INTRODUCTION
Glucoma is the second leading cause of vision loss in the world. Characterized by progressive and irreversible vision loss, it affects more than 60 million people world-wide [1]. The pathogenesis of this disease is not fully understood however glaucoma has been positively correlated with elevated pressure inside the eye. Patients with glaucoma show significant deformations in the optic nerve head at the posterior pole of the eye. These results suggest the onset of this disease may be mechanical in nature, and thus we choose to study the eye as a mechanical structure to gain insight into the onset of glaucoma [2].

As mentioned, the optic nerve head is the specific region of interest in terms of studying glaucoma. It is the complex structure at the back of the eye that allows passage for the optic nerve and central retinal vessels. The optic nerve head has several structures within it, many of which have been studied in depth. Due to limitations in imaging capabilities, the blood vessels that nourish the optic nerve head have not been studied much. This will be the focus of this project.

These vessels provide nourishment to the tissues of the optic nerve head and are critical to tissue health. It has been shown that in patients with glaucoma, blood flow to the optic nerve is reduced [3]. We believe that the elevated pressure conditions present in patients with glaucoma may impact these vessels, and thus is worth investigation.

Previous imaging techniques that have provided insight into the optic nerve head require analysis to be done ex-vivo. A new imaging technique, optical coherence tomography, has been developed that can generate 3-D images of the optic nerve head non-invasively. This technique has not yet been used to quantify the blood vessels in the optic nerve head. Our study will aim to do this.

OBJECTIVE
The objective of this study will be to develop a method of marking the locations of the blood vessels of the optic nerve head and show that this method is repeatable. The motivation for this study is to show that this technique of marking is repeatable, so it can be used to analyze the effects of pressure on the vasculature of the optic nerve head non-invasively. This technique has not yet been used to quantify the blood vessels in the optic nerve head. Our study will aim to do this.

SUCCESS CRITERIA
The success criteria of this study will be to develop a method that can produce repeatable results. The goal is to see less than 5 percent error in the diameter of the vessel markings. We also aim to see that the number of vessels identified through each set of markings is the same and their locations are comparable.

METHOD
Four images of two monkey optic nerve heads, generated from optical coherence tomography, were analyzed with respect to their vasculature network. Using the program FIJI, the vessels were marked on every 8th slide of the image stack (761 slides) using the elliptical tool. Each image was marked once, and then the process was repeated until each image had three sets of markings. To minimize remembered effects, and to get a more accurate representation of the true repeatability, the markings on the same image were not done immediately following each other so that the marker did not apply bias from previous trials.

The outer wall of the vessels were marked where they were visible. Vessels were identified by a defined wall, and a shadow cast beneath them. Also they characteristically exhibited hyper-reflective regions on the top and bottom due to the interaction between light and moving blood [4].

Once all the markings were collected, the three sets for each image were overlaid and the markings were checked, by inspection, for number and location of the vessels marked. The results for this step were not quantified. This evaluation was done to ensure the test for diameter repeatability would not be affected by repeatability of the number of markings.

The markings were then imported into the 3-D visualization software, AVIZO. Here, the vessel network was visualized and can best be described as a series of tree branches. Five individual markings were selected from each branch for diameter measurements. Diameters were measured using the 3-D measurement tool in a projected view from the top of the network. The corresponding markings on sets 2 and 3 of the same image were measured for their diameters as well. For the two images from monkey 1, 35 diameters were measured (7 branches), for the two images from monkey 2, 45 diameters were measured (9 branches). Corresponding diameters between sets were then compared.

RESULTS
The diameter of each marking was compared to the average diameter measured at that location (from the three sets). The absolute value of the error was normalized by the average, and then the normalized error of all the markings of a set were averaged to find the average percent error within each set of markings (12 sets total). The average error across all 12 sets was then averaged to find the average error of the method.

Figure 1 is a histogram showing the distribution of the average percent errors within each image set. The average error was located at 5.4%, and the median at 5.5%.
The average error across all sets was determined to be 5.4%. The minimum and maximum of the 12 sets were 4.2% and 6.3% respectively. The maximum error of an individual diameter to the average at that location was 26.3%. The equivalent minimum was 0.04%.

**DISCUSSION**

The objective of this project was met. The observed percent error of 5.4% was slightly higher than our goal but sufficient to claim this method is repeatable and can be applied to future studies. We were also pleased with the consistency of location and number of vessel markings between sets.

The measured variability will need to be considered when determining the experimental effect of pressure manipulations in future studies. In other words we will need to see a certain magnitude of change in a parameter to say it was due to the pressure conditions and not an artifact of our measured variability.

In the case of the 26.3% error, the vessel was a smaller one, in a less clear region of the periphery. Errors this high were not common but tended to be located on the smaller vessels in the peripheral regions of the scan where the image clarity was compromised. To modify the protocol we could raise the clarity criteria so that we only mark vessels we feel very confidently are visible and defined, but in doing so we would be sacrificing many data points that could help contribute to our findings. The balance between quality of the data and the quantity of the data is something the researchers marking these scans must keep in mind. However, as the imaging technology continues to improve, the quality of the scans we are marking will get better and the repeatability of our measurements will continue to increase.

As mentioned earlier, the primary limitation in our study was the inherent blurriness of the images. Vessel walls were rarely well defined and this blurriness was the primary source of our measured variability. On top of that all markings and measurements were done by hand, limiting the speed of data collection. Due to the fact that all vessel diameters were measured by hand, only select vessels, 5 per branch, were measured. There are more diameters that can be measured and compared in the future if the process can be automated, that project is currently in progress. It would also be interesting to see if we could quantify the repeatability of location using centroid positions rather than just checking visually. Also, investigating the local repeatability between the central vessels, which tend to be well defined, and the peripheral vessels, which tend to be harder to see, may be a project worth pursuing in the future.

Now that this method has been shown to be acceptably repeatable we can use this method to investigate how the vessels of the optic nerve head are affected under various pressure conditions. Using this method, over 30 images have now been marked at various pressure conditions for the optic nerve head of 3 monkeys. The development of this method has translated directly into this new project which is now underway, investigating the effects of pressure on blood vessels of the optic nerve head which may lead to new insights into the cause and progression of glaucoma.

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