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Project: Improving the Multicolor Atlas for Neuronal Identification in C. elegans for Freely Moving Imaging

As an MSRP student in Steven Flavell's laboratory, my research focused on improving the multicolor atlas for neuronal identification in *C. elegans* for freely moving imaging. This project aimed to enhance our

understanding of how neurons across the brain produce behavior and how this is influenced by an animal's internal state. While brain-wide calcium imaging in freely moving *C. elegans* is possible, there remains a need for a strain that combines naturalistic behavior with reliable neuronal identification. Using the innovative NeuroPAL strain (Yemini et al., 2021), neurons can be identified by their genetic expression using a color-coded system of 4 fluorophores: TagRFP-T panneuronally, as well as CyOFP1, mTagBFP2 and mNeptune2.5 in distinct subsets of neurons. However, annotating neuronal identity requires extensive manual curation, and not all neurons are identifiable in every animal. Additionally, the high number of transgenes expressed in the NeuroPAL strain hampers normal growth and behavior. To address these issues, we aimed to create a new tool by crossing two strains: a base strain that expresses brighter TagRFP-T panneuronally and CyOFP1 in a genetically defined subset of neurons, and an assistant strain that identifies the remaining neurons using mTagBFP2 and mNeptune2.5.



Carolina with her mentor, Di Kang (Candy). Photo courtesy of Mandana Sassanfar

This summer, I worked on developing this two-color base strain, and characterized the growth rate and locomotion pattern of this base strain in comparison with wild-type animals. The identification of all neurons of this base strain based on their brightness, size, and spatial location during freely moving imaging can be achieved with an artificial neural network, which learns from human annotations of the four-color images of the crossed strain. This new approach will enable us to study the function of the *C. elegans* nervous system in a more accurate and high-throughput fashion.



Carolina presenting at the MSRP Poster Session. Photo courtesy of David Orenstein



MSRP Poster Session, August 2024. Photo courtesy of David Orenstein