Overview: Research in the Nance Lab is dedicated towards engineering tools to understand and treat neurologic diseases. This project work focuses on engineering a tissue model of age-related cognitive decline to the explore mechanisms that underly development of pathological aging. In a broader sense, we are interested how aging mechanistically renders the brain more susceptible to sustained injury or irreversible disease, as well as the trajectories of these pathologies within the brain. Similar to how clinicians use diffusion MRI-based imaging to detect early onset age-associated neurodegenerative diseases, I map diffusion characteristics in 2D brain tissue cultures to study extracellular/structural mechanisms of neurodegeneration. It is critical to benchmark diffusion studies with verified biomarkers of cognitive decline and associated neurodegeneration; however, this remains a relatively undeveloped space in tissue models of brain disease. With background materials provided for how neurodegeneration is typically benchmarked in other model types, you will aim to propose and engineer biomarker quantification in tissue models following full training on culturing and characterization techniques.

Goals for winter quarter 2022:

1. Gain experience working with tissue culturing and downstream quantification methods such as confocal microscopy, immunofluorescence histochemistry, gene expression profiling and separation chromatography
2. Based on knowledge and experience with the above techniques, as well as a list of 5 verified neurodegeneration biomarkers from the literature, identify 3 potential biomarkers for neurodegeneration in tissue models and propose evidence-based experiments for evaluating their presence in tissue model of age-related neurodegeneration. You will be provided an example and template for protocol design.
3. Aim to implement protocol and generate dataset for at least one neurodegeneration biomarker in age-related neurodegeneration model (may carry over into spring 2022)

Work expectations:

- 3 hours / week, which can be dedicated time for planning experiments, organizing/visualizing data, running experiments, or building your slide deck for a presentation in lab group meeting. You are welcome to take on more hours of extracurricular research work case-by-case as you see fit, but we'll brainstorm how to spread out objectives over the 10 weeks to maximize productivity without compromising time spent prioritizing schoolwork/other extracurricular responsibilities.
- We'll shoot for half-hour check-ins every week to start, can be more/less frequent depending on how things go
- For science communication practice, you'll either present in lab meeting, or if not scheduled for this quarter, you'll prepare a 1-page summary of your findings and interpretations and translate this to a slide deck for a future presentation

Training: no prior experience is required for tissue culturing or any of the downstream quantification methods (above), although some background from current/past coursework in engineering, introductory biology or chemistry may be useful. The aim for this quarter is that you receive lab training in parallel with learning science communication and experimental design. In the lab, you will receive full hands-on training at a comfortable pace. We will first cover all our lab safety protocols and expectations. Tissue culturing is the foundation of our experiments, so we will introduce tissue culturing step-by-step. Following comfortability with tissue culturing, we will

begin covering quantification methods such as immunofluorescence microscopy for visualizing cells and tissue structures, RT-qPCR (reverse transcriptase quantitative polymerase chain reaction) for gene expression profiles, and liquid chromatography/mass spectrometry for analyzing composition of samples, all based on potential biomarkers for quantifying neurodegeneration in brain tissues.

Objectives and experiments:

1. Review literature on neurodegeneration studies and biomarkers in a variety of models. To aid you in learning how to approach scientific literature, a list of 5 neurodegeneration biomarkers (and sources) will be provided for you. We will go over strategies for reading and analyzing these articles, as well as how to maintain a document/database of key findings and references. This is meant to be exploratory and skills to build upon throughout the quarter and beyond.
2. In parallel with (1), become familiar with tissue culturing protocols and downstream quantification methods. The goal here is that your training informs your knowledge of which biomarkers may be most efficient to translate to the tissue model.
3. Choose 2-3 biomarkers from your literature readings that you think would be suitable and valuable for benchmarking neurodegeneration in the tissue model. For each biomarker, write up a 1-page (max) proposal for how you would try and implement these in slice cultures. This is intended to be an engineering/design exercise and there's no expectation to get it right on the first try! Part of the point of science research (and especially engineering) is troubleshooting, and you must start somewhere. Proposals should include the following information and follow the general format (you will be provided a sample!):
    i. Literature background – discuss use and implementation of X biomarker in X model(s) and provide a brief description of quantitative results
    ii. Translation to tissue model – explain how quantification of X biomarker could be adapted to brain slice culture. This section should include a brief description of experimental procedure and methods used (immunofluorescence microscopy, RT-qPCR, etc.).
    iii. Hypothesis – what do you expect? For example, "I hypothesize that immunofluorescence of neurofibrillary tangles will increase in response to higher or longer sustained doses of neurotoxin" or "The expression of X gene is positively correlated with neuronal death at 6 days of neurotoxin exposure".
    iv. Limitations and alternatives – what are the assumptions you are making about the functionality of this protocol? How might this protocol be limited, or fail? What are some alternative approaches you could engineer to better quantify X biomarker?
4. Towards the end of the quarter, or into next, give it a shot! We'll review your protocol(s) together and you'll have the opportunity to generate a dataset for one neurodegeneration biomarker.