Longitudinal profiling of mitochondrial membrane potential in C. elegans.

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Caenorhabditis elegans

Model for biological systems

- Extensive knowledge of genome
- One of simplest organisms similar to humans
- Ability to be mass produced
  - Produce 300 progeny per generation

Commonly used for aging research

- Short lifespan with distinct larval stages
- Important to measure a change in mitochondrial membrane potential ($\Delta\Psi_m$) with respect to age
  - Difficult to measure $\Delta\Psi_m$ in *C. elegans*

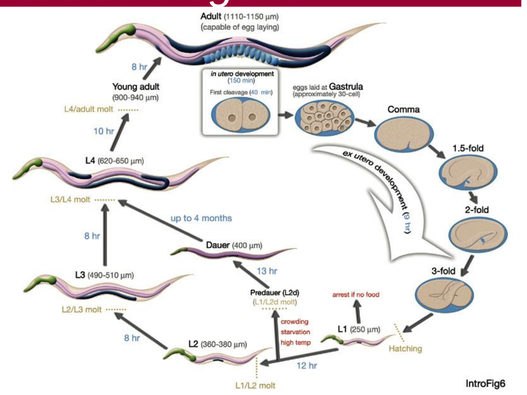


Figure 1. Life cycle of C. elegans, with four larval stages, time elapsed, and average length.[1]

Goal

Create a method to perform high-throughput quantitative longitudinal profiling of the mitochondrial membrane potential of *C. elegans* using a ratiometric fluorescent dye.

Method

Fluorescent Dyes

C. Elegans were treated with two kinds of fluorescent dyes

- JC9: ratiometric fluorescent dye that can emit both red and yellow fluorescence[2]
  - Monomer form emits yellow fluorescence: measure of dye uptake into cell
  - Aggregate formed by high membrane polarization emits red fluorescence
  - Ratio of red/yellow intensities gives relative $\Delta\Psi_m$
- mCherry: red fluorescent dye
  - Used to identify longitudinal positions of tissue regions with tissue-specific promoters
    - Pharynx-specific dye: ex-myo-2p::mCherry
    - Neuronal-specific dye: ex-rab-3p::mCherry
    - Intestinal-specific dye: ex-vha-6p::mCherry
- All C. elegans experiments performed by Dr. Joe Daniele in the Dillin Lab at UC-Berkley.

COPAS INTSTRUMENT

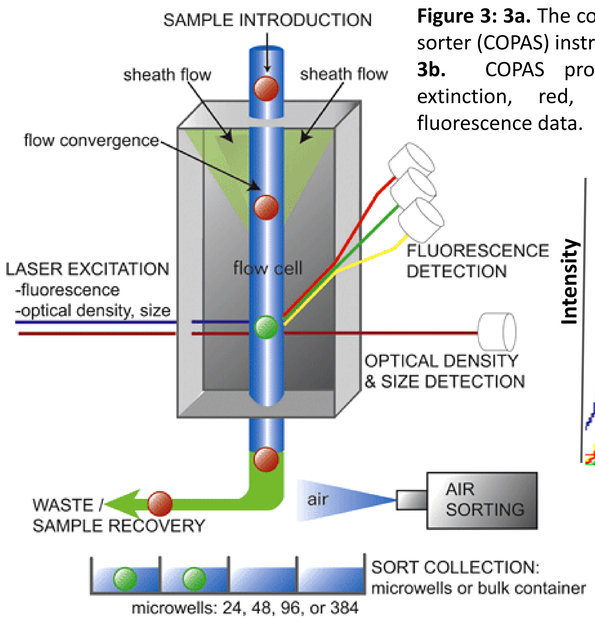
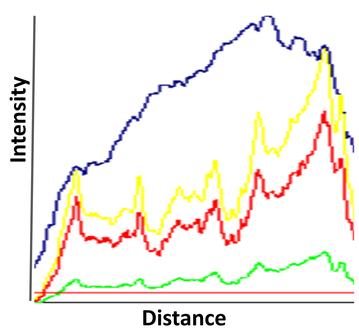
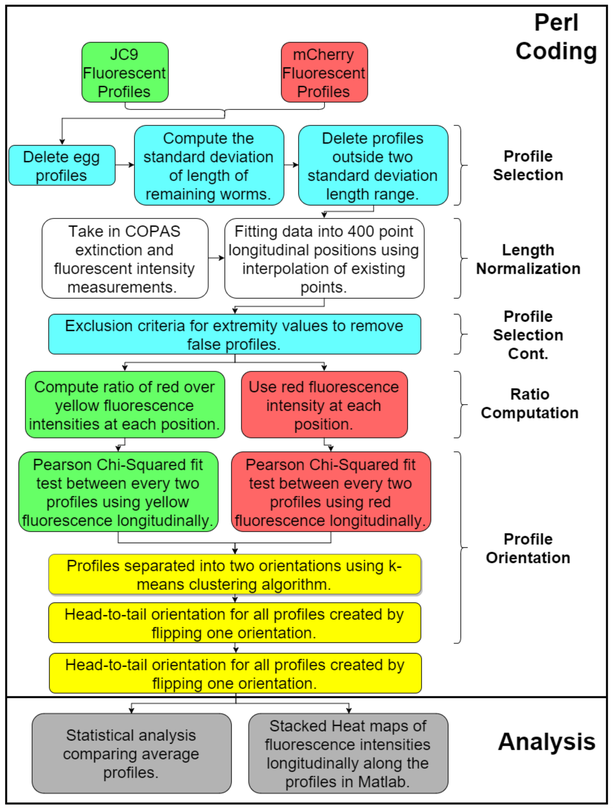


Figure 3: 3a. The complex object parametric analysis sorter (COPAS) instrument.[3]

3b. COPAS profile plot: Blue line indicates extinction, red, yellow, and green indicate fluorescence data.



Coding



Low JC-9 Yellow Fluorescence "Flatline" Profiles

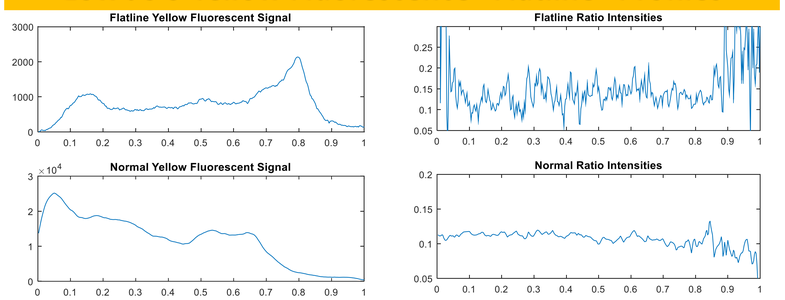


Figure 4: Length of Day 2 adult worms

- Low yellow fluorescence when there is little JC-9 uptake into the mitochondria
  - Causes any slight deviation in red fluorescence to cause major changes in calculated ratio of $\Delta\Psi_m$
- Up to 30% of worms in certain trials show this trend

Profile Orientation

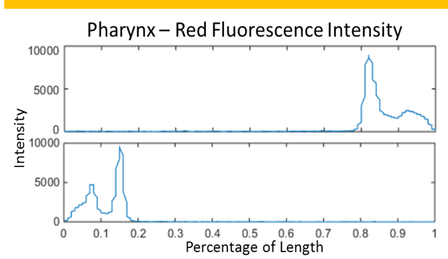


Figure 5

- Profile from COPAS ordered randomly
- Orientation system flips first profile
  - Chi-square value low when red intensity on opposite longitudinal positions

Results

TISSUE-SPECIFIC ORIENTATION VALIDATION

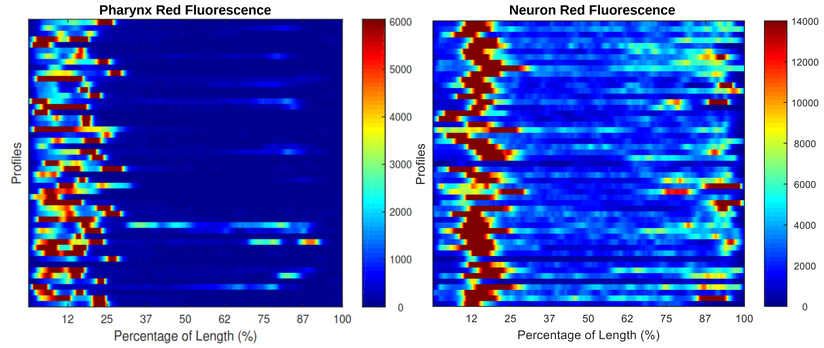


Figure 6. Heatmap of pharynx and neuron-specific profiles

- Used to find tissue regions and validate the orientation system works properly

Stacked/TISSUE-SPECIFIC ORIENTATION VALIDATION

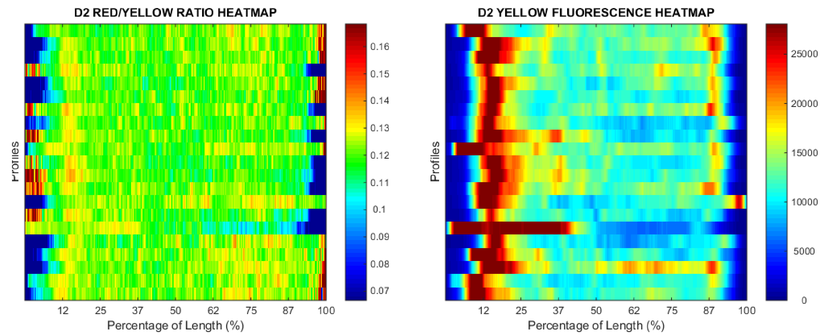


Figure 7. Heatmap of day 2 adult *C. elegans* red/yellow ratio and the yellow fluorescence intensity used for orientation

- Program has the ability to process hundreds of profiles
  - First 20 profiles displayed above
- Orientation system works effectively: high intensity yellow fluorescence in the front of all worms.
- Identify heightened ratio values near the pharynx location and between the 60-80% percentage of length.

Conclusions

- Program eliminates erroneous profiles, standardizes profile length, computes relative mitochondrial membrane potential, reorients profiles to face head-to-tail for all profiles, and prints heatmaps.
- Application:
  - Model *C. elegans* as standard profiles along the length of the profiles.
  - Analyze the effects of ageing on specific tissue regions
  - Statistical comparison of average profiles from different trials to find differences
- Future Work: Finish paper on this method for publication and start work on organelle isolation in C. elegans.

References

1. "INTRODUCTION TO C. Elegans ANATOMY." *Worm Atlas*. N.p., 2006. Web. 14 Apr. 2015.
2. Wolken, Gregory G., and Edgar Augusto Arriaga. "Simultaneous Measurement of Individual Mitochondrial Membrane Potential and Electrophoretic Mobility by Capillary Electrophoresis." *Analytical Chemistry* (2014) *86* (9), 4217-4226.
3. "COPAS." *Gene Company:*. N.p., 21 May 2008. Web. 19 Apr. 2014.