Unbiased rapid batch quantification of retinal gene therapy immunohistochemistry

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Purpose
Multiple methods and formulations exist for transduction of the murine retina. However, quantification methods of transduction efficiency often involve time-intensive manual counting of individual cells or regions of interest (ROIs) that introduce bias, or custom programs that require knowledge of complex coding languages. Here, we present a rapid and reliable batch quantification method using a user-supervised program with a simple interface in the open-source FIJI/ImageJ software.

Methods
Murine eyes were injected intravitreally with different formulations of AAV9 with GFP cDNA versus balanced salt solution (BSS) as a control. Mice were culled and eyes enucleated at 47 days post-injection. Eyes were fixed and immunohistochemically (IHC) stained to enhance GFP. Z-stack images were acquired using confocal microscopy at 5 fixed locations along each retina section at 40x magnification (n = 175). Images underwent batch processing and analysis using our program in FIJI/ImageJ. Program workflow is shown in Figures 1 and 2.

Results
Our program successfully processed images from over 175 unique retinal locations in a single batch within minutes. Mean pixel intensity values showed a significant difference in GFP expression between different AAV9 groups. Retinas injected with BSS were negative for GFP expression.

Conclusions
Our program automatically measures differences in GFP expression and allows user supervision of ROI

generation. This has three advantages. 1) Avoidance of manual selection bias. 2) Rapid quantification of large image sets. 3) User feedback to avoid artifact. The open-source nature of the program allows broad application with the widely used FIJI/ImageJ software. This tool can be easily incorporated into existing workflows for bulk quantification of IHC with sagittal retinal sections.

Layman Abstract (optional): Provide a 50-200 word description of your work that non-scientists can understand. Describe the big picture and the implications of your findings, not the study itself and the associated details.

Scientists often compare treatments to find the most effective options for patients, using various tools to investigate their effects in the lab. One such method involves injecting different treatments into the eyes of mice and studying the resulting changes. By taking high-resolution images of thin slices of mouse eyes, researchers can observe changes at the cellular level. However, analyzing these images manually is time-consuming and subjective.

Our program automates this process, analyzing large sets of images and assigning numerical values to represent changes in each one. These numbers make it easy to compare the effects of different treatments. For example, we used this tool to evaluate how different gene therapies are absorbed and utilized by eye cells.

This program is versatile and can measure a wide range of changes in the eye. It decreases subjectivity and dramatically speeds up analysis, processing in minutes what would otherwise take days. Its simplicity and efficiency make it a valuable resource for labs studying eye treatments.