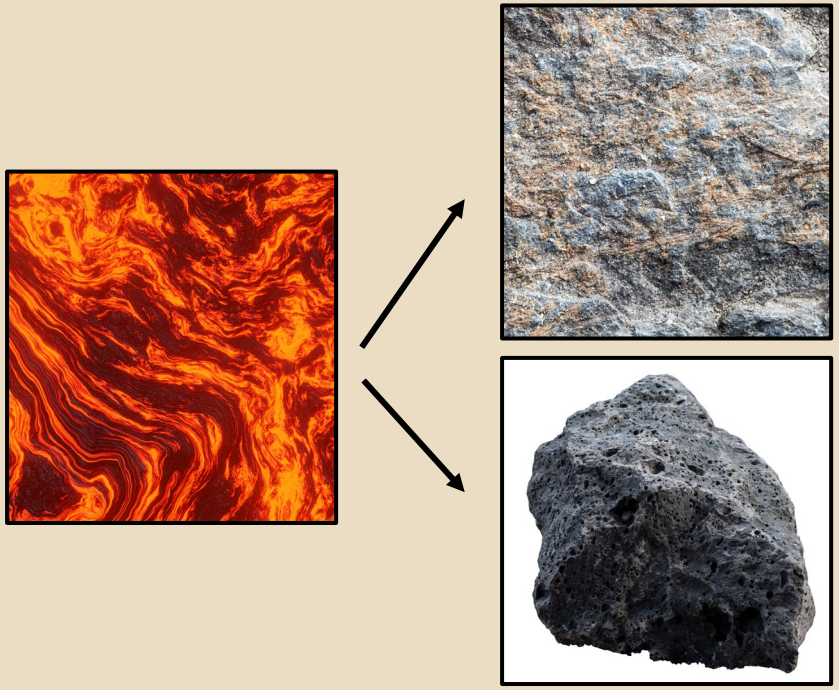
The Structure and Dynamics of Mitotic Waves forming the *Drosophila* Blastoderm

Harrison Oatman

Many natural phenomena feature changes in density



Increasing the density of a system is simple

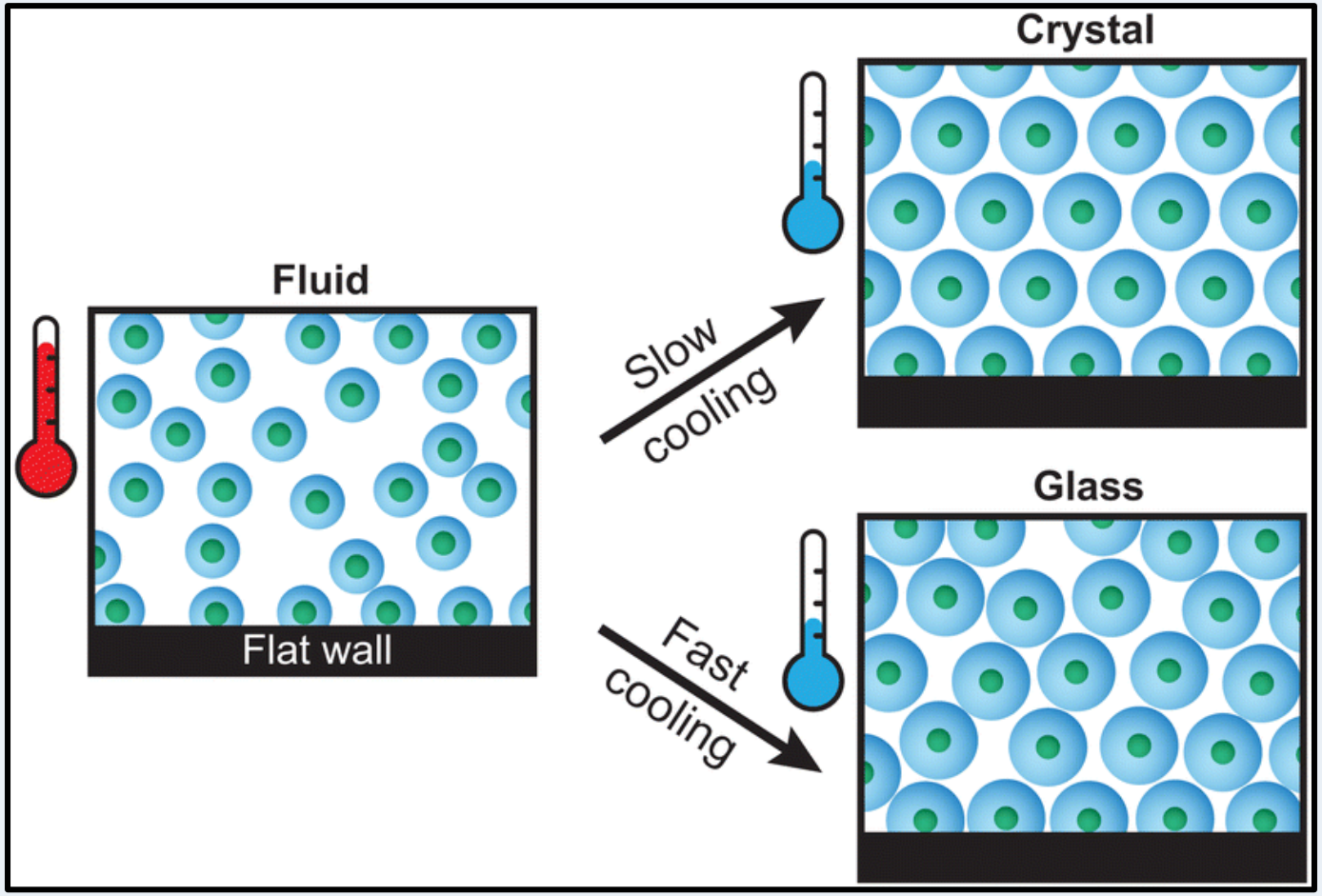
$$\rho = \frac{N}{V}$$

Decrease V

Increase N

...but the approach matters!

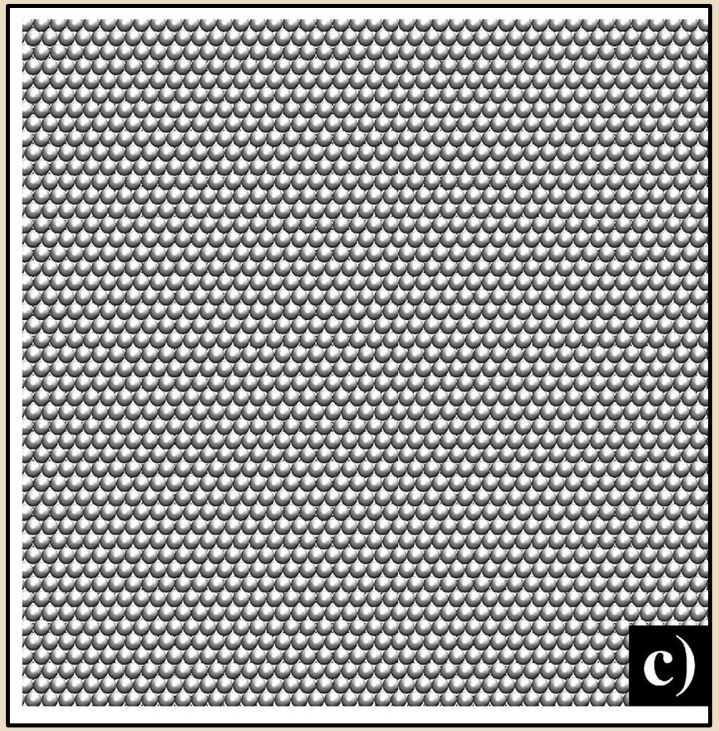
Decreasing V: rate of cooling affects the resulting structure



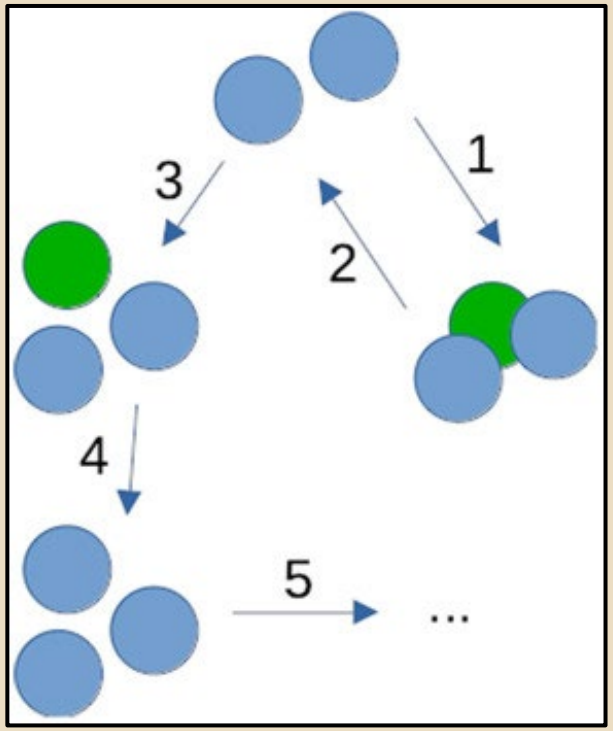
Schelling et al. 2024

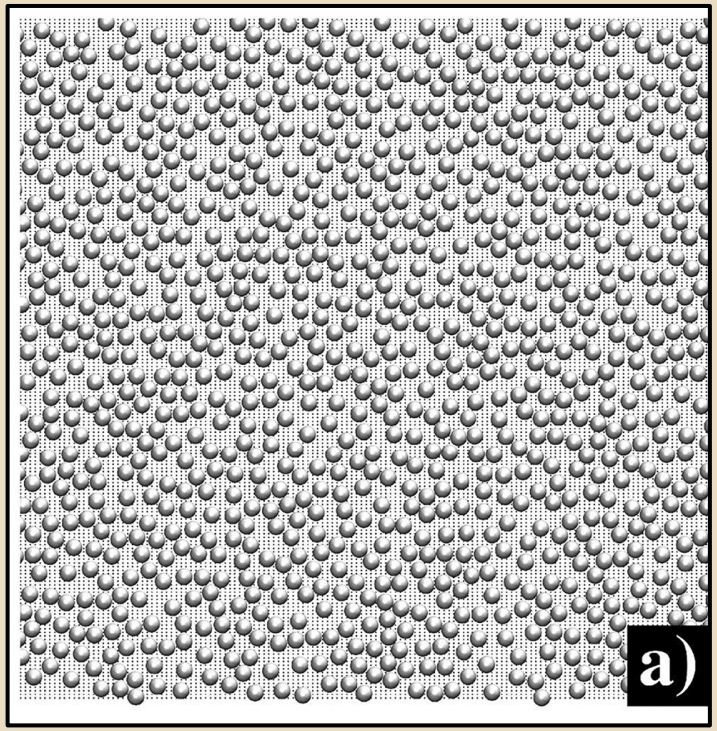
Increasing N: random sequential adsorption produces a less than maximal coverage

coverage ≈ 0.909



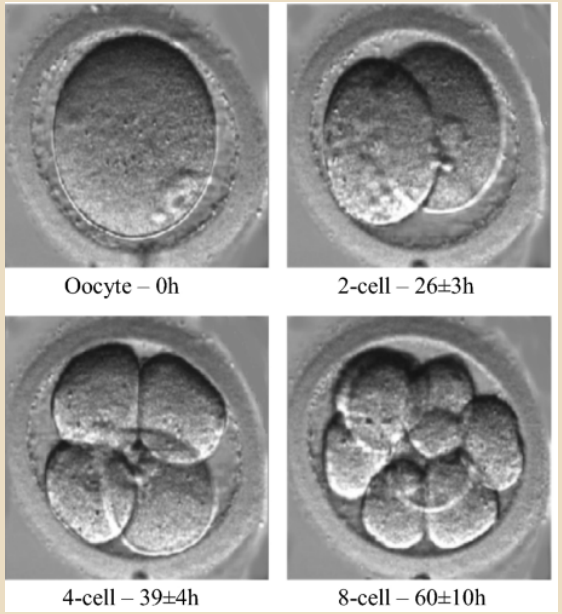
coverage ≈ 0.547



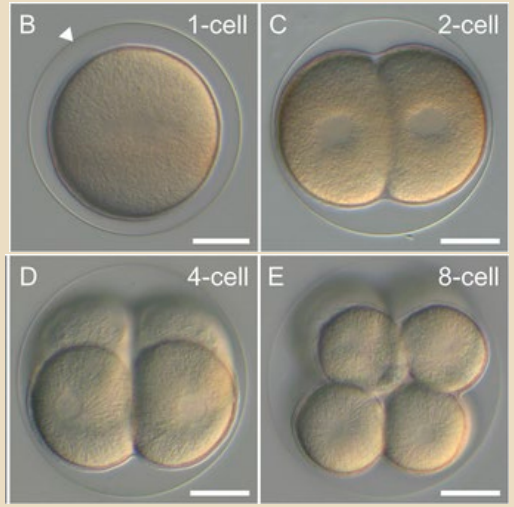


Kubala et al. 2022

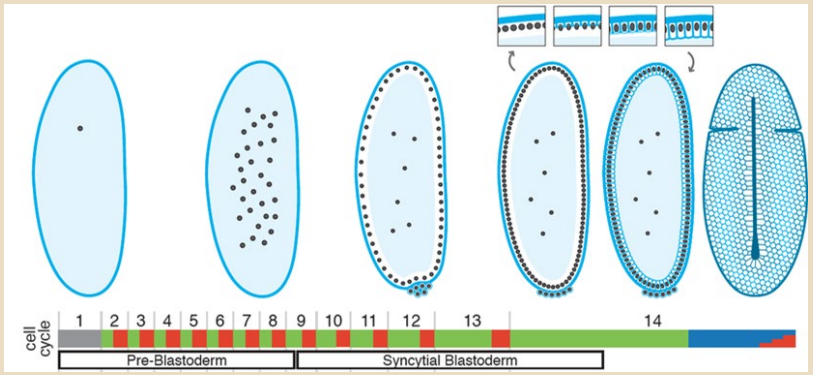
Densification of nuclei is a universal task of multicellular embryogenesis



Malmsten et al. (2020)



Formery et al. (2022)

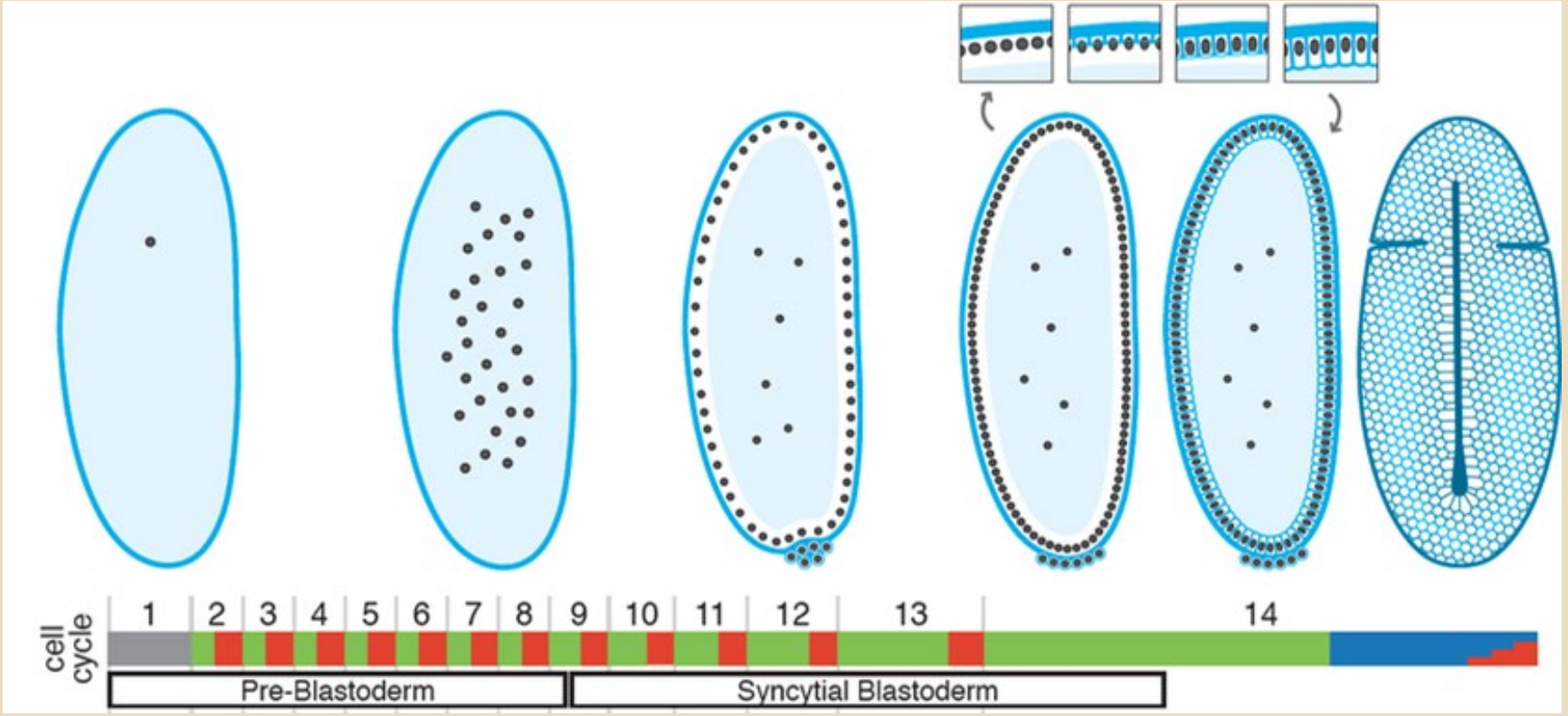


Farrell and O'Farrell (2014)

How does the densification approach affect later embryonic structure?

Drosophila development begins with 13 nuclear divisions

- 9 divisions take place in the yolk
- Nuclei migrate to the surface of the egg, and undergo 4 more divisions
- These divisions form a blastoderm containing ~6000 nuclei



Farrell and O'Farrell (2014)

1941

STUDIES ON THE CYTOLOGY AND EARLY EMBRYOLOGY OF THE EGG OF DROSOPHILA MELANOGASTER [1]

MORRIS RABINOWITZ

Washington Square College, New York University

TABLE 2

Temperature: 24°C. Total number of eggs: 354

STAGE OF DEVELOPMENT	NUMBER OF EGGS	MEAN AGE (IN MINUTES)
Telophase of 2nd maturation division to conjugation of pronuclei	13	15 ± 1.21
1st division of cleavage nuclei	10	23 ± 1.72
2nd division of cleavage nuclei	14	34 ± 1.72
3rd division of cleavage nuclei	17	47 ± 1.47
4th division of cleavage nuclei	10	53 ± 1.05
5th division of cleavage nuclei	18	60 ± 0.57
6th division of cleavage nuclei	22	70 ± 0.58
7th division of cleavage nuclei	14	78 ± 1.28
8th division of cleavage nuclei	31	93 ± 1.13
1st division of blasteme nuclei	82	99 ± 0.60
2nd division of blasteme nuclei	97	109 ± 0.51
3rd division of blasteme nuclei	24	120 ± 0.90

1983

STUDIES OF NUCLEAR AND CYTOPLASMIC BEHAVIOUR DURING THE FIVE MITOTIC CYCLES THAT PRECEDE GASTRULATION IN *DROSOPHILA* EMBRYOGENESIS

VICTORIA E. FOE AND BRUCE M. ALBERTS

Department of Biochemistry and Biophysics, University of California at San Francisco, San Francisco, California 94143, U.S.A.

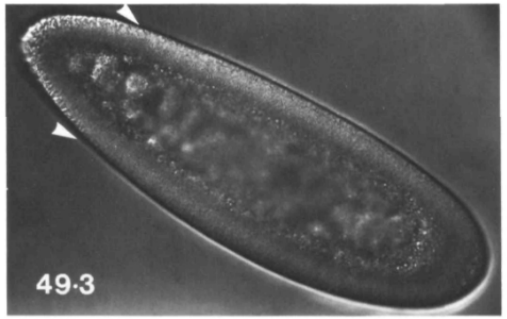


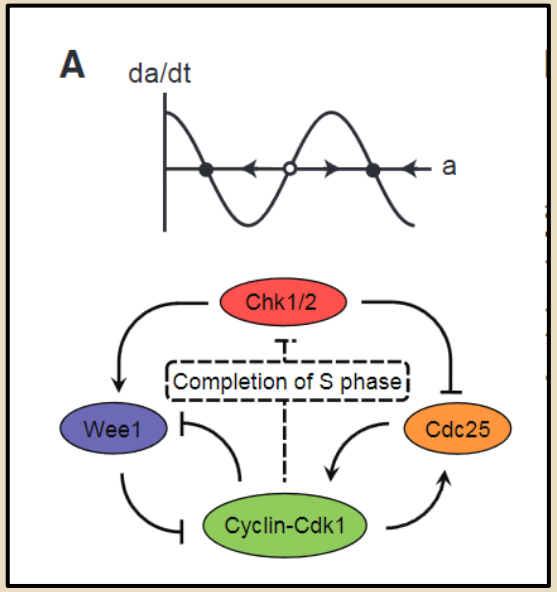
Fig. 7

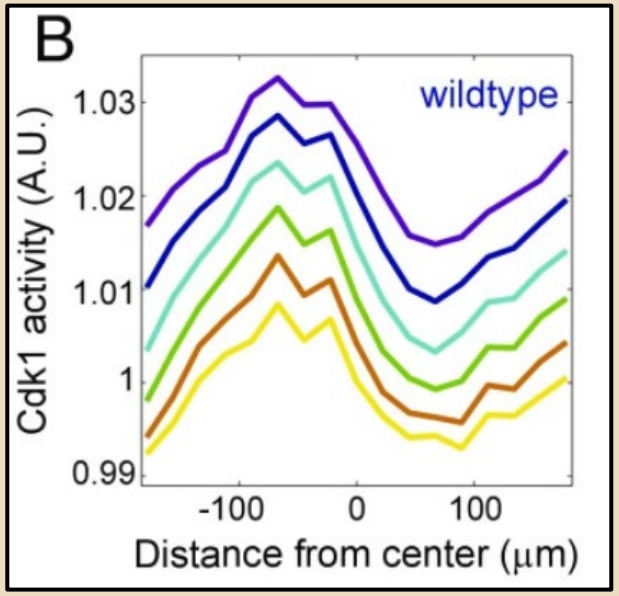
Previous works have investigated the chemical interactions underlying the mitotic waves

Proc Natl Acad Sci U S A. 2018 Feb 15;115(10):E2165–E2174. doi: 10.1073/pnas.1714873115

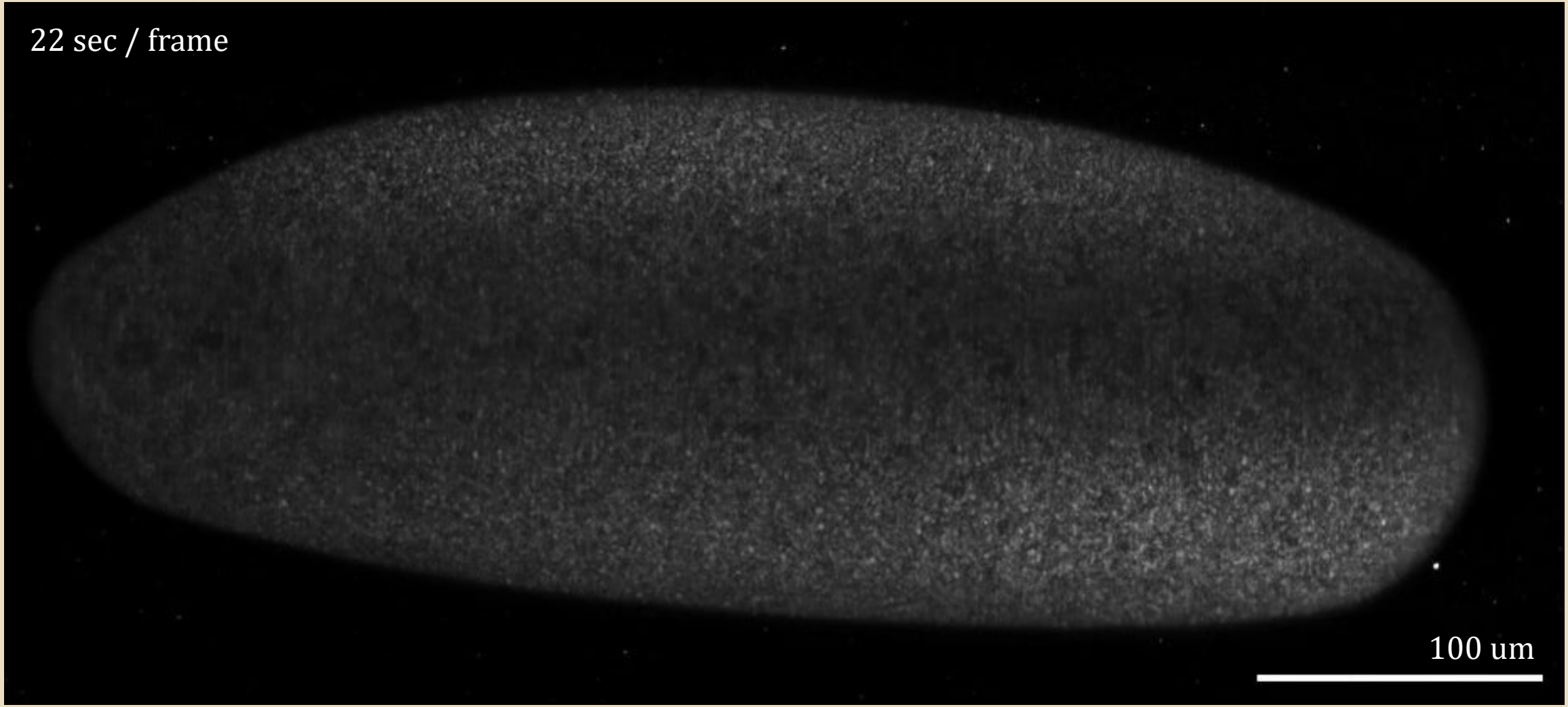
Mitotic waves in the early embryogenesis of *Drosophila*: Bistability traded for speed

Massimo Vergassola [a,1], Victoria E Deneke [b], Stefano Di Talia [b,1]





Mitotic waves are an embryonic-scale phenomenon that takes place at the per-nucleus level

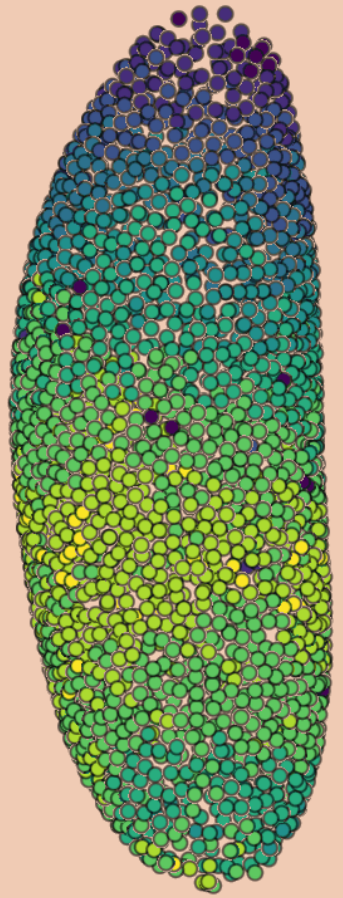


How do mitotic waves direct *Drosophila* blastoderm formation?

I. What is the trajectory by which mitotic waves traverse the *Drosophila* embryo?

II. How do mitotic waves affect the final packing of nuclei?

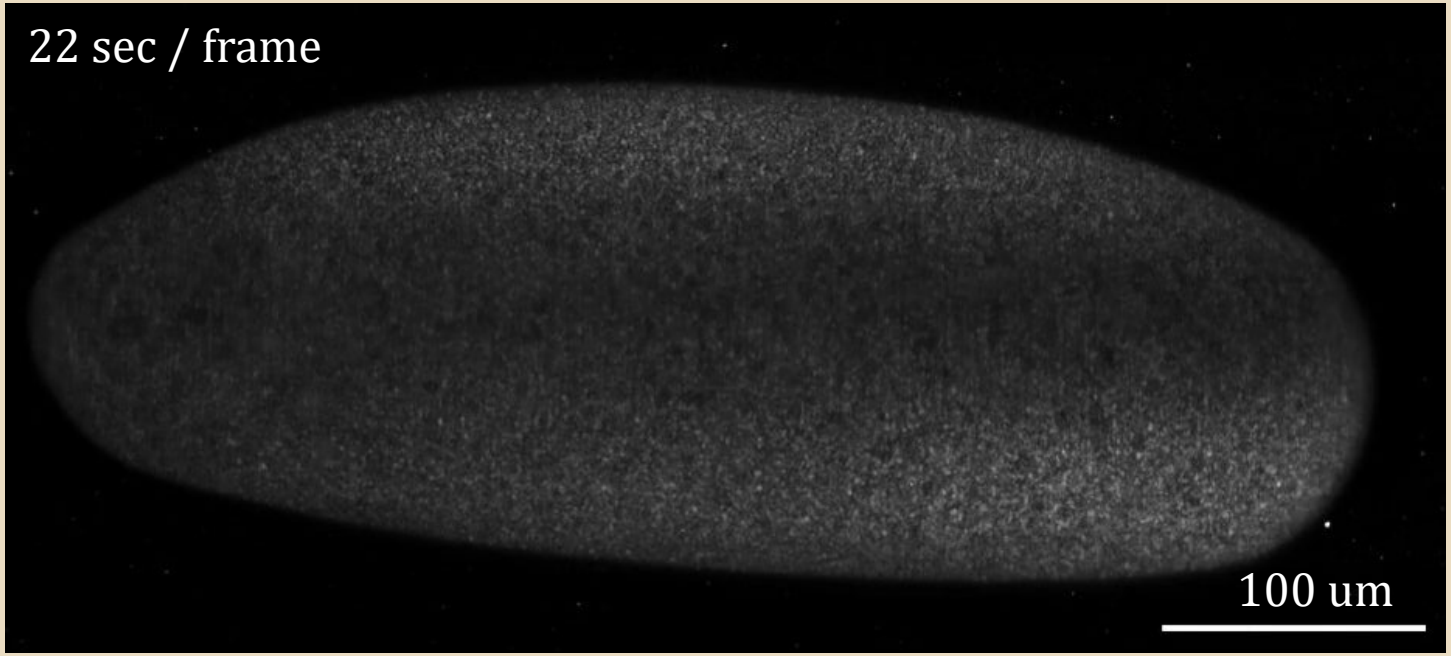
III. How do the waves respond to genetic perturbation?



Division times (darker = earlier)

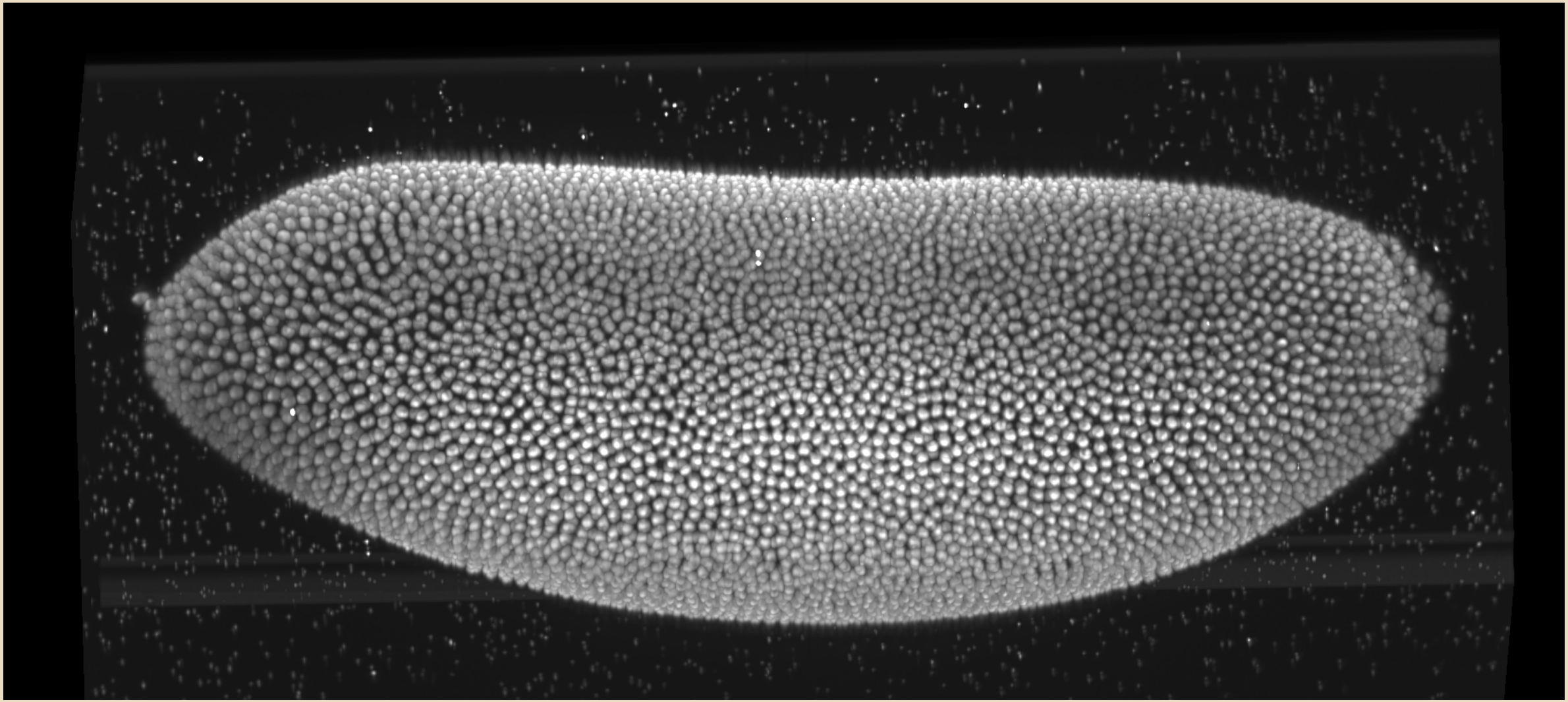
Light sheet imaging produces highly resolved, isotropic, *in toto* data

Liu Yang

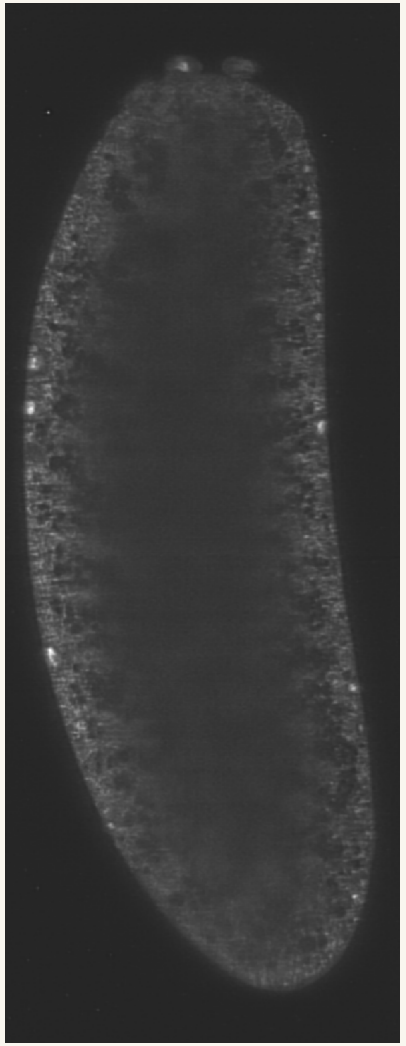


- Light sheet microscopy
  - Two cameras x two angles per time point = 4 views
  - 20-22 seconds / time point (about as fast as possible)
- Embryos expressing h2a-GFP
  - Start when nuclei first visible (NC8) through NC14
  - Cooled to 18 C to slow down development

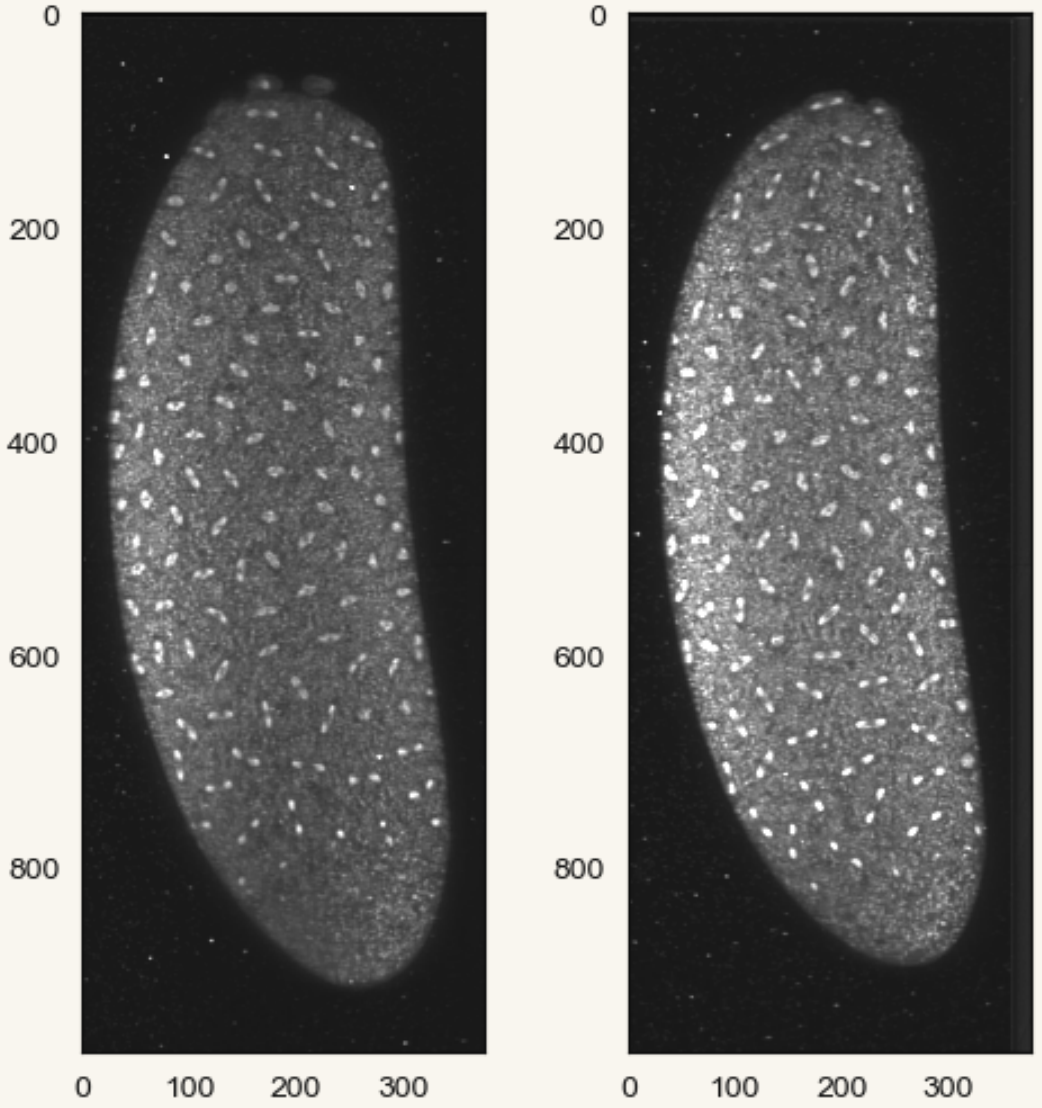
Raw data (final frame)



Single slice

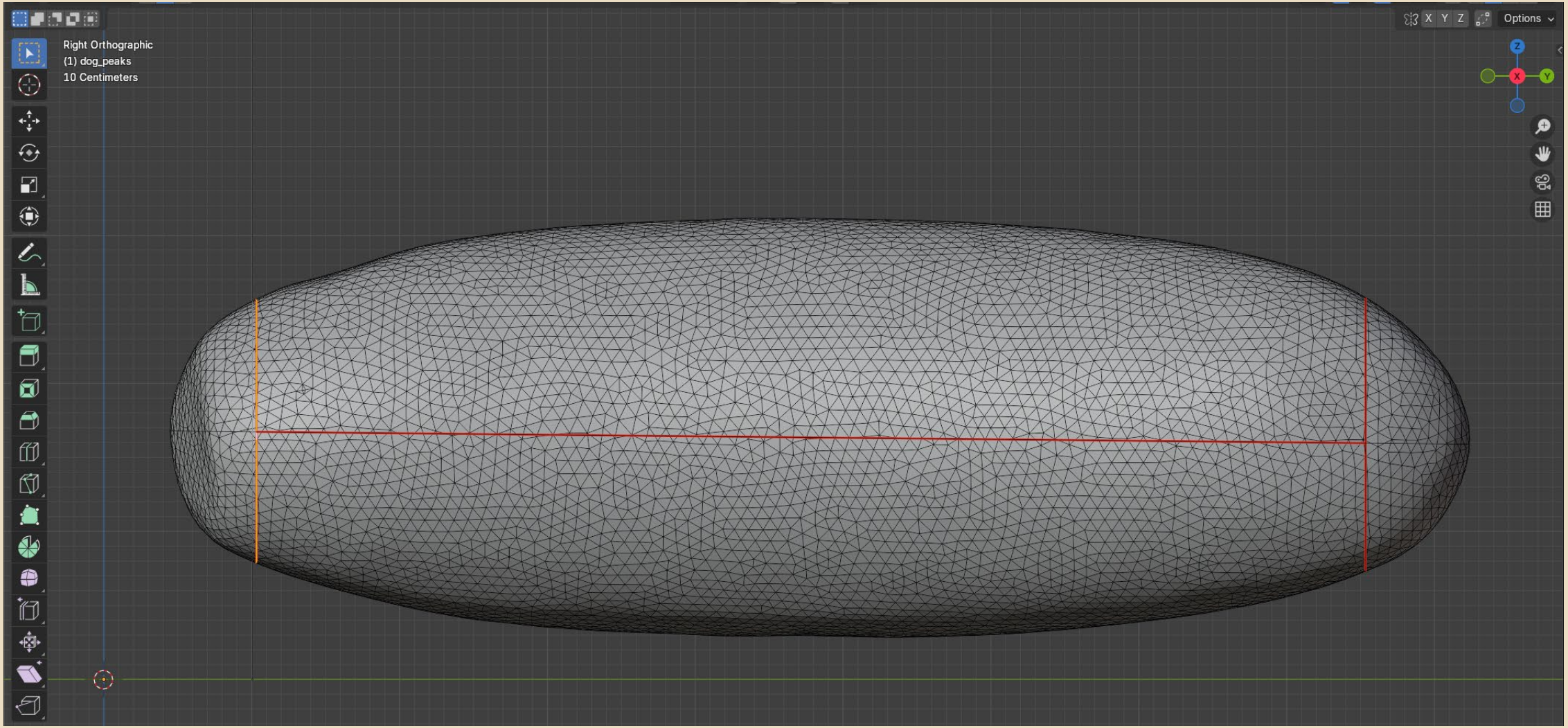


Max projected



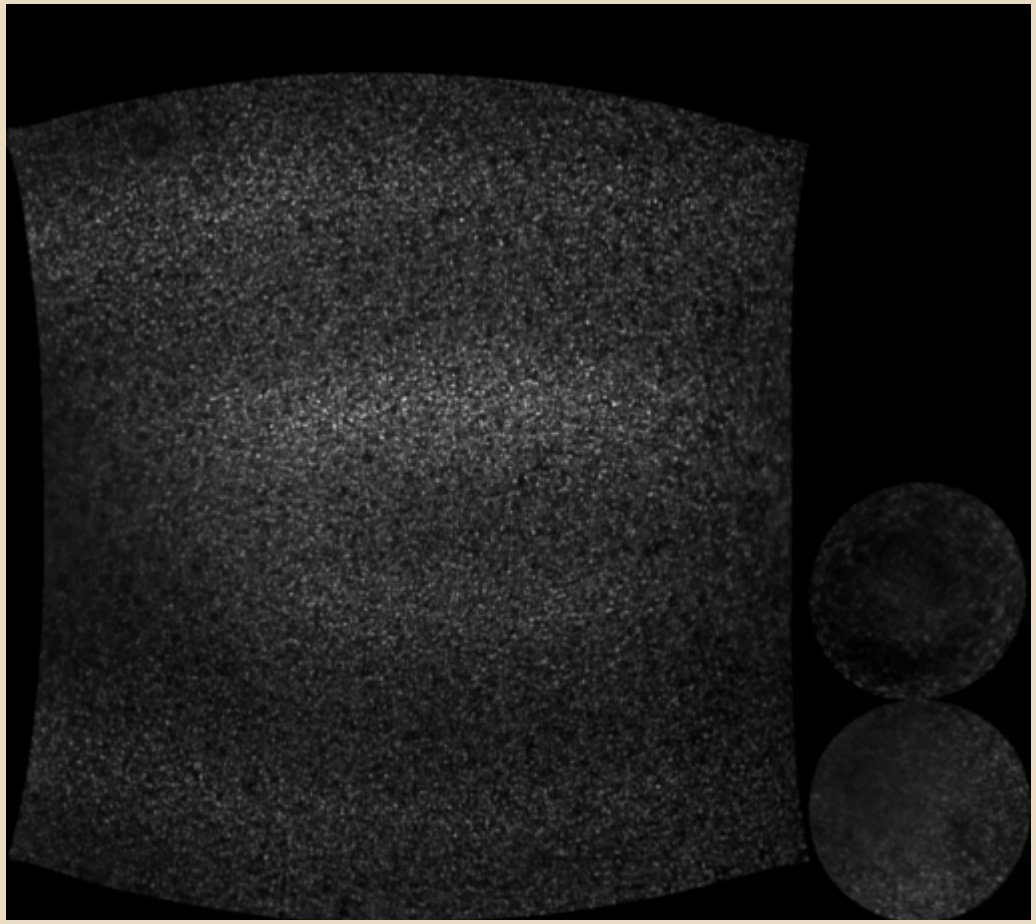
Choosing the best data transformation

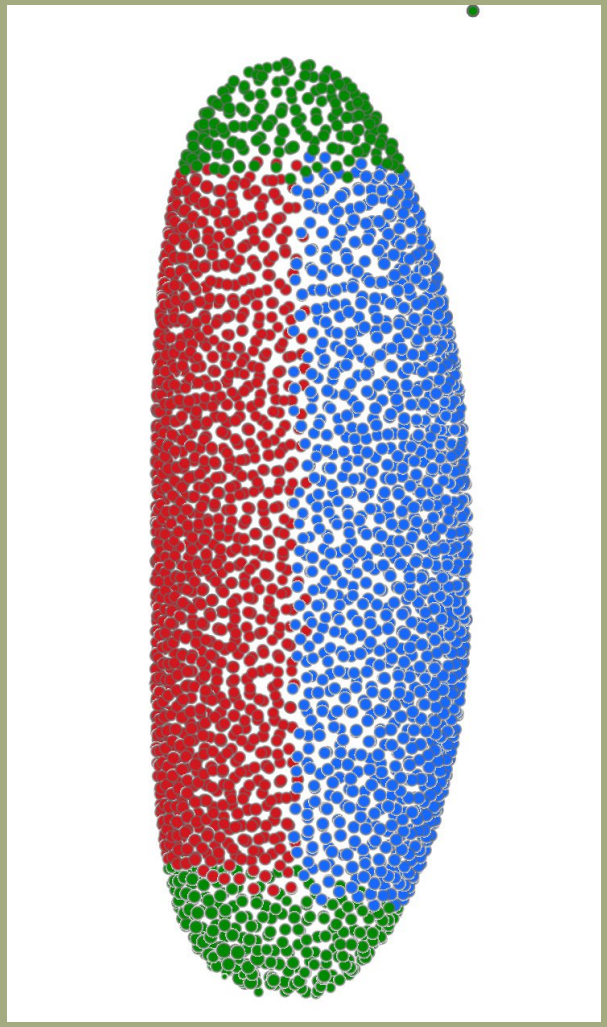
Embryos are converted to meshes (blender)



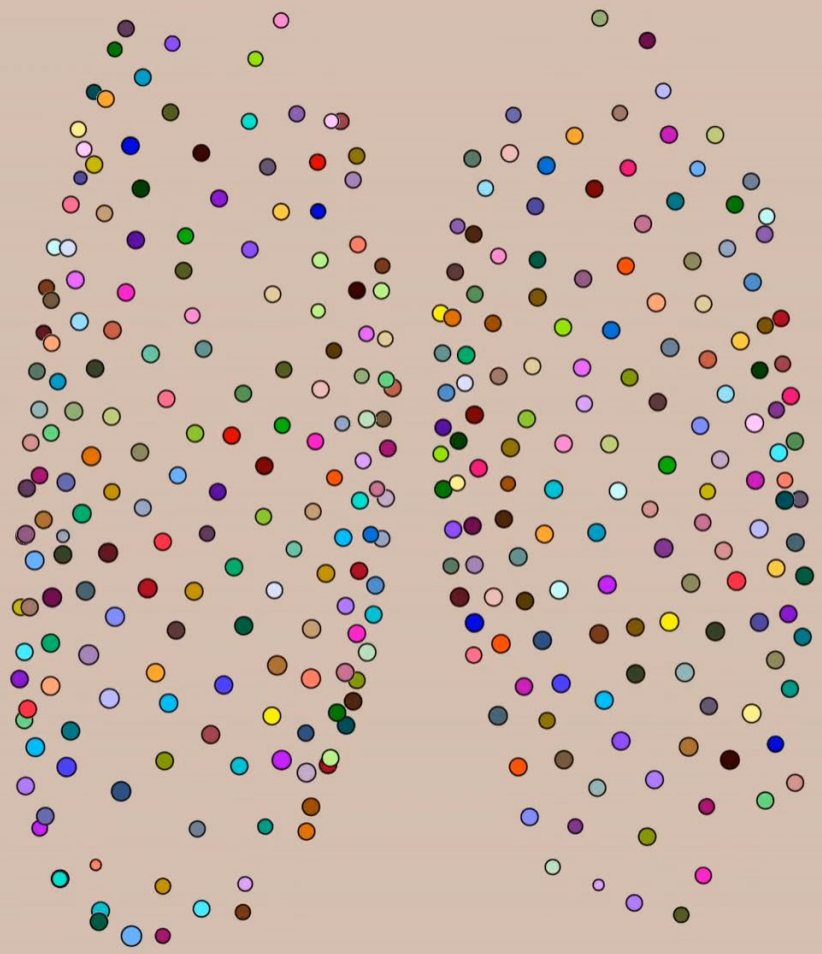
Klaussen et al. 2025 (preprint)

Segmentations are carried out in 2d using Cellpose-SAM





Nuclei are tracked via a two-step algorithm



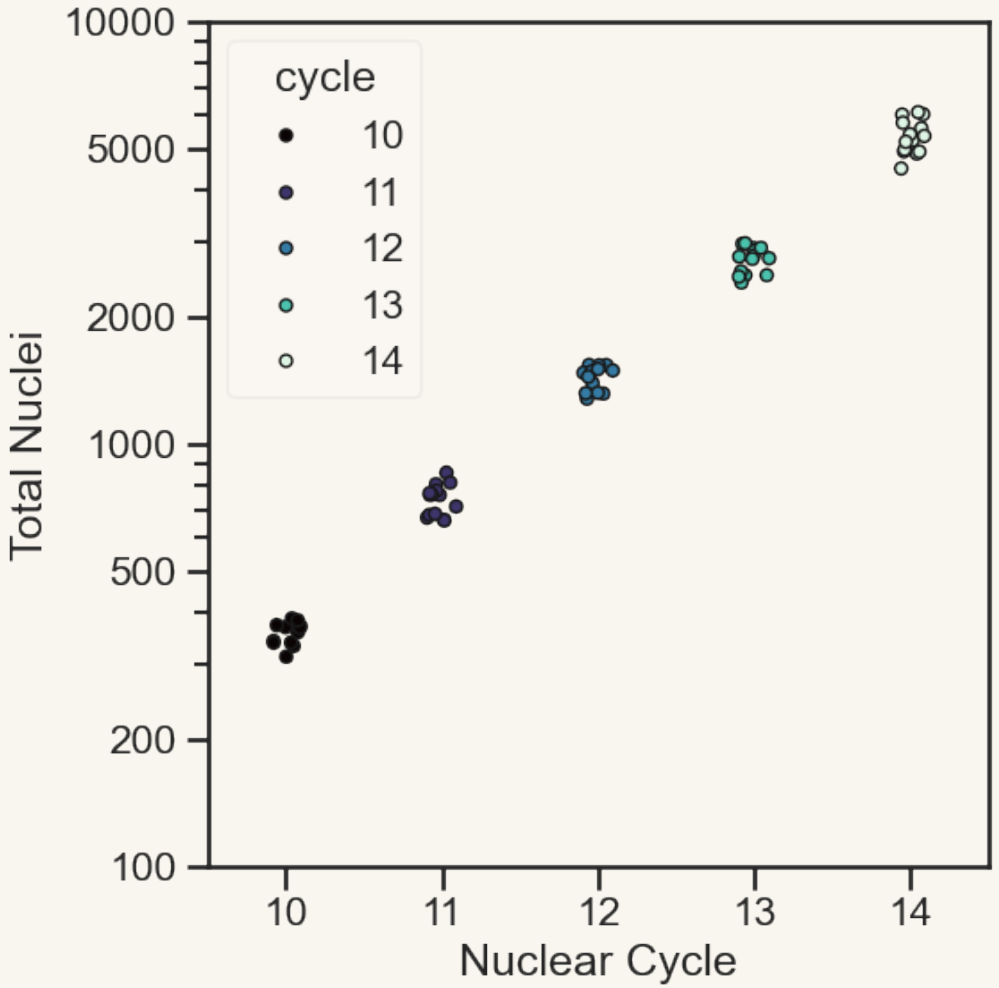
Step 1: Tracking without divisions

- LAP tracking using 3D coordinates (implemented by Trackmate)

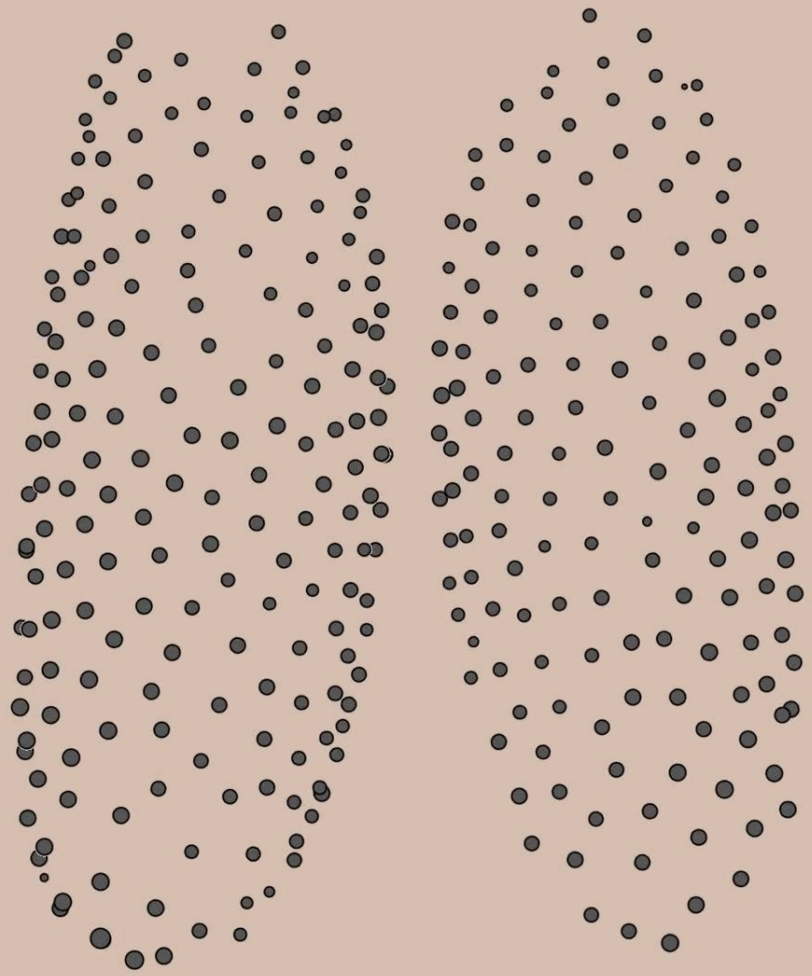
Step 2: Assign daughter nuclei to parents

- Split the problem up into one problem per M phase
- Assume that daughter nuclei move in opposite directions during anaphase

Thousands of nuclei are tracked per embryo

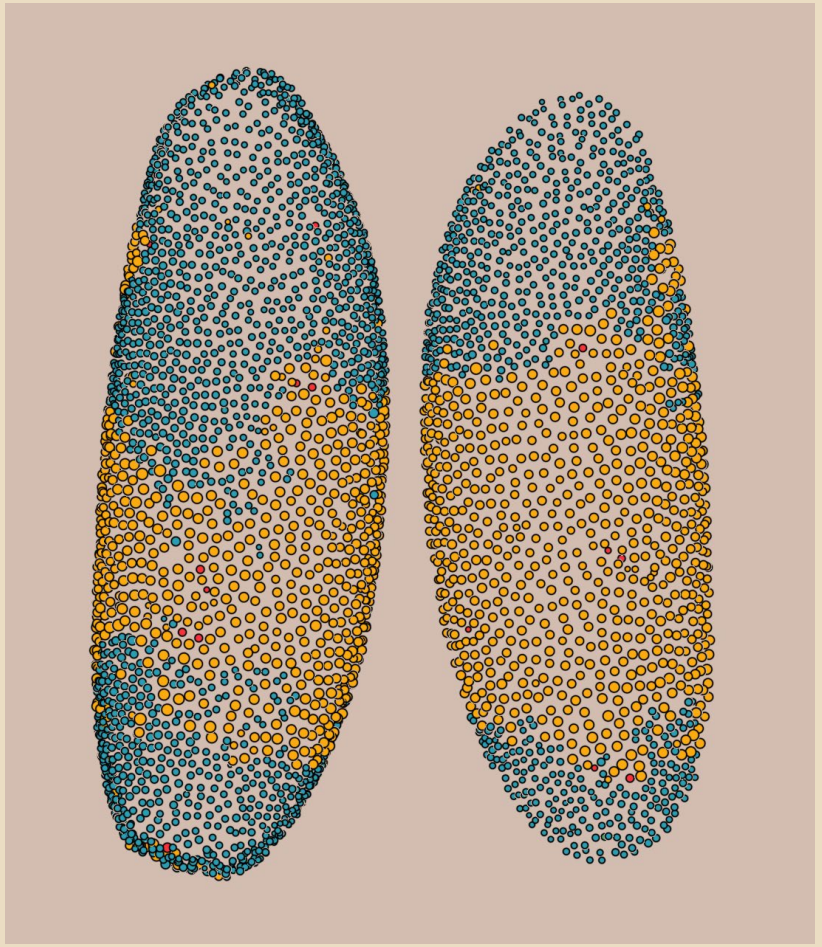


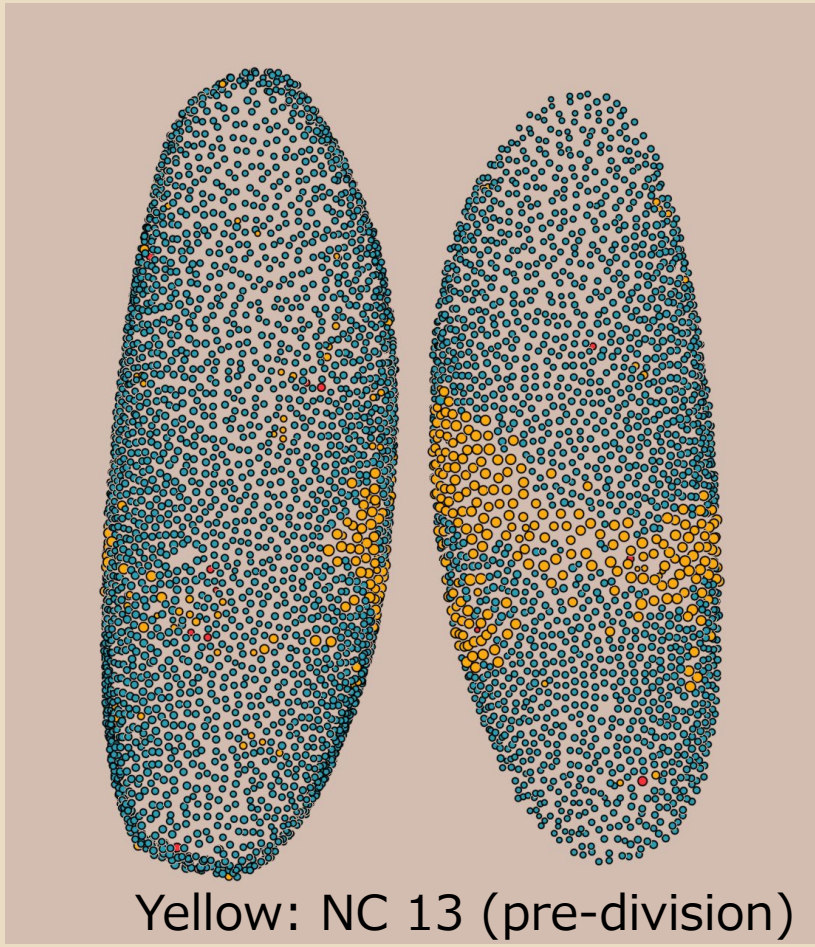
Individual nuclear divisions can be tracked across the embryo



Gray: NC 10
Green: NC 11
Red: NC 12
Yellow: NC 13
Blue: NC 14

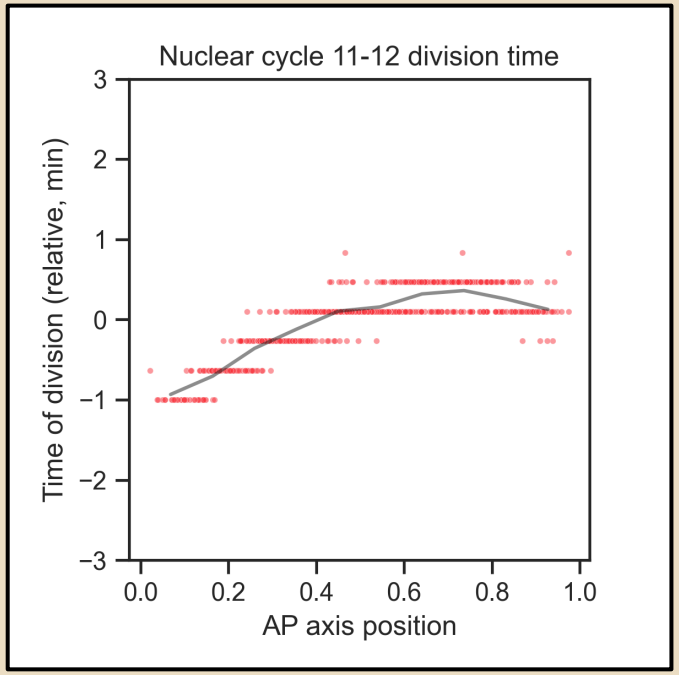
Individual nuclear divisions can be tracked across the embryo

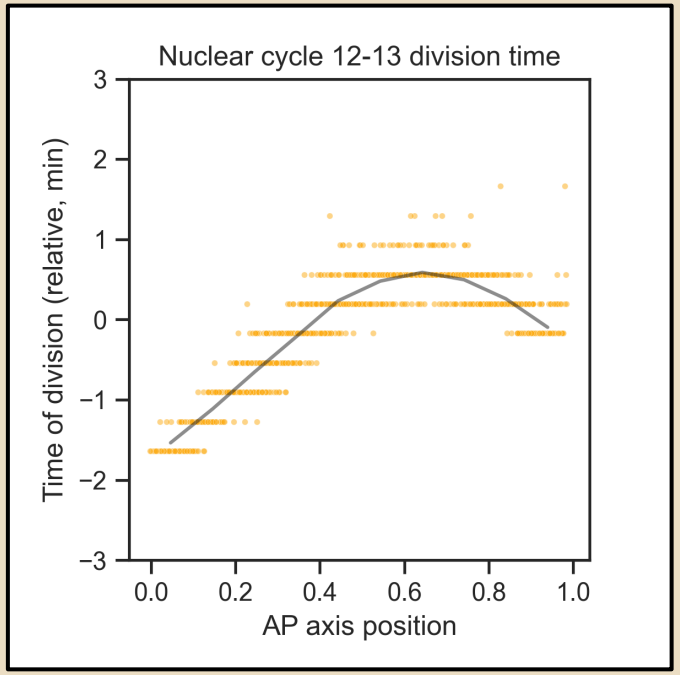


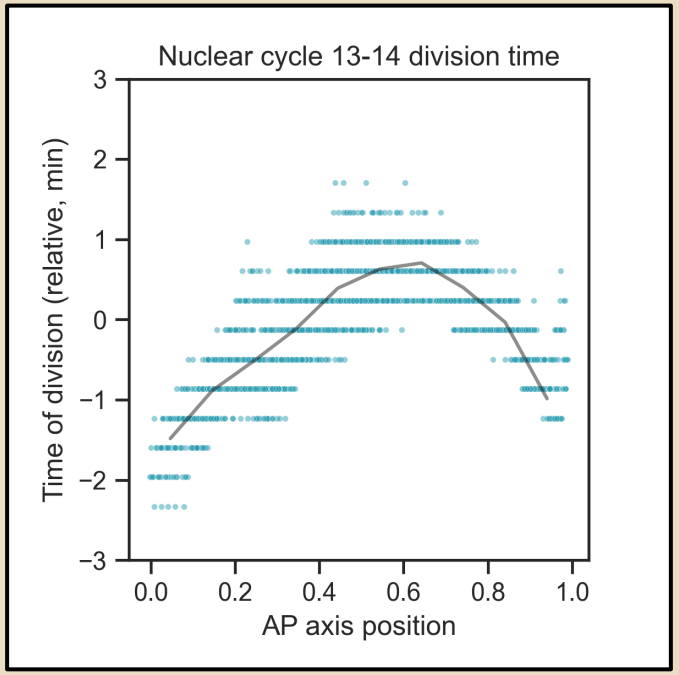


Yellow: NC 13 (pre-division)
Blue: NC 14 (post-division)

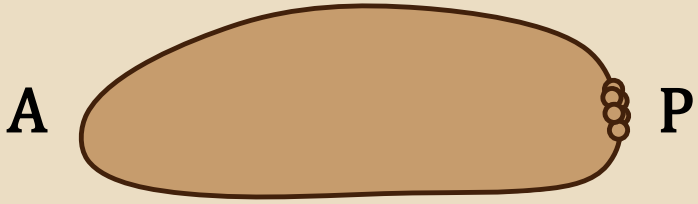
Nuclear division times vary across the AP axis



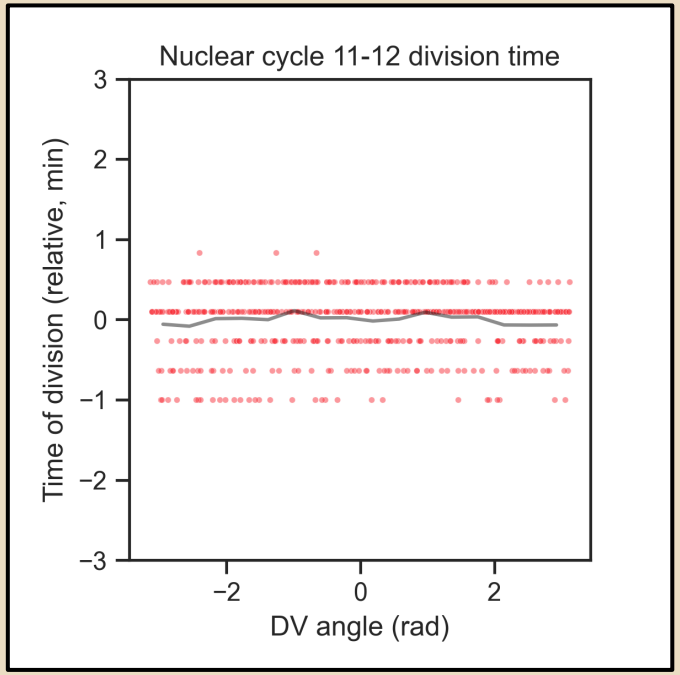


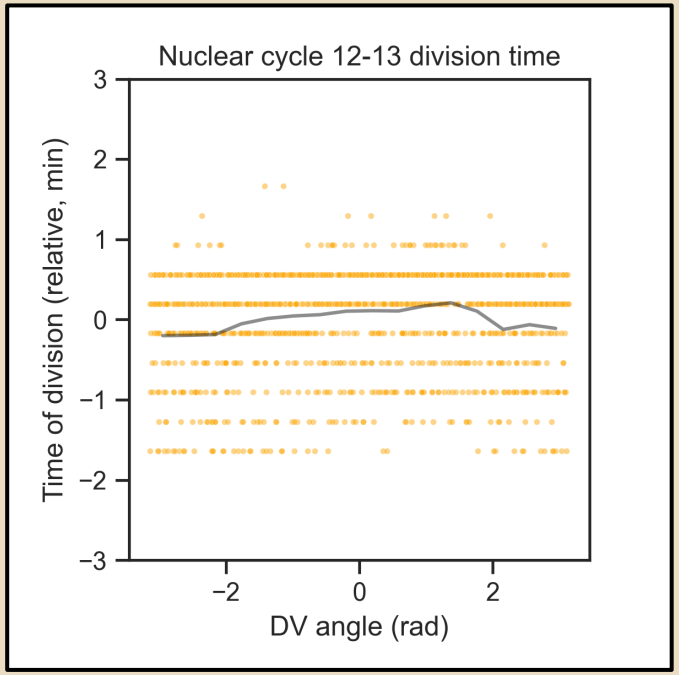


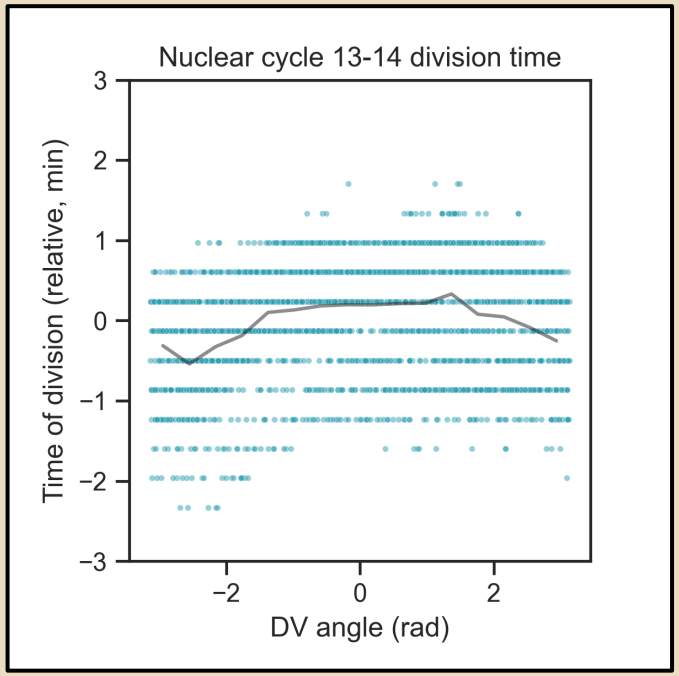
Each dot: one nucleus dividing

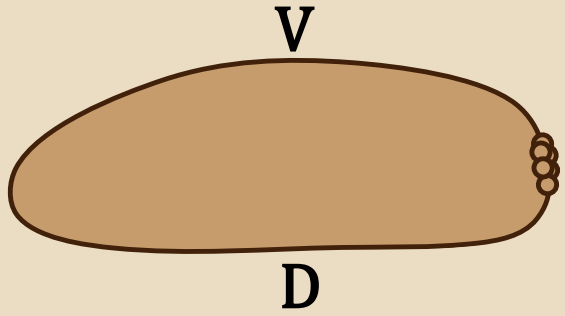


Nuclear division times vary less across the DV axis

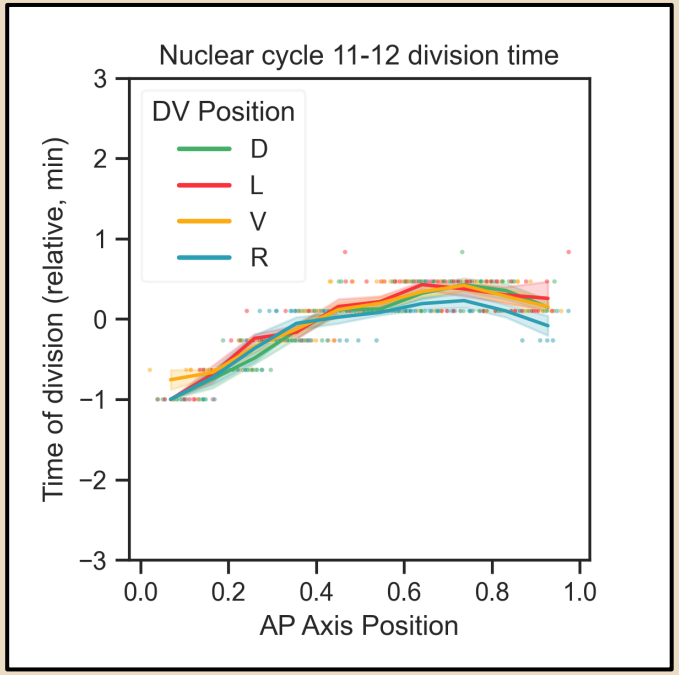


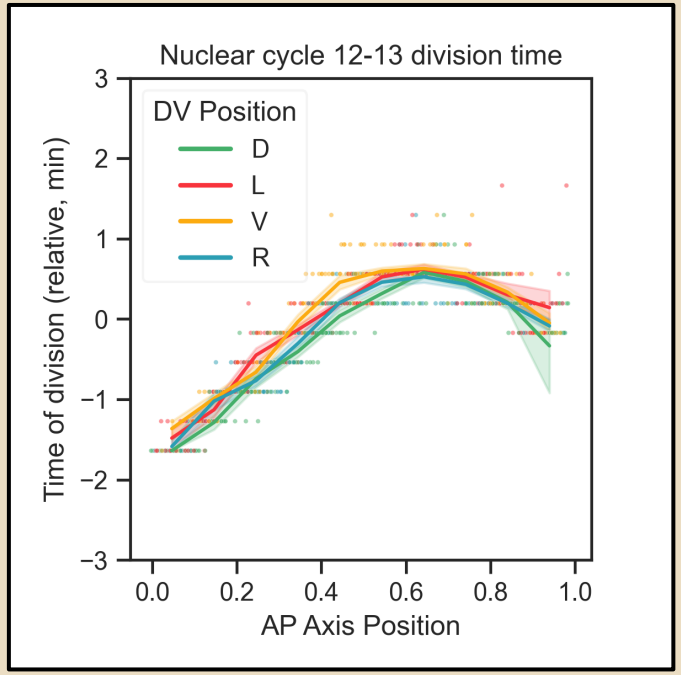


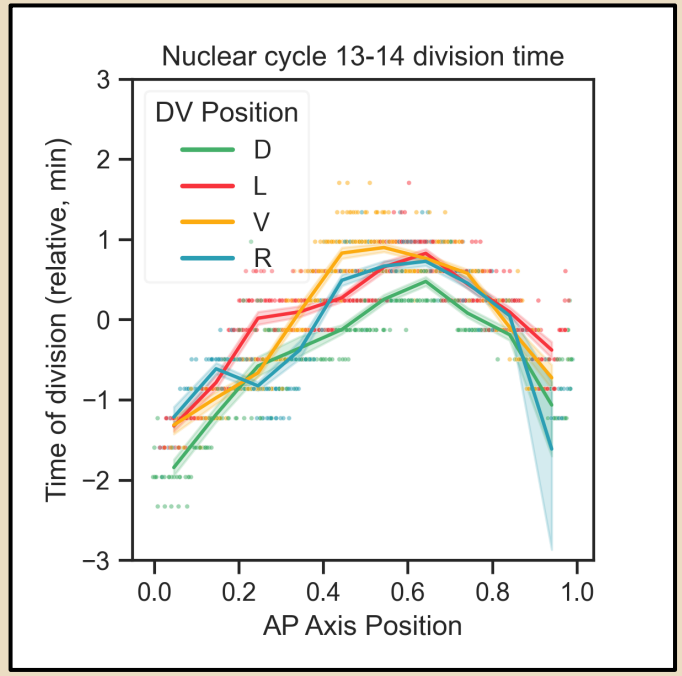


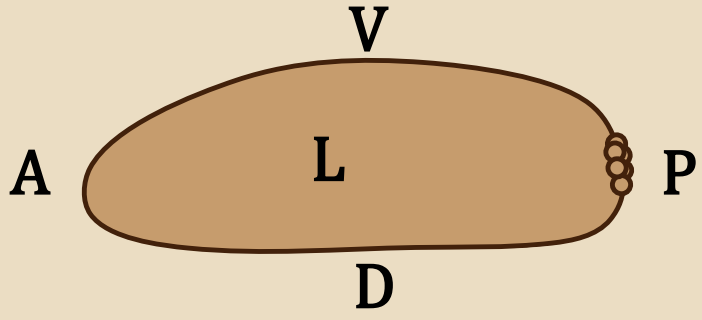


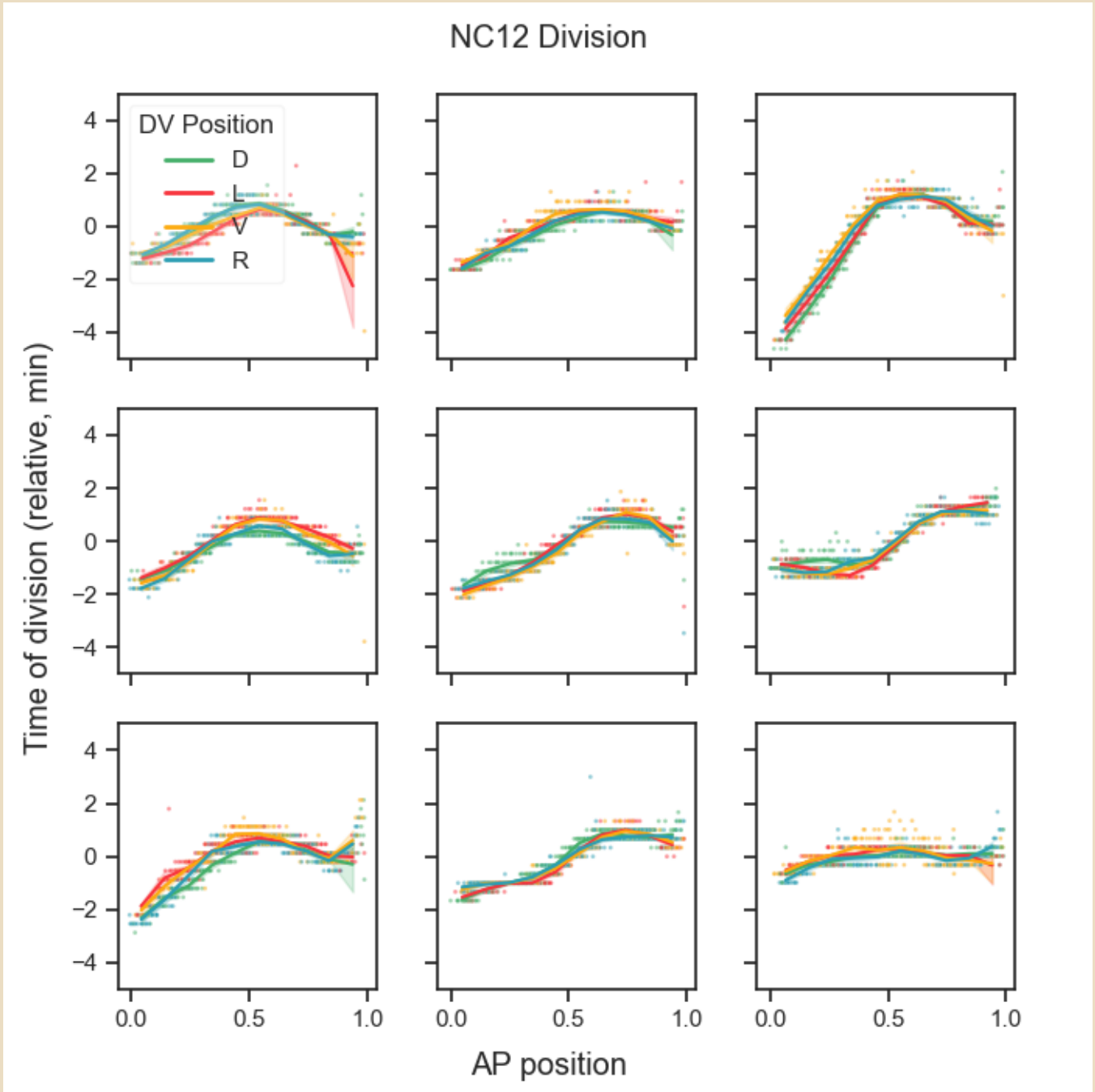
All sides of the embryo show similar mitotic waves



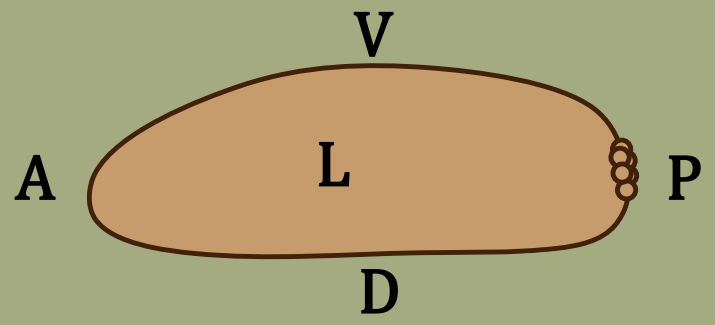








This strong AP axis relationship holds over many embryos

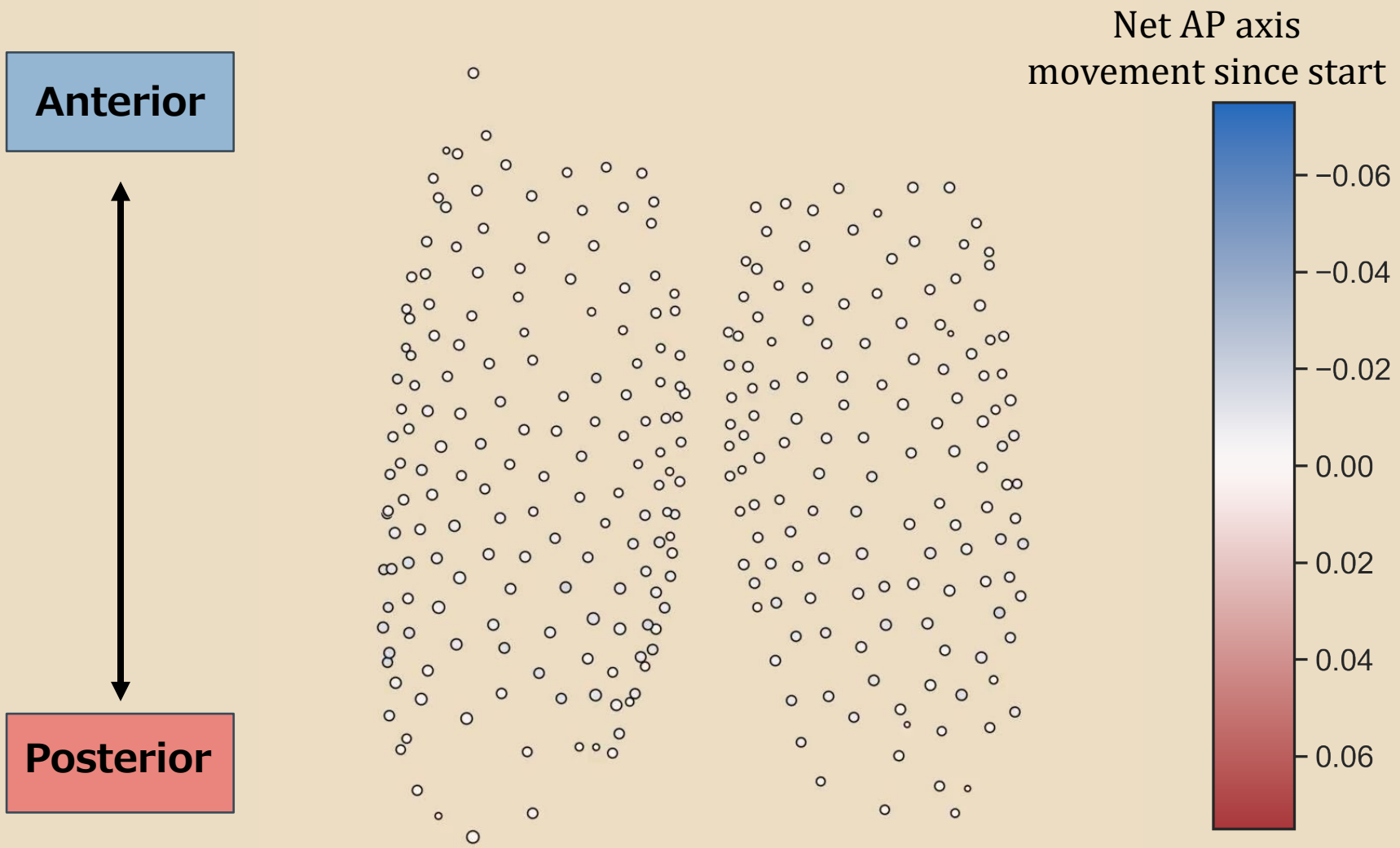


Properties of mitotic waves

I. Mitotic waves traverse the embryo primarily along the AP axis.

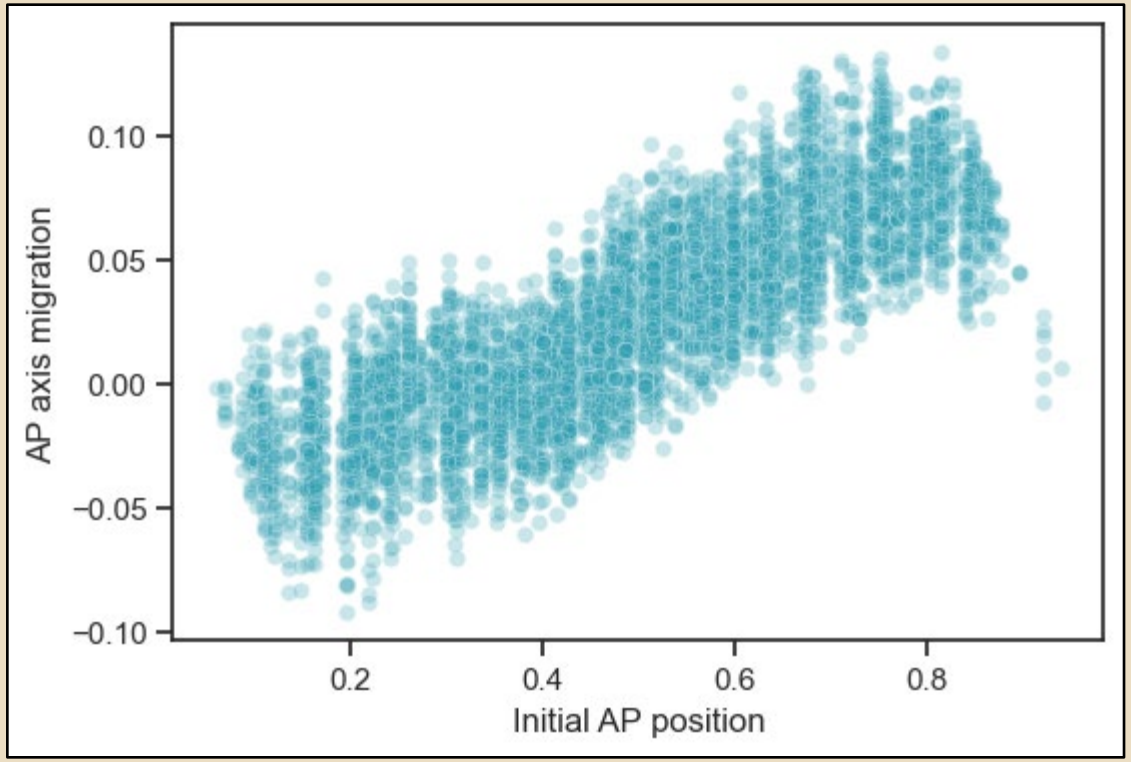
II. Mitotic waves begin at the poles and move towards the center of the embryo

Nuclei spread out along the AP axis over divisions

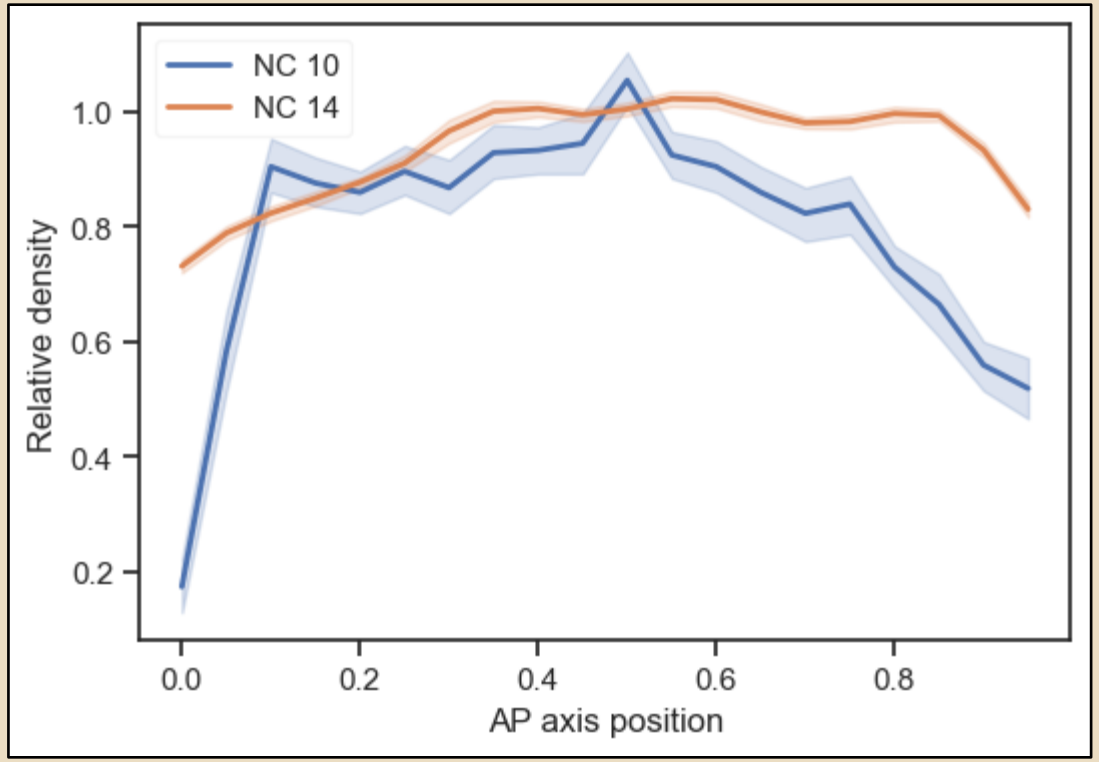


Nuclear spreading generates a more uniform density across the embryo

Initial position vs. net migration

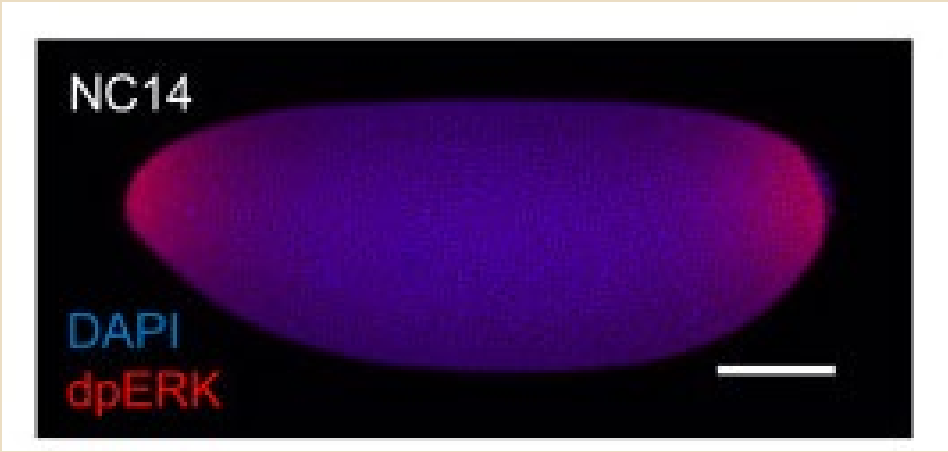


Initial vs. final density profile



ERK activity influences timing of mitotic entry

Erk is active at the poles of the embryo



Developmental Cell

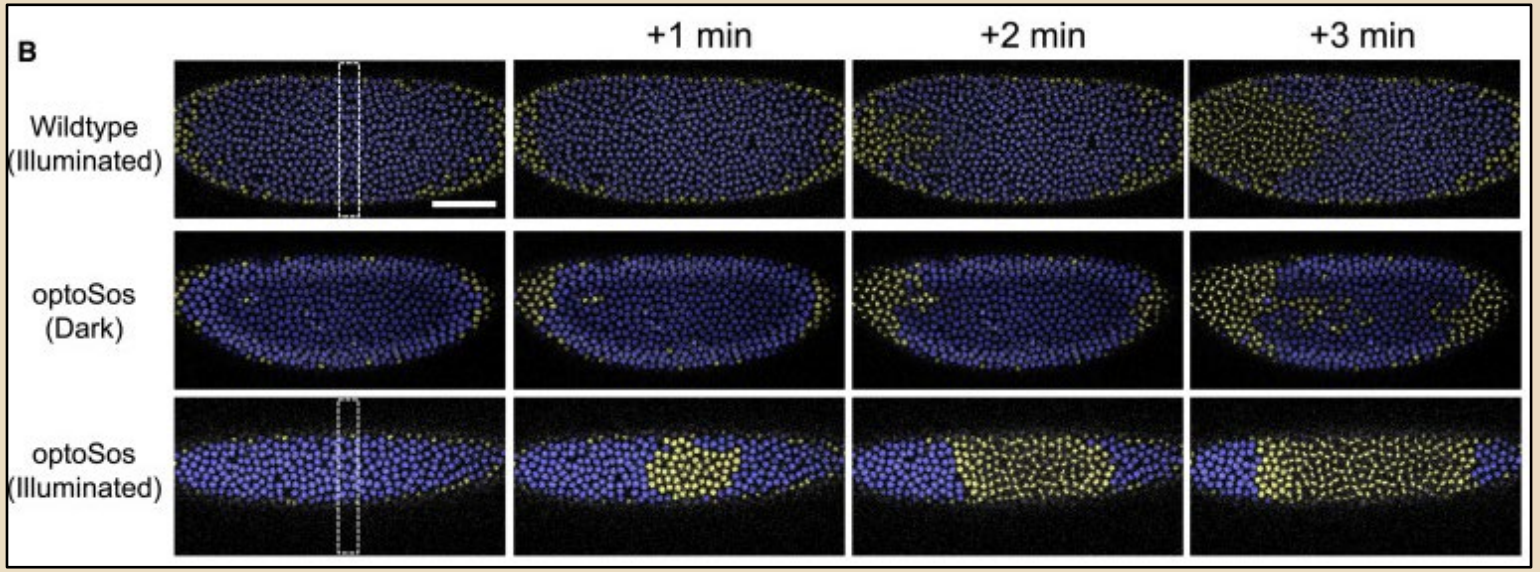
CellPress

Volume 59, Issue 23, 2 December 2024, Pages 3061-3071.e6

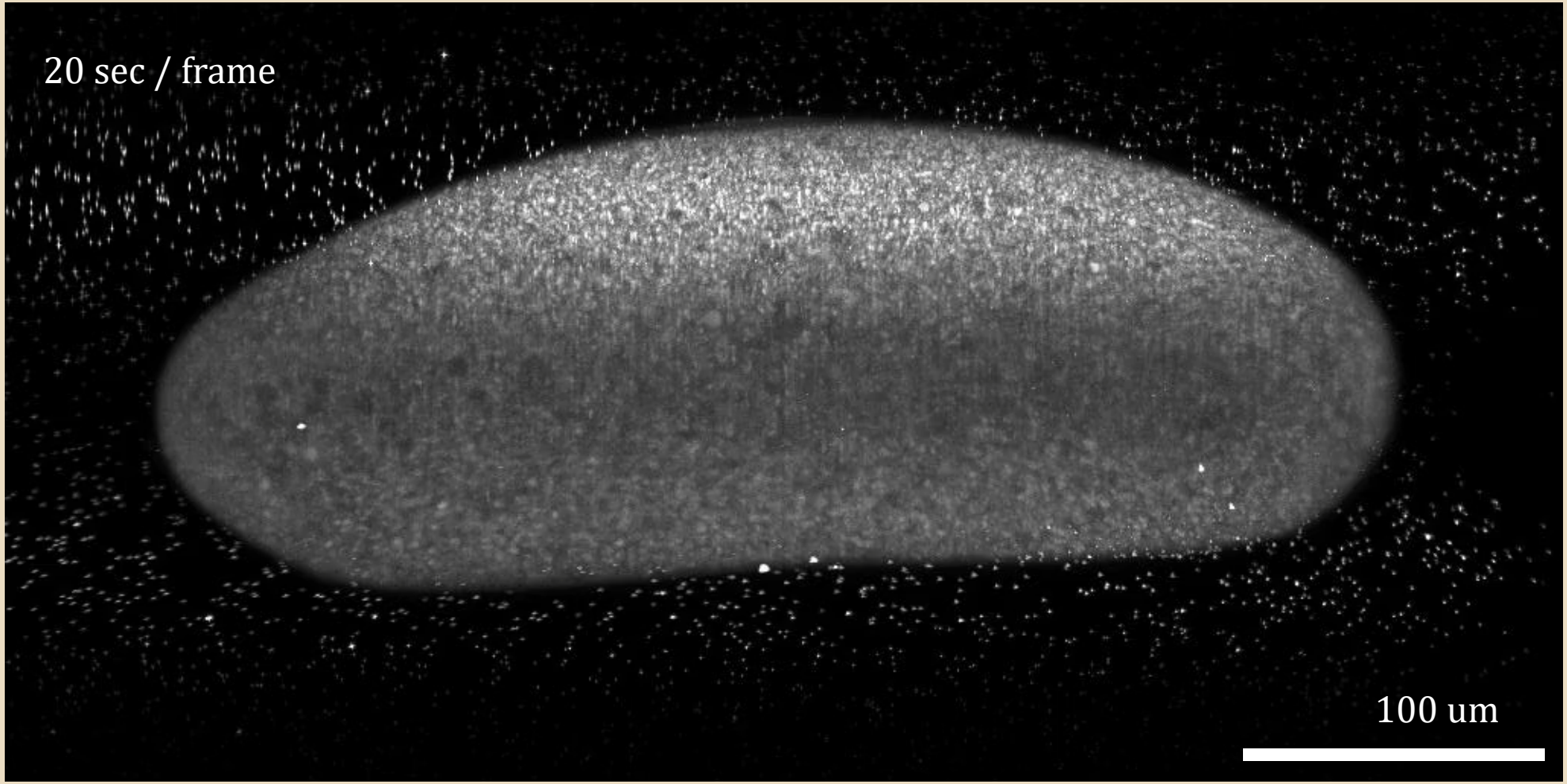
Short article

ERK synchronizes embryonic cleavages in *Drosophila*

Liu Yang [1 8], Audrey Zhu [1 2 8], Javed M. Aman [1 3], David Denberg [1 4], Marcus D. Kilwein [5], Robert A. Marmion [1], Alex N.T. Johnson [1 2 5], Alexey Veraksa [6], Mona Singh [1 3], Martin Wühr [1 2 5] ✉, Stanislav Y. Shvartsman [1 4 5 7 9] ✉



In Trk null embryos, mitotic waves travel slowly to the posterior



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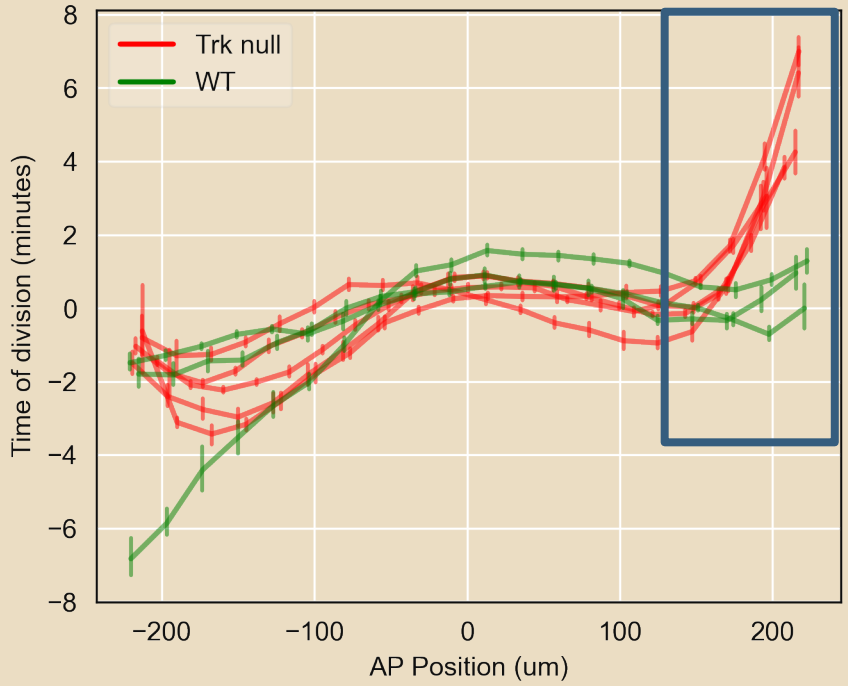
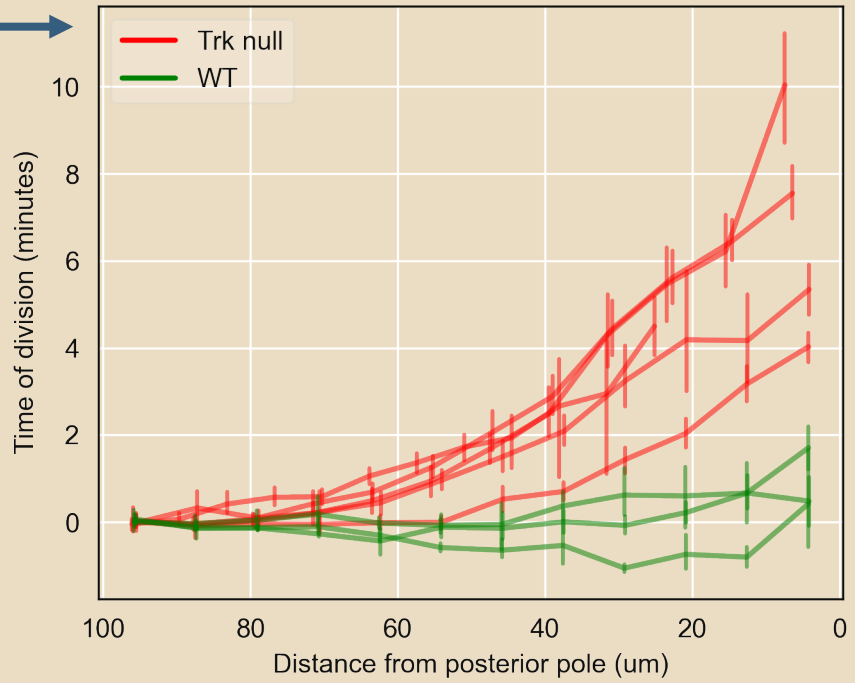
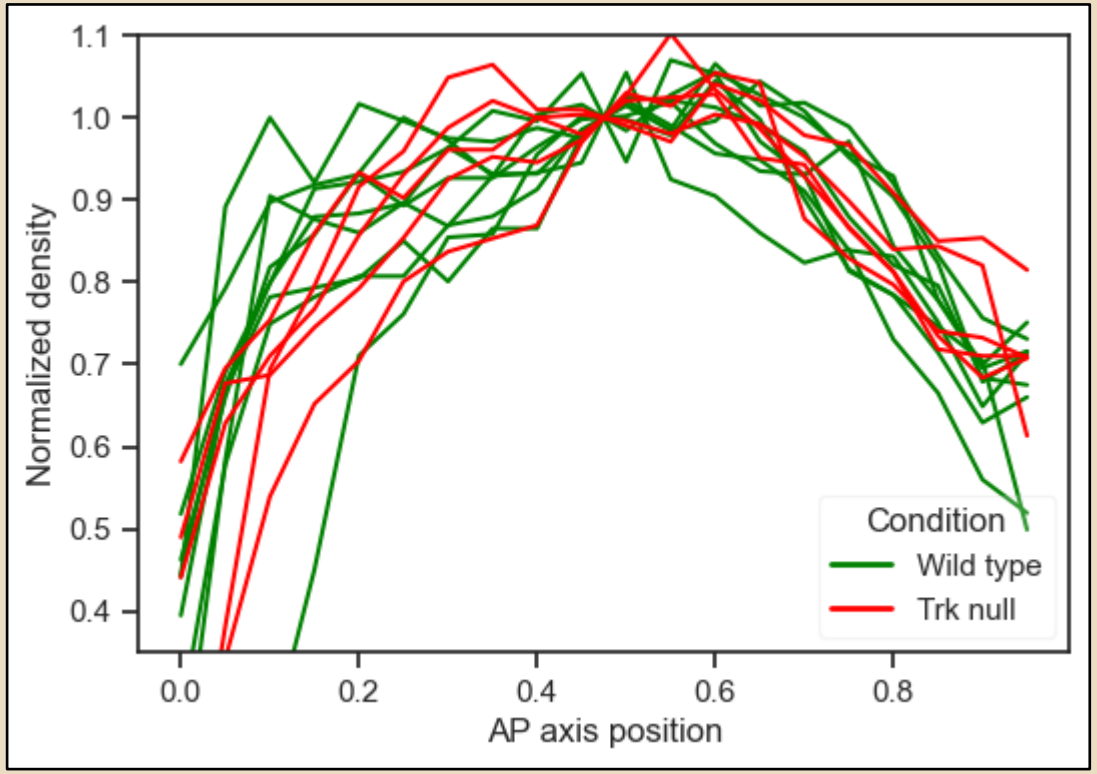




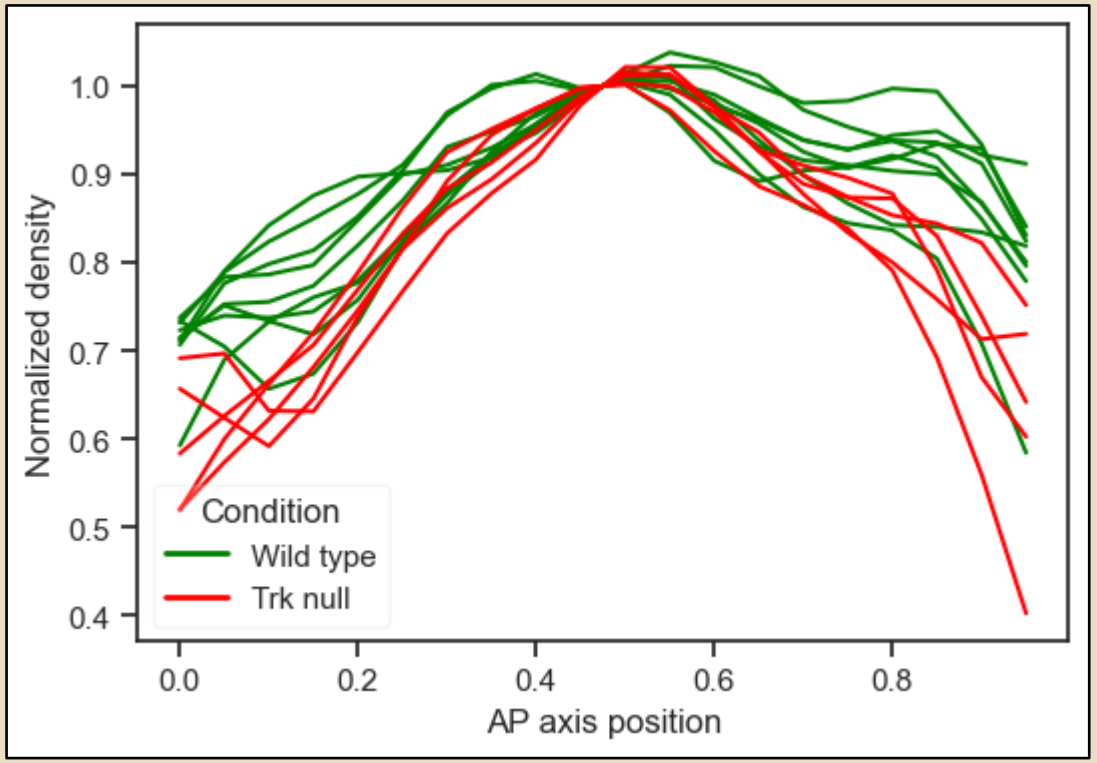
Trk null: ~0.25um/s

Trk null embryos produce a less uniform distribution of nuclei

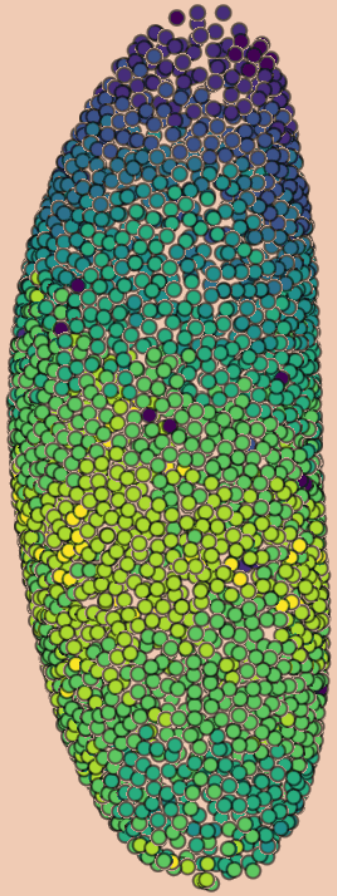
Initial Density Profiles



Final Density Profiles



Conclusions



I. Individual nuclear tracking enables precise description of blastoderm formation

II. Mitotic waves produce thousands of new nuclei while simultaneously organizing density at an embryo scale

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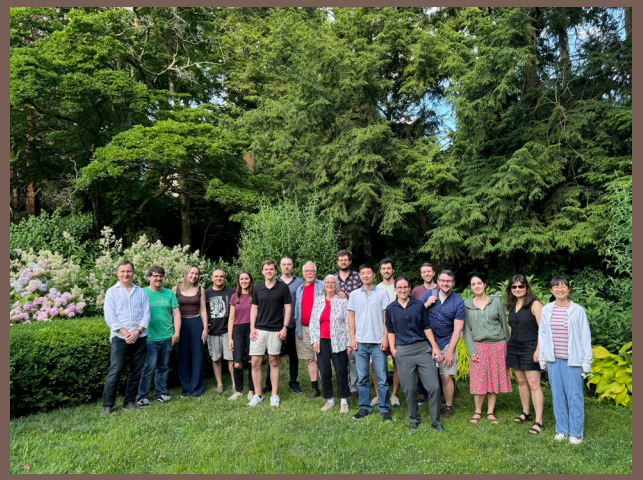

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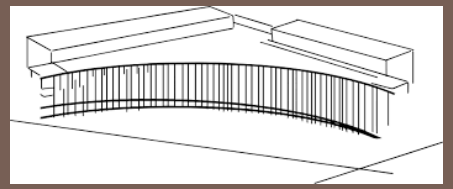

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