# Capturing stress-induced changes to developing *C. elegans* neuron structure with light microscopy

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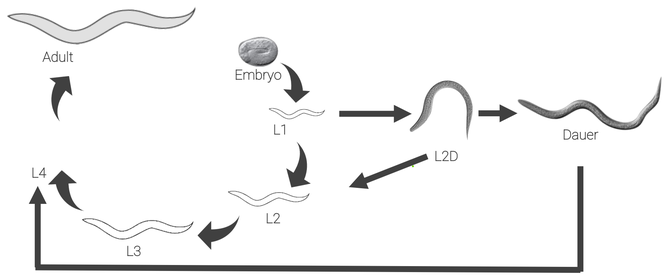
## Introduction

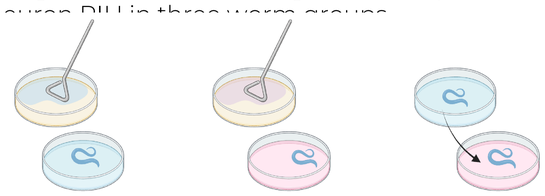
- The model organism *Caenorhabditis elegans* offers a useful lens for understanding the plasticity of life to environmental stressors




- In crowded conditions, developing *C. elegans* diverge into a hibernation-reminiscent larval stage called 'dauer' [1]
- Electron Microscopy (EM) has revealed significant changes to neuron morphology in dauers, perhaps underlying their altered behaviour
- The limited number of EM datasets available leave several important questions remain unanswered:
  I. The exact timeline by which neuron morphology changes arise during dauer development
  II. How stereotyped these changes are
  III. Whether the changes persist upon recovery

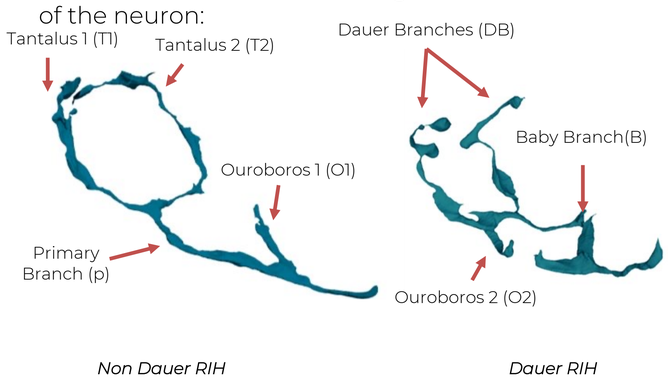
## Methodology

- Light microscopy (LM) is less time and resource-intensive than EM due to its coarser resolution [2]
- We gather LM data tracing the development of the neuron RIH in three worm groups



*Dauer Condition Media worms (DCM)* | *S-complete Media (SC) worms (WT)* | *Worms recovered from DCM to SC (Recovered)*

- Preliminary differences in branching between non-dauers and dauers found using EM reconstructions of the neuron:

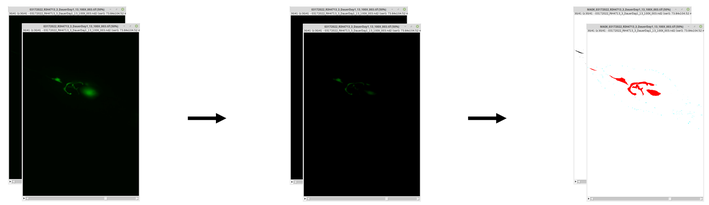



*Non Dauer RIH* | *Dauer RIH*

- I analyze the development of these characteristic 'dauer' features in order to answer the questions posed above

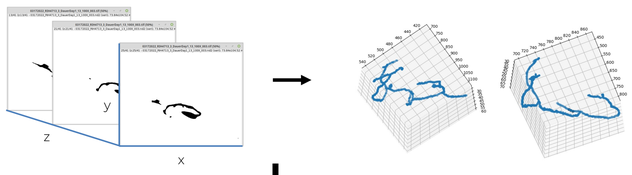
## Pipeline for LM image processing

Pre-processing with ImageJ

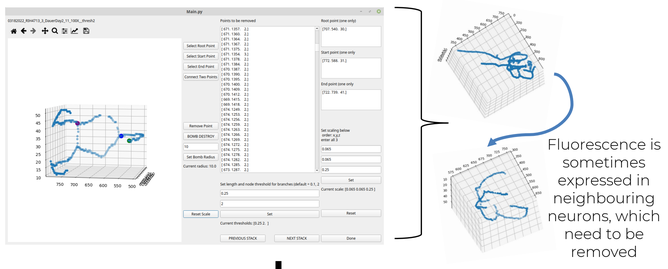


Original Image Stack → 3D Gaussian Blur + Background subtraction using sliding paraboloid → Thresholding and removal of small particles/noise

3D Skeletonization using Scikit-Image

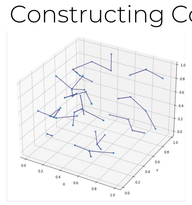



GUI 1: Cleaning and Primary-Branch Selection



Fluorescence is sometimes expressed in neighbouring neurons, which need to be removed

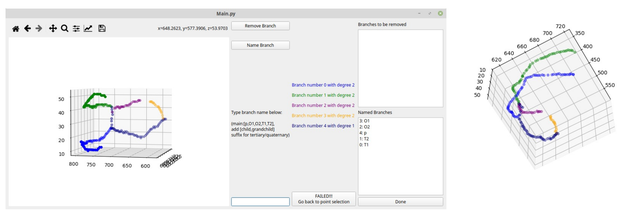
Constructing Connected Graph + Analyzing Branching



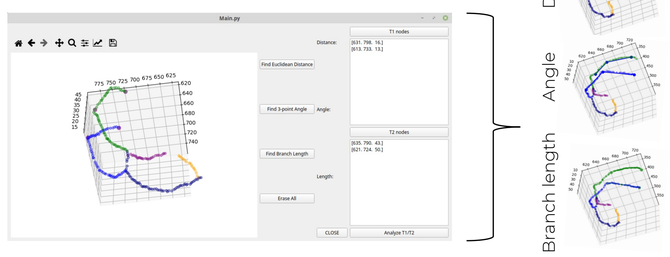
K-nearest neighbours algorithm [3] (k=3) is used to connect points into the neuron skeleton.

All branches found are characterized relative to the primary branch by features such as branch degree, length, and location

GUI 2: Branching Checkpoint and Naming

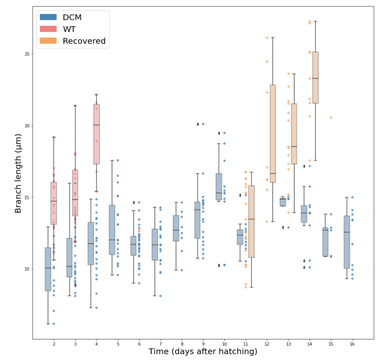


GUI 3: Semi-Automatic Branching Analysis



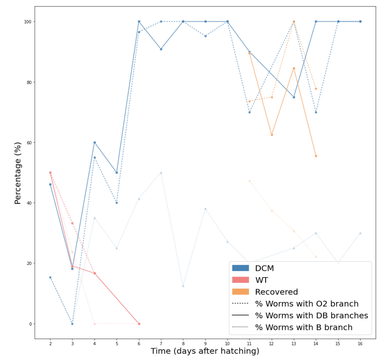

## Preliminary Results

Fig 1. Primary branch length of RIH neuron across development of stressed *C. elegans*



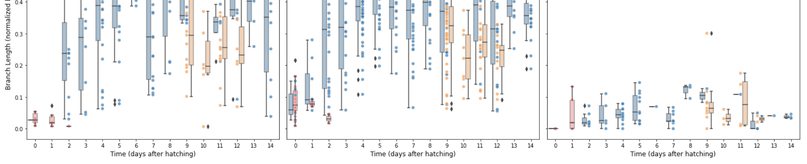

*C. elegans* grown in DCM have stunted growth compared to WT. DCM worms returned to WT conditions recover quickly.

Fig 2. Percentage of *C. elegans* with dauer features across development




O2/DB branches are indicative of dauer worms. A spike in development of dauer characteristics occurs ~day 4 after hatching.

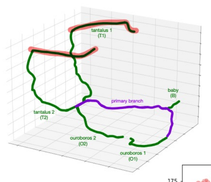
Fig 3. Length of characteristic dauer branches over time




Characteristic O2/DB branches grow longer as dauers develop. These branches persist in recovered worms but appear to recede slightly

### Categorizing "DB" branches

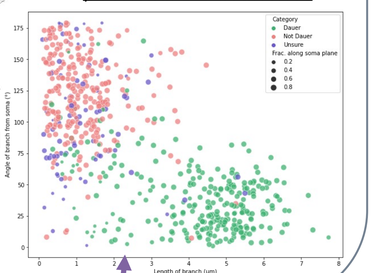
- The characteristic DB branches come off T1/T2 and appear to reach back towards the soma

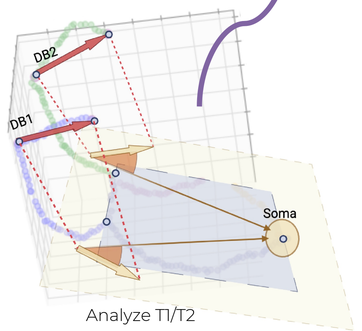



- In older dauer worms, the DB branches are unmistakable

Fig 3. Classifying potential DB branches

- In young worms, I use features to classify child branches of T1/T2 as being DB branches




Analyze T1/T2

Characterizes the projection of dauer branches onto the plane of the neuron soma

## Conclusion

- Utilizing my pipeline, I find that the onset of neuron morphology change occurs early in dauer development, around day 4 after hatching
- These precede other recognized whole-body dauer modifications [1]
- These morphological shifts are largely preserved across dauers
- While these changes recede over time in recovered worms, they remain observable.
- Broadly, future work using my pipeline might investigate the morphology of other neurons in various genetic mutants of *C. elegans*, ultimately connecting phenotypic manifestations to both environmental influence and molecular mechanisms.

## Acknowledgements

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## References

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[3] Cover, Thomas M.; Hart, Peter E. (1967). Nearest neighbor pattern classification. *IEEE Transactions on Information Theory. 13* (1): 21–27. doi: 10.1109/TIT.1967.1053964. S2CID 5246200.