Developing a high-content, whole organism behavioral screening platform for plant-based compounds

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Motivations:

- Plants have evolved complex chemical strategies to influence animal behavior and ward off predators and parasites.
- Humans have cultivated plants with this strategies and have used them therapeutically.
- Plant compounds have provided insight into the molecular basis of neural communication and starting points for treatments of nervous system disorders.
- Ion channels modulated by G-proteins in chemosensation are conserved between human and *Caenorhabditis elegans* (1).

Goal: To leverage medicinal plants and animal behavior to identify new chemical and molecular entry points for the treatment of neurological and psychiatric diseases.

How do worms sense chemical compounds in their environment?

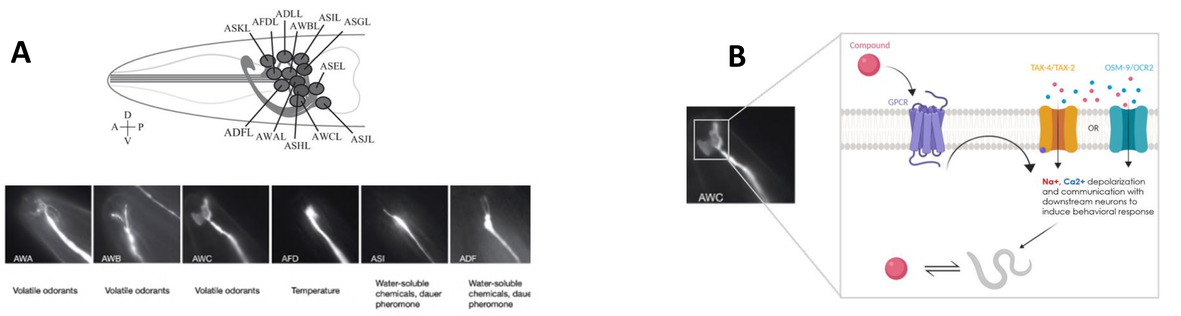
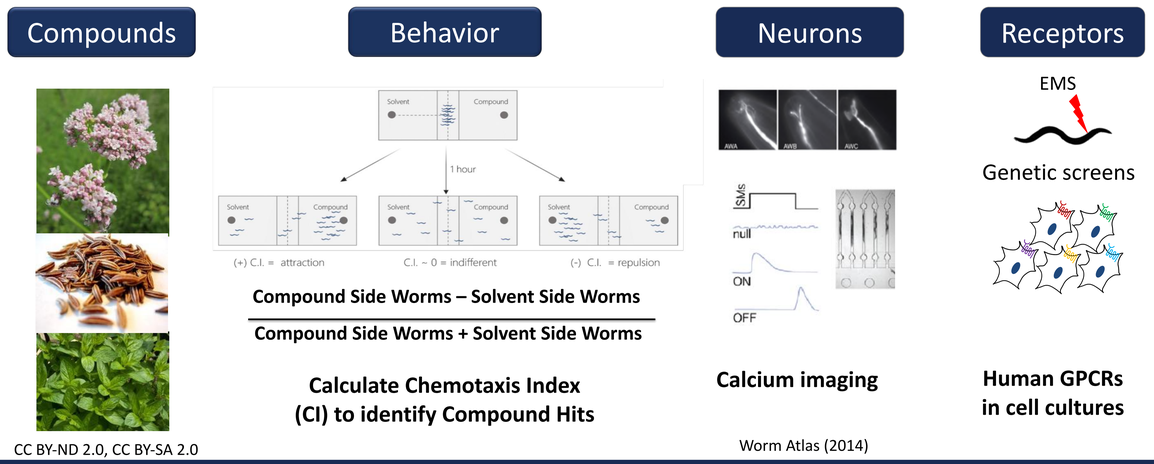


Figure 1: (A) Diagram and images of the anterior neurons responsible for chemosensation. **(B)** Chemosensory signaling is activated when a compound binds to a G-Protein Coupled Receptor (GPCR) resulting in a secondary signaling cascade

Figures adapted from Bargmann, Wormbook (2006) and Worm Atlas (2014)

NeuroPlant Concept



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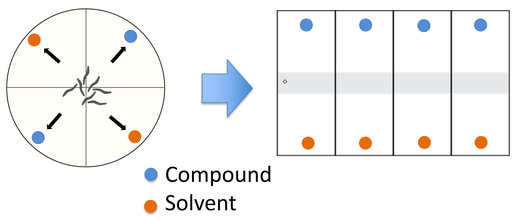
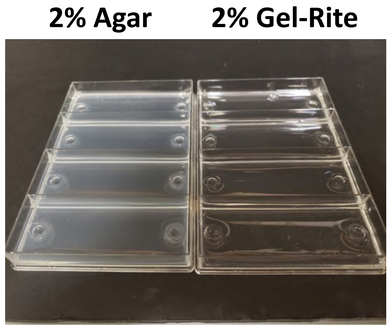
Worm Atlas (2014)

Transforming Classic Chemotaxis Assays for High-Throughput Capability

Moving away from a classic round plate (2), we utilize a 4-well plate with a standardized 96-well footprint. This allows simultaneous runs of multiple worm strains and seamlessly integrates with automation.



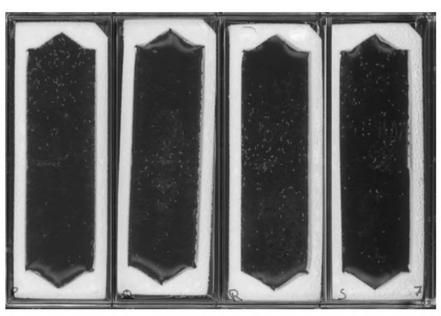
2% Agar **2% Gel-Rite**

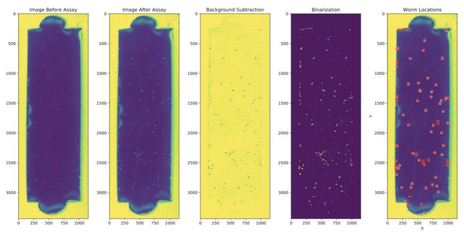


Using Gel-Rite instead of agar improves optical clarity and allows us to capture images on a scanner from the bottom up.

Addition of foam inserts:

- Prevents exit of worms from arena.
- Provides contrast for better adaptive thresholding values.
- Creates positioning markers for compound placement accuracy.



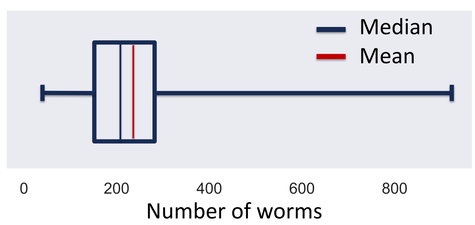


We are creating an algorithm to automate worm counting and CI calculation by incorporating:

- Adaptive thresholding
- Background Subtraction
- Filtering by size

Improvements Between Screens

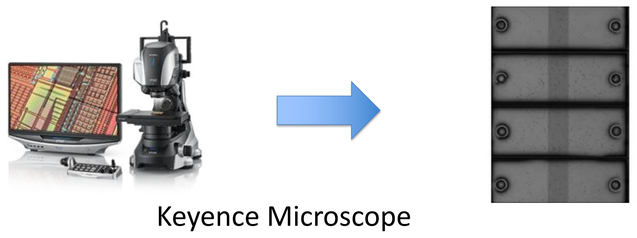
Worm Dispensing



Dispense Method: Manual Pipette

Image Capture

Imaging Time: 1 Plate/15 min
File size: 600 MB/image
*Worms need to be anesthetized

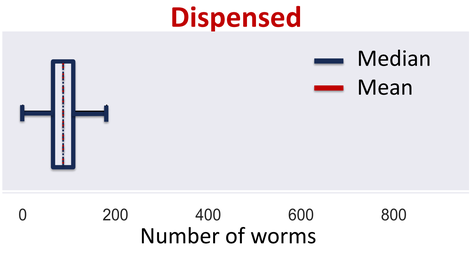


Keyence Microscope

Personnel

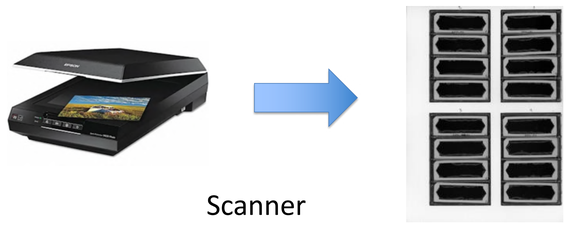
X 2 Weeks

Increased Uniformity of Worms Dispensed



Dispense Method: Liquid Handler

40-Fold Increase in Image Capture Speed
Image Content: 4 plates/1.5 min
File size: 143 MB/image
*No anesthetization needed



Scanner

50% Reduction in Personnel Needed

X 3 Weeks

Future Directions

- Redesign foam inserts to reduce variability in chemotaxis 'starting zone' introduced during drying step.
- Calibrate worm/liquid dispensing ratio to increase mean and reduce variation in total # of worms dispensed.
- Create Virtual Machine to coordinate simultaneous image capture by 4 separate scanners.
- Run screen with single strain to identify lower limit of personnel required for screen.
- Further investigate hits to identify responsive neurons and GPCRs.

Conclusions

First Screen:

1. Imaging was found to be the rate limiting factor during August screens.
2. We obtained 3 "hits" out of 10 compounds screened.
3. The ion channels involved in GPCR mediated signaling have been identified for these 3 compounds.

Second Screen:

1. Imaging speed increased 40-fold and is no longer the rate limiting factor.
2. We were able to **double** the total number of compounds screened.
3. Total number of personnel needed was reduced by half.

Citations

1. Bargmann, C.I. Neurobiology of *Caenorhabditis elegans.* Science. 1998 Dec 11;282(5396):2028-33.

2. Bargmann, C.I. Chemosensation in *C. elegans* (October 25, 2006), WormBook, ed. The C. elegans Research Community, WormBook, doi/10.1895/wormbook.1.123.1, http://www.wormbook.org.

Affiliations and Acknowledgements

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