore axon number is a primary outcome measure to determine if glaucoma is present. However, counting every axon is inefficient, so we also tested whether the sampling counting method could give equivalent results compared to the whole counting method.

**Methods:**

Optic nerves were dissected from control and glaucoma mice. They were fixed in 4% paraformaldehyde for 24 hours then post-fixed in 2% paraformaldehyde + 2% glutaraldehyde. Both optic nerves were osmicated, embedded in Polybed 812 and sectioned on an ultramicrotome (1μm). The sections were mounted on slides and stained with ρ-phenylenediamine. The optic nerve sections were imaged on a Zeiss microscope at 100x magnification. Photoshop® was used to enhance the image by changing the contrast and brightness to better capture the axons. Finally, Bioquant® measurement filters and editing tools were used to capture and quantify the axons.

**Results:**

There was an average 47.5 (0.194%) axon count difference between the whole counting method and the sampling counting method. Using the sampling counting method, a 6695 (36.8%) average axon count difference was found between the control optic nerve and the experimental (glaucoma) optic nerve. There was an inter-rater variability of 9.20% when using the whole counting method compared to 5.65% when using the sampling method.

**Conclusions:**

The sampling counting method was found to be a much more efficient method with similar accuracies when compared to counting the whole optic nerve section. Experimental optic nerve has significantly fewer axons than the control optic nerve**.** The inter-rater variability was significantly less when the sampling method was utilized. Given these observations, Bioquant® was determined to be a viable resource for counting axons**.**