

A computational pipeline to analyze high-throughput imaging of nematode embryogenesis

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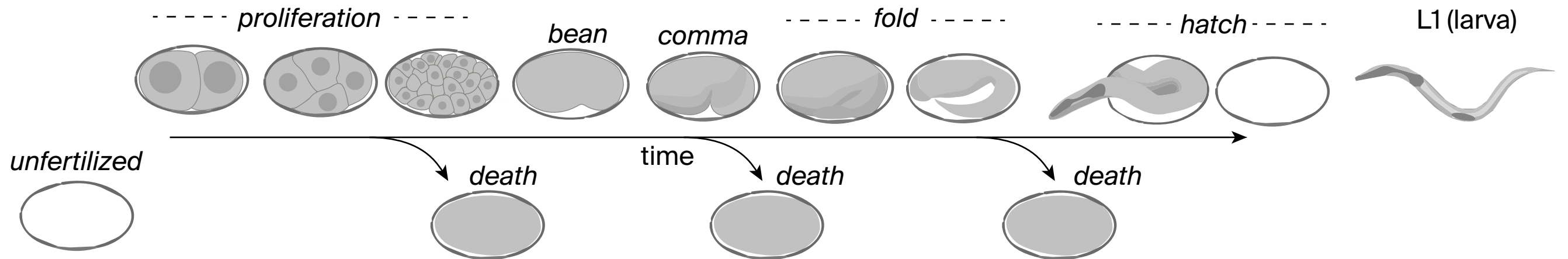


Background

Unlocking high-throughput developmental biology through deep learning

We describe here the generation of a trained classifier that can be used to identify nematode embryo stages from microscopy time-course data sets. We hope this tool will be useful to biologists using *C. elegans* or other free-living nematode species to interrogate embryonic development, reproductive success, or tracking developmental outcomes following perturbations.

This is the initial version of this tool, which will allow the user to go from imaging nematode embryos to classifying developmental stages and quantifying the frequency of successful versus unsuccessful developmental outcomes. We are interested in expanding the functionality of this resource in future versions. **We welcome your input and would be excited to incorporate user feedback to improve the functionality of our classifier.**



During embryogenesis, multicellular organisms pass through discrete developmental stages, including fertilization, cleavage, morphogenesis, and organogenesis, ultimately hatching into their environment. Animal development is characterized by sets of shared and species-specific features. For example, following fertilization, most animal embryos undergo a series of rapid cell divisions. At some point during this cleavage period, cells undergo a suite of morphogenetic changes as embryo patterning results in tissue-layer organization through the process of gastrulation. While embryos from many different organisms may share similar-looking cleavage stages, within specific lineages there are often unique morphologies characteristic of distinct taxonomic groups – animal embryos that look similar at cleavage stages might look very different during gastrulation. These species-specific differences only compound as development continues. **Thus, there is a need for automated tools to classify key embryonic stages to unlock high-throughput approaches to developmental biology.**

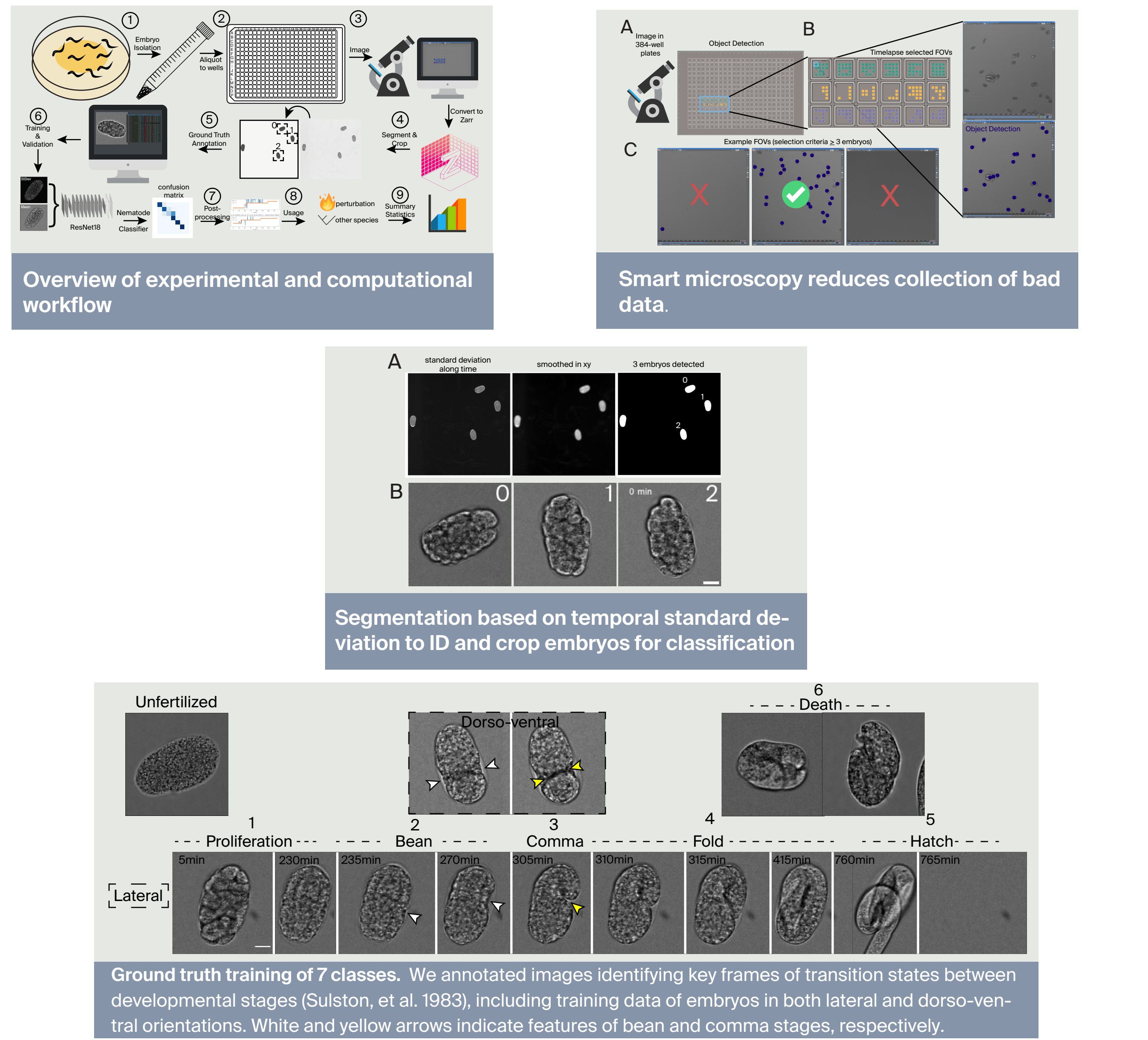
Here, we selected nematode embryogenesis for our first effort at building a classifier using deep learning methods based on label-free imaging. Free-living nematodes, including the well studied *Caenorhabditis elegans*, undergo rapid development passing through **proliferation**, **bean**, **comma**, **fold** stages until they **hatch** about ~14 hours after fertilization, making them useful for this effort.

Key questions:

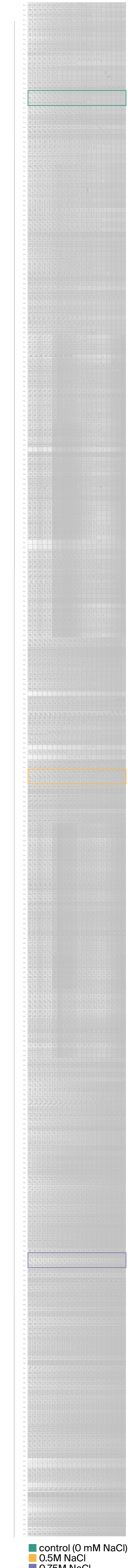
- 1. Could we train a robust classifier to recognize developmental stages of nematode embryos irrespective of embryo orientation, and with varying brightfield input data?
- 2. Could we teach our classifier to identify embryonic lethality or **death** during development?
- 3. Do we need to train new models to accurately classify other nematode species beyond *C. elegans*?

Approach

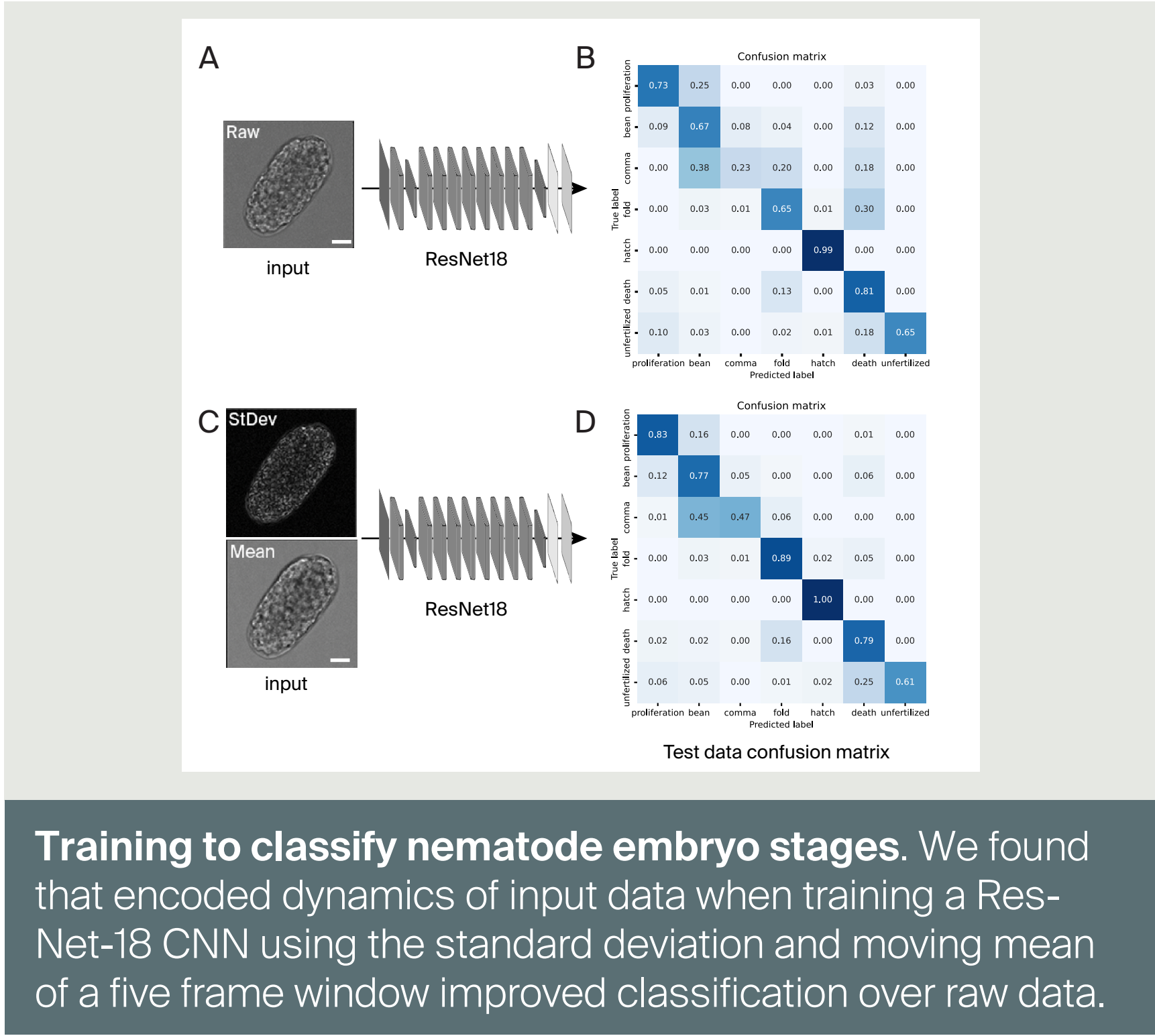
Our workflow combines smart microscopy with high-throughput imaging, segmentation, cropping and key frame annotation to train a classifier



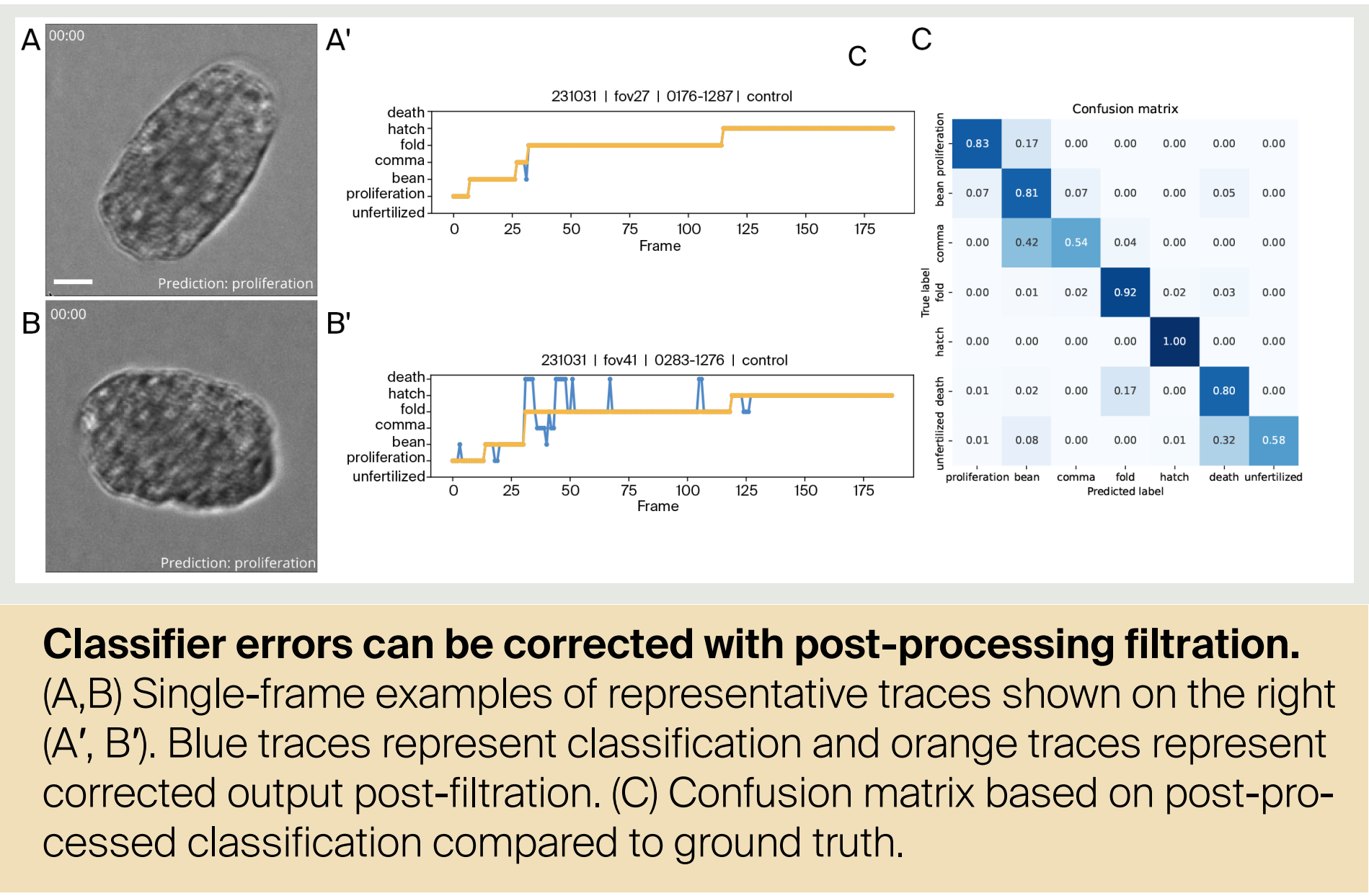
The osmotic shock experiment



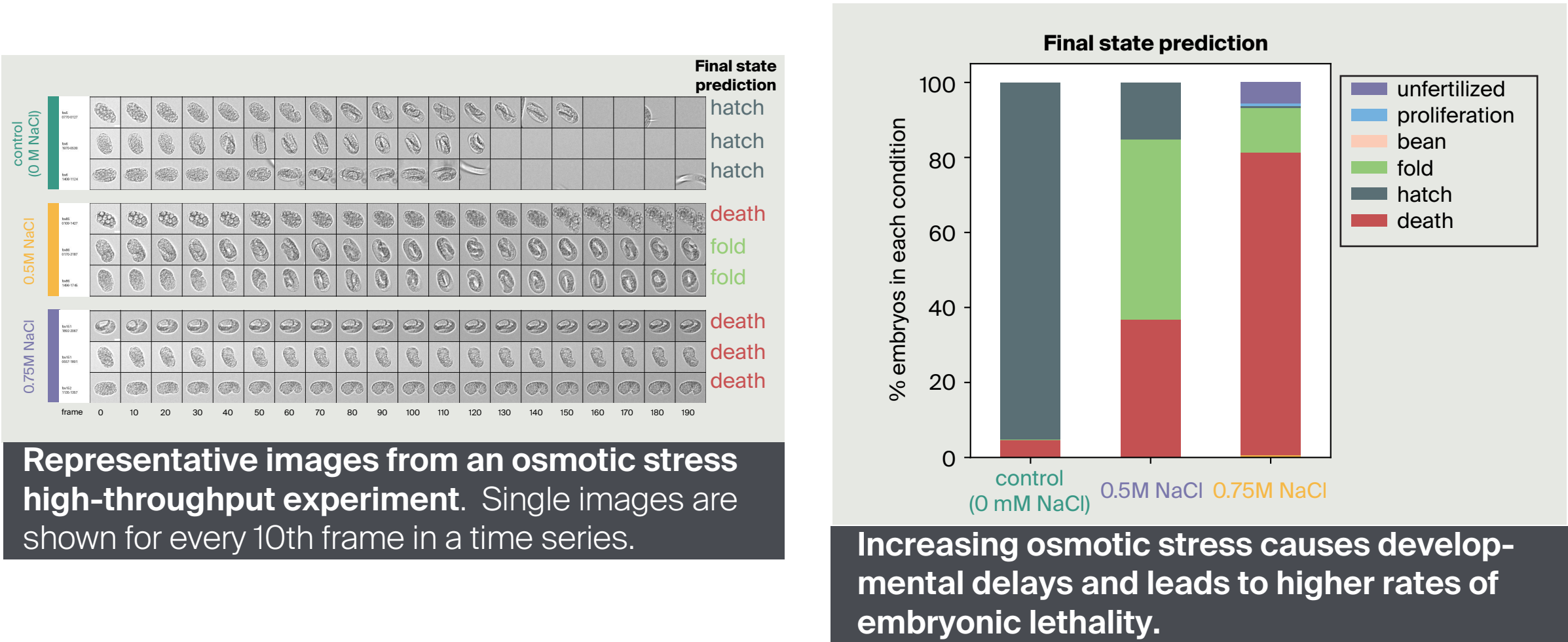
We leverage dynamic information to train a neural network to classify developmental stages



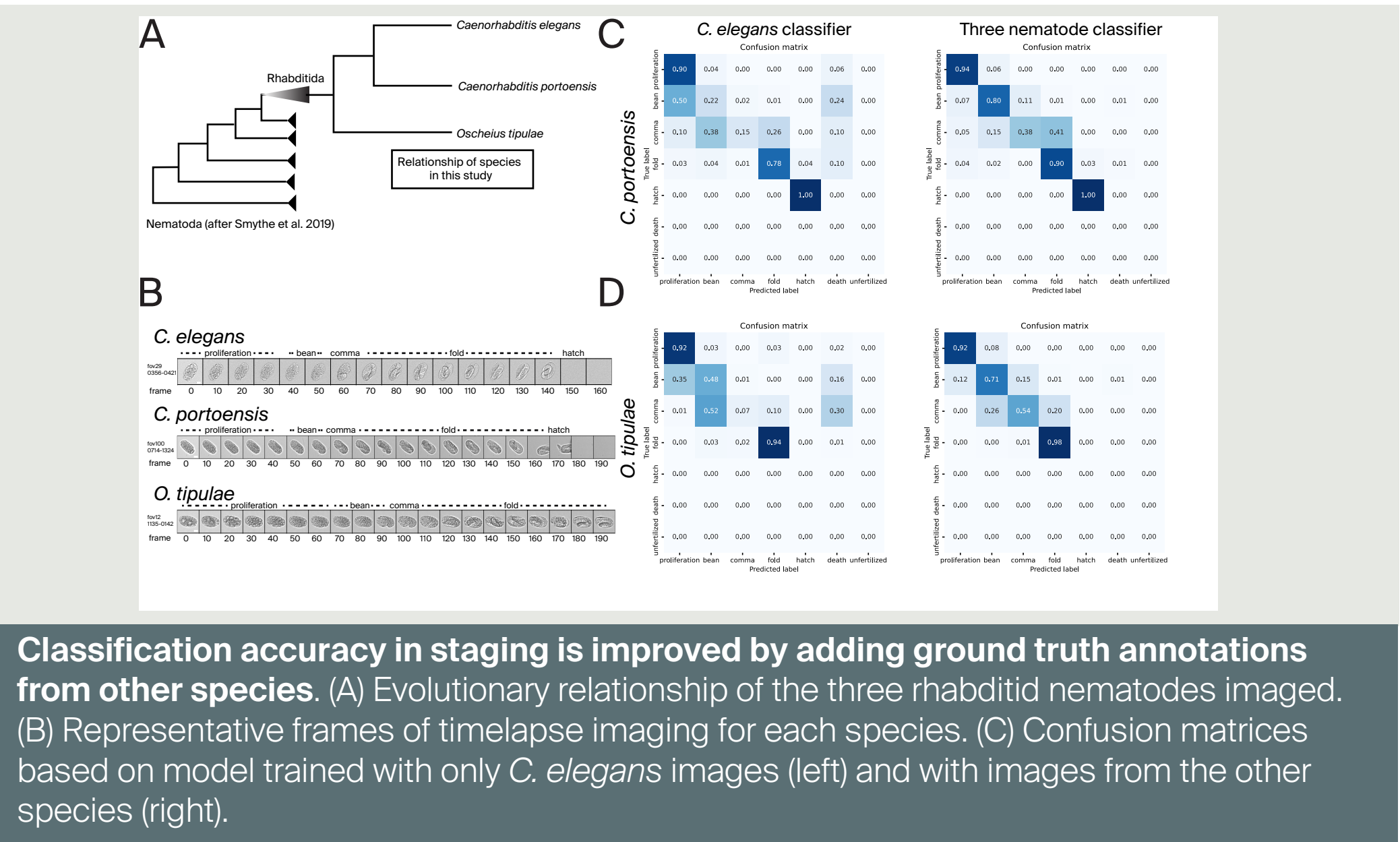
Post-processing corrects some classifier errors



Classifying high-throughput experimental data



We had to re-train models to make them more generalizable to other nematode embryos



Re-visiting our original ?s

- 1. Could we train a robust classifier to recognize developmental stages of nematode embryos irrespective of embryo orientation, and with varying brightfield input data?
Mostly YES! We needed a post-processing filtration step to increase accuracy. Morphogenesis stages are HARD (especially comma), but if we bin bean and comma together, we get robust results.
- 2. Could we teach our classifier to identify embryonic lethality or **death** during development?
This worked at about ~80% accuracy, but might be improved by incorporating images of embryonic lethality from other perturbations beyond osmotic stress (e.g., mutant alleles, RNAi, etc).
- 3. Do we need to train new models to accurately classify other nematode species beyond *C. elegans*?
Yes! Our classifier was pretty good at predicting early and late developmental events without re-training (e.g., proliferation and fold and hatch, but performance was enhanced by adding in images from the other species.

Next Steps

We built a classifier using a **supervised** learning strategy, with ground truth annotations on a subset of image data. Moving forward, we are interested in exploring other learning strategies, including **self-supervised** learning, to eliminate the need for labor-intensive manual annotation and to facilitate the extension of this work to other imaging modalities (e.g., phase contrast, DIC, fluorescence) or other species.

- I'd appreciate feedback on any of this work, but I'm especially curious about the following:
- What would make this tool more useful for you to use in your own work?
- What would be the (computational? user interface?) barrier for you to use this tool?

Leave Feedback!

Comment on the pub:

Automated classification of time-course imaging data applied to nematode embryogenesis



bit.ly/nematode-classifier

#WormClassifier

All other published work: research.arcadiascience.com

A NOTE ON SHARING WITH US!

Part of our mission is to share as much useful research as we can.

If you choose to share a protocol or other useful information with us after viewing this poster, please understand that we may act upon this knowledge and share it when we publish our work. We publish quickly on an independent platform, so this may happen soon after you share, and we cannot wait for you to publish elsewhere.

If you decide to share anyway, yay! That's what science is all about. If your input is useful, we will include you as a contributor to the publication and explain that your role was in providing "Critical Feedback," likely with an additional description of what you shared.

tl;dr – If you're not ready for everyone to know about something, please refrain from sharing it with us.

Contributors (A–Z)

- Prachee Avasthi • Supervision
- Ferdinand Mert Celebi • Critical Feedback, Supervision
- Keith Cheveralls • Data Curation, Software, Formal Analysis, Investigation, Editing, Visualization
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- David Mets • Critical Feedback, Editing
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- Harper Wood • Investigation

References

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- Smythe et al. 2019 doi.org/10.1186/s12862-019-1444-x