**Motivation:** Developing my passion for research and engineering was not my initial plan coming to UW, as I initially joined Professor Nance's lab with the goal to pursue a medical degree. With an attraction to studying pathology - the study of diseases - to enrich the lives of those in the same way UW medicine helped my family, I found myself interested in how research is involved in the medical field. As I met more people from different backgrounds and got to work in different areas, I realized that not all research involved white lab coats. In the Nance lab, I saw graduate students combine current technology and developing lab techniques to collaborate towards one goal - to develop nanotherapeutics for the brain - through emerging technologies of data science.

Working with my graduate mentor, Hawley, I became fascinated by cell morphology - cell shapes and shape changes - and the power of data science to analyze features of healthy and diseased brain cells. Rather than manually counting and segmenting the brain cells over hours, code expedited this process to a few seconds. An automated methodology also created a reproducible pipeline that was versatile for other researchers to use and apply to quantify a variety of features. I would like to build onto this existing package by exploring the following questions: **How can we build on previous research to quantify microglia features of microglia branching by giving it the ability to specify certain characteristics? When analyzing microglia across species, how do we determine if certain microglia characteristics are specific to a certain species?**

**Background:** Immunofluorescent images are a common way to analyze preclinical models of brain disease. To study the brain's immune response, microglia – the immune cells of the brain – are typically stained and viewed with immunofluorescent imaging. Often, after images have been acquired microglia are qualitatively analyzed by the eye. Rather than qualitatively analyze their

morphology, I plan to build on a method developed by the Nance lab that can quantify important features of microglia with a focus on branching - arm like protrusions from the cell body.

Microglia have a range of functional states dependent on their local environment to keep the brain environment healthy. Microglial functionality and microglia morphology (shape) are highly correlated. When microglia are in their normal surveillance state, they have a ramified or branched state with a small cell body [5]. In the presence of injury or disease, they can migrate and change their shape, increasing their cell body size and changing their number of branches [5]. By taking images of microglia in healthy and diseased brains, we can gain insights into their functional state and their local environment.

Previously, research within the Nance Lab has focused on quantifying microglial features such as area, perimeter, or circularity - examining the cell as a whole [3]. In my research, I plan to expand upon previous work by adding additional branching features to the quantification pipeline. Looking at the number and length of branches around each cell gives us information on the functional state of the cell. Healthy microglia have fewer and more extended branches [4]. Microglia with short branching and uneven branching are less able to perform the functions required to maintain a healthy brain environment [5].

Much fundamental research about microglia involves the usage of animal models. There are many different species used to model brain disease in therapeutic trials and preclinical research studies which have limited - directly comparing microglia response in one species to another. For example, in the field of neonatal brain injury in which we do our research, three common models to test therapeutics are the rat and ferret, representative of a small animal model, and the sheep, representative of a large animal. The rat and sheep display a range of similar microglial morphologies but have unique differences in branch features such as increased

branching in the sheep and longer branch length in the rat [1]. The ferret is an emerging small animal model to study neonatal brain injury due to the similarity in brain gyrification and white matter to gray matter ratio seen in humans [3]. Qualitatively, the lab has seen differences in branching in ferrets and rats [3, 7]. In this proposal, I will investigate the species-dependent effect on the microglial shape.

**Methods:** I will analyze microglia shape using images of these cells obtained from the neonatal human-term equivalent rat (postnatal day 10, P10), ferret (P21), and sheep (P90). Tissue samples are provided by lab members and existing collaborators, and a lab member with expertise in imaging microglia will collect the images using a Nikon A1R confocal microscope (**Figure 1a**). I will analyze microglia skeletonization - branching of microglia - by building out the microglia quantification pipeline [2]. This pipeline includes image acquisition, image segmentation, image skeletonization, and data analysis. I will compare (1) male versus female within a single species and (2) species versus species of microglia images from the cortex, hippocampus, thalamus, and white matter [3].

To perform my analysis, z-stack images of microglia at 40x magnification obtained from the confocal will be converted from a .nd2 file format to a .tiff file format [6] (**Figure 1b**). I will then binarize the image using the Otsu threshold, an algorithm for binarizing images (**Figure 1c**). Comparing the raw to the binary image, I will identify any small holes or errors that Otsu has recognized as microglia and refill or erase them manually through Fiji. I will save the segmented images as a .png file [6]. If this method does not work, I will be able to do the segmentation by hand of a sample, which is the industry standard for microglia research. Afterward, I will skeletonize the image in scikit-image using the built-in skeletonization function which takes a bulkier shape and finds the minimum outline or basic lines of the shape (**Figure 1d**). To quantify

the skeletonized images, I will use a python package called Skan (**Figure 2a**). Skan can measure branch features of the cell skeletons [2]. I will then collect data that includes: the number of branches, branch length, and the number of nodes - which measures the breaking points in each cell. I will plan to analyze a minimum of 200 cells per region. Statistical analysis will be performed using GraphPad Prism [3]. Vertical scatter plots of different regions will be displayed comparing the number of branches, branch length, and the number of branch nodes in the rat, ferret, and sheep with markers indicating the sexes (**Figure 2b**).

**Impact:** The support of Mary Gates' research scholarship will allow me to commit more time to this project as a prospective Ph.D. student interested in computer engineering and data science. After graduation, I plan to pursue a career as a research scientist. Later, hope to go back to school and teach or lead a research group like Professor Nance - mentoring students pursuing higher education through my expertise working in both academia and industry.

This project will allow me to combine data analysis and wet lab concepts as I will collect cell images, write python scripts, and visualize quantitative data. Identifying a concrete hypothesis, collaborating with researchers, and writing for grants will leave an impression and allow me to have a smoother transition to higher education.

My project will also apply to a larger cross-species project where the lab will continue to compare microglia cells of different animal models. This would be beneficial to the field as researchers would be able to differentiate between species or treatment-specific characteristics when comparing animal models. I will be meeting with Hawley biweekly and will be communicating with Dr. Nance through our weekly lab meetings to get any feedback on the progress of my project. I will also be able to build on my soft skills to communicate technical terms by presenting at lab meetings and the Undergraduate Research Symposium.

## Figures:

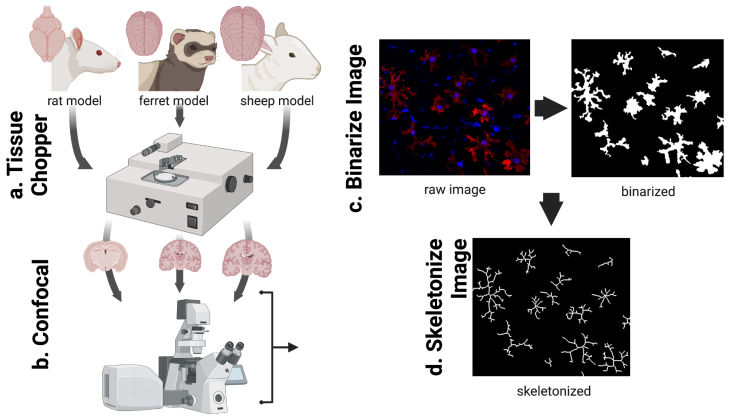


**Figure 1: Schematic of microglia skeletonization pipeline.** (**a**) Brains are collected and cut into slices. (**b**) Image fluorescent imaging is applied. (**c**) Image is segmented using Otsu method. (**d**) Image is skeletonized using scikit-image. Image adapted from Ref [2]. Created with BioRender.com.

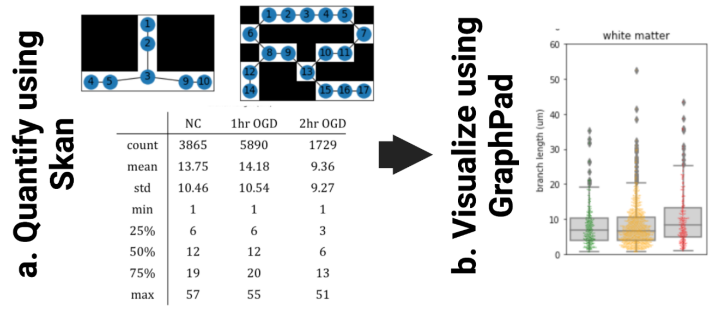


**Figure 2: Schematic of data analysis workflow.** (**a**) Images are quantified using Skan. (**b**) Data is visualized as a vertical scatter plot. Image adapted from Ref [2]. Created with BioRender.com

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