**Progress Report April 2025**

- Project Title: **Cyst Nematode Single-Cell Omics**
- Lead PI: Thomas Baum

The Baum lab has made substantial headway into the milestones laid out for Year 1 of our project on "Cyst nematode single-cell omics." For Objective 1, we have successfully established an inoculation method to localize SCN-infected root regions, utilizing the aid of a 3D-printed inoculated chamber developed in the Baum lab. Using this new method, we can routinely inoculate young soybean root radicles with large numbers of penetrative juveniles and concentrate the resulting infective nematodes into a region of heavily infected soybean root tissue that can be rapidly harvested at set timepoints. By utilizing our method for focusing the infected tissue, we have subverted, for the moment, the need to incorporate a fluorescent tag into the system to identify and retrieve syncytium-specific nuclei. Instead, we are opting to collect all the nuclei from the highly infested root region and utilizing the power of next generation sequencing to rapidly sequence all the nuclei. We then identify the syncytium-specific nuclei by utilizing gene tags unique to our syncytial tissue.

Working in collaboration with Mark Libault at the University of Missouri, Khalid Meksem at Southern Illinois University and Tarek Hewezi at the University of Tennessee, we have designed an experiment where we inoculated and harvested soybean roots at two time points and utilizing both a susceptible and resistant soybean variety against an avirulent SCN population. We have collected nuclei from the infected regions for all these conditions, as well as from mock inoculated control roots for those same conditions, all with replicates, and sequenced them in a pilot experiment. The data from this experiment are currently being analyzed, the results of which will educate our next steps for Objective 1.

For Objective 2, we have made great strides toward our ability to sequence SCN gland cell nuclei on a large scale. Our lab has previously established the ability to routinely extract whole gland cells and sequence small populations of these to generate small scale transcriptomic analysis of these cells. Recently, we have had a breakthrough in scale. By using a combination of established nematologic methodology, as well as existing nuclei extraction buffers and techniques, we have shown the ability to extract, isolate and detect gland cell nuclei from these very same gland cells. More importantly, this can be done at scale, meaning we can perform this technique on large numbers of nematodes, starting with pre-parasitic juveniles, which are easily obtained, and from which, we can generate pools of thousands of gland cell nuclei.

An existing hurdle in this approach is that these gland cell nuclei are present in a mixture with other nuclei from the nematode body. However, we can use the unique fact that our gland cell nuclei are much larger (on the order of 5 to 10 times) than non-gland cell nuclei to our advantage and apply the established technique of cell/nuclei sorting to our pool of nuclei. Utilizing our Flow Cytometry Facility on campus, we have successfully sorted nematode nuclei and identified unique regions in our sort that represent enriched regions of gland cell nuclei, which can be isolated and collected based on their unique properties. We have been able to collect pools of nuclei consisting of nearly 90% gland cell nuclei. Next steps for us for Objective 2 will be to generate next-generation sequencing libraries for these gland cell nuclei and sequence those libraries to verify identity using gene tags that are unique to gland cells. Furthermore, utilizing gene tags that differentiate between the two types of gland cells, dorsal and subventral, each with unique developmental functions, we can sort our transcripts by gland cell type. This will further enhance the functionality of our resulting single nuclei transcriptomic analysis of the gland cells. Finally, by employing this technique on the differential SCN life stages, we will finally be able to generate a complete picture of the gland transcriptome over the SCN life cycle, allowing us to identify several unique potential targets to attack in engineering resistance against SCN.

**Supporting Figures**

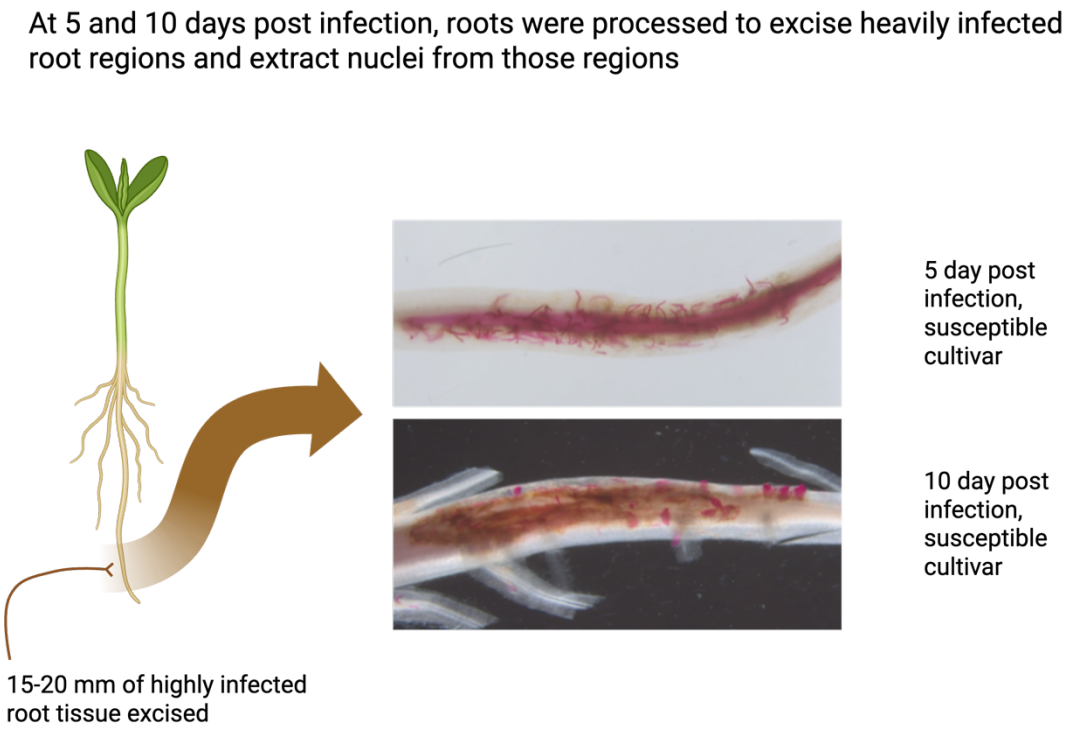


***Figure 1:*** Soybean roots were infected using our 3D-printed tools to localize nematodes to small regions, which allowed the harvest of a high percentage of cells from developing syncytia.

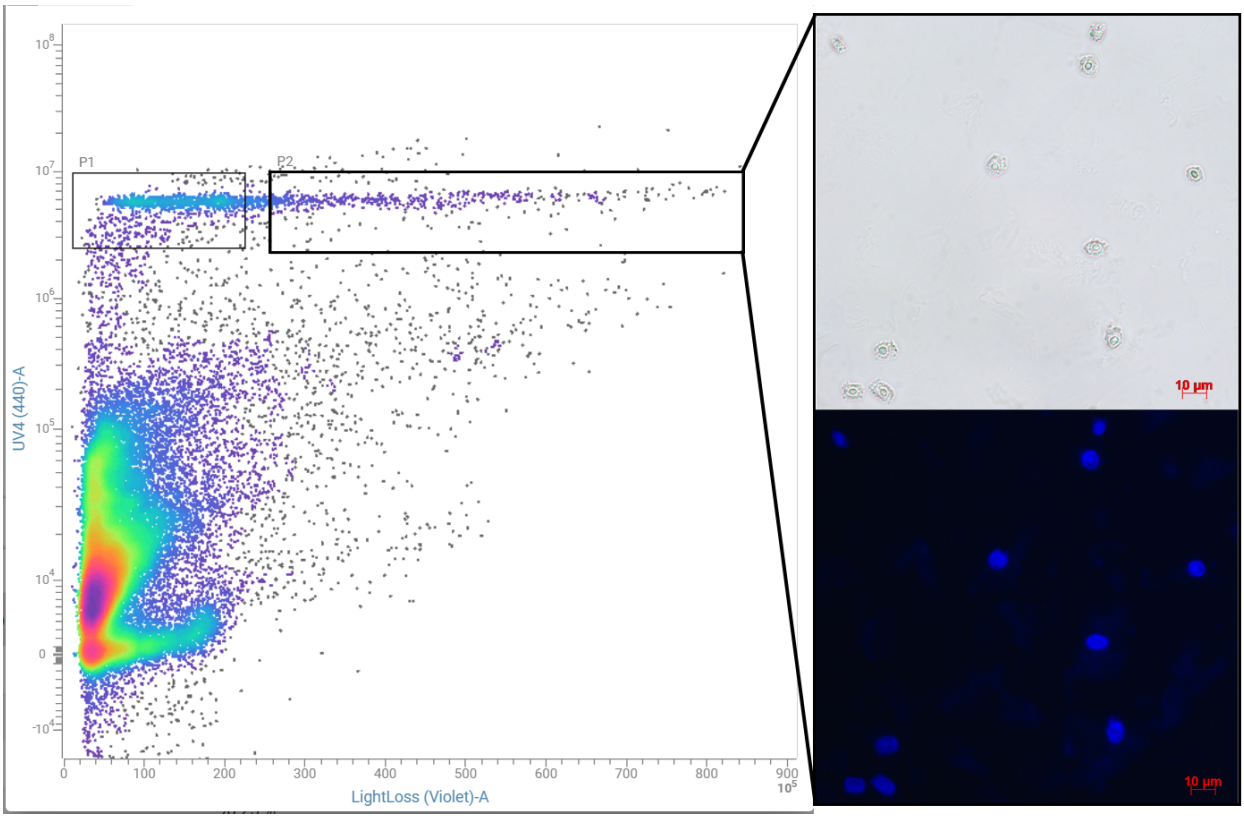


***Figure 2:*** Nematode nuclei were harvested and subjected to cell sorting using the ISU Flow Cytometry facility. We were able to identify nuclear fractions that were highly enriched for gland cell nuclei.