Live cell imaging and tracking for studying transcription

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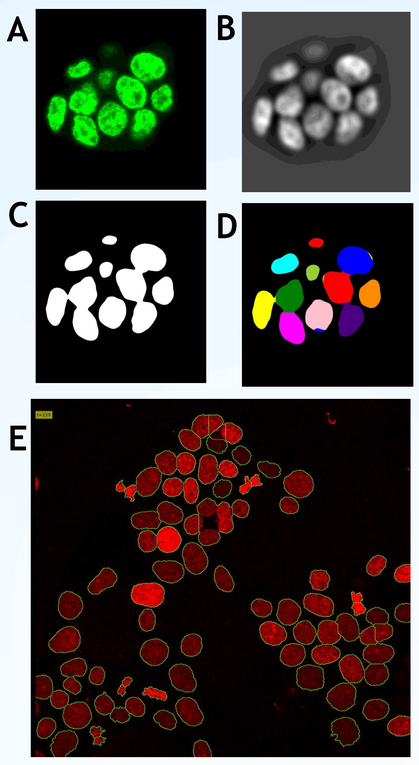
I. Summary

- **Part 1:** Image analysis to identify and track nuclei/cells from fluorescence microscopy time-lapses
- **Part 2:** Analyzing tracking data giving the intensity of transcription spots of two alleles of the *hunchback* gene in each nucleus of the developing Drosophila embryo. **Goal:** quantify the transcriptional noise and look for a possible temporal correlation in the expression of the two alleles.

II. Image Analysis : Segmentation

Segmentation method is specific to the type of image being analyzed (harder in 3D).

Fig II. A- D : 3D image (z-stack, 32 slices) of mouse embryo containing 15 cells, nuclei stained with GFP; implementation in Python using scikit-image.



Segmentation steps :

- Raw image (**A**)
- Filter via difference of Gaussians (**B**)
- Convert to black and white mask via thresholding
- Clean mask by filling holes and removing small objects (**C**)
- Apply marker-based watershed algorithm to the mask (Markers derived from distance transform of the mask) (**D**)

We then tried to automate segmentation of a time lapse of mouse ESC culture as the cells move and divide. We did not achieve an accurate segmentation at every time-point.

Fig II. E: Example of poor segmentation. Frame displaying both over and under segmentation.

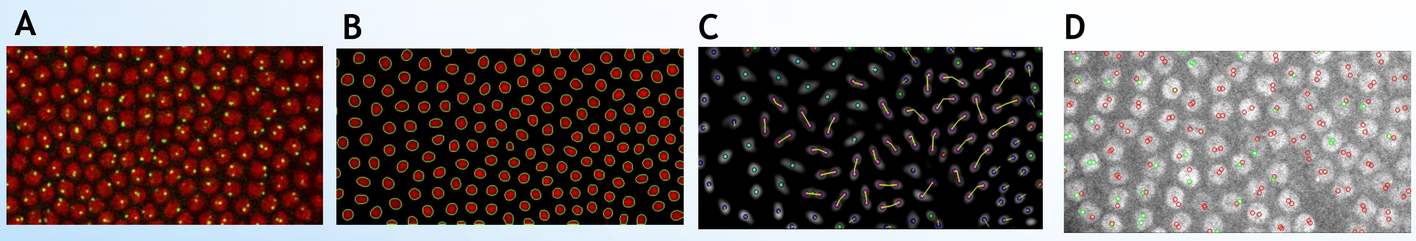
III. Image Analysis: Tracking

The lab developed a pipeline to segment+ track nuclei as they move and divide from 3D time-lapse of the developing Drosophila embryo. Accurate segmentation is possible due to good spacing between nuclei and uniformly distributed intensities.

Tracking method (simplified): For each frame:

- Calculate centroids of nuclei from segmentation
- Link nuclei from one frame to the next by solving a partial linear assignment problem allowing dividing nuclei to be assigned to its two daughters in the next frame (Hungarian algorithm)
- Cost function is distance between centroids

Fig A-D: Tracking results on embryogenesis time-lapse (see **IV. Experimental setup**). Images do not correspond to the same time-point.



(**A**) Raw image , nuclei (red), Hunchback-P2 transcription sites (green)
(**B**) Segmentation, nuclei boundaries in green
(**C**) Tracking information, e.g velocity vectors, pairs of daughter nuclei (pink), nuclei about to divide (green)
(**D**) Hunchback-P2 transcription spots identified as intensity peaks within each nucleus, also tracked

IV. Hunchback-P2 transcription in the Drosophila embryo

Hunchback is a maternal effect gene important for patterning of anterior parts (head and thorax) of the *Drosophila* embryo. Drosophila embryogenesis has been shown to be very precise. To understand why, we might study the noise in Hunchback expression.

Experimental setup:

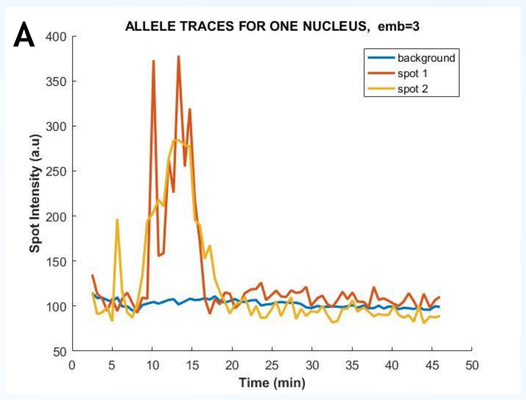
-Live image embryos during last several nuclear cycles (NC) with confocal fluorescence microscope
-Visualize nuclei with histone-RFP expression
-visualize hunchback P2 promoter transcription sites with MCP-GFP (two sites per nucleus)
-track spots through each cycle (MATLAB pipeline developed by the lab)

V. Tracking Data Analysis

- Time series data for 14 embryos extracted from tracking pipeline
- Before pooling data, spatial alignment along the anterior-posterior (AP) axis and temporal alignment (start time at NC14 mitosis)
- Mean background intensity subtracted from spot intensities

Fig A: Transcriptional activity of two spots in a single nucleus

Fig B: expression levels in a single embryo, in a given AP position bin (size 2.5% egg length). (binned mean in red) . Estimated NC14 peak is 10-12 min after mitosis.



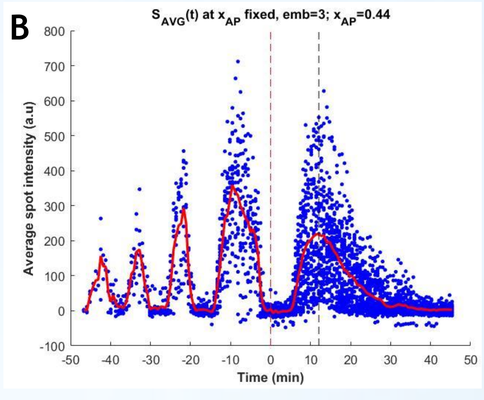


Fig C-G: Average spot intensities in nuclei at the peak of NC14 (AP position bins are 2.5% egg length)

(**C**) Raw spot intensity data as a function of position along the AP axis

(**D**) Mean expression profile for each embryo and for pooled data. We identify three regions: (I) high, (II) transition, (III) low.

(**E**) Standard deviation as a fraction of mean (roughly 40%)

(**F**) Decomposition of variance. Embryo to embryo variability represents 10 to 20 percent of total variability in region (I).

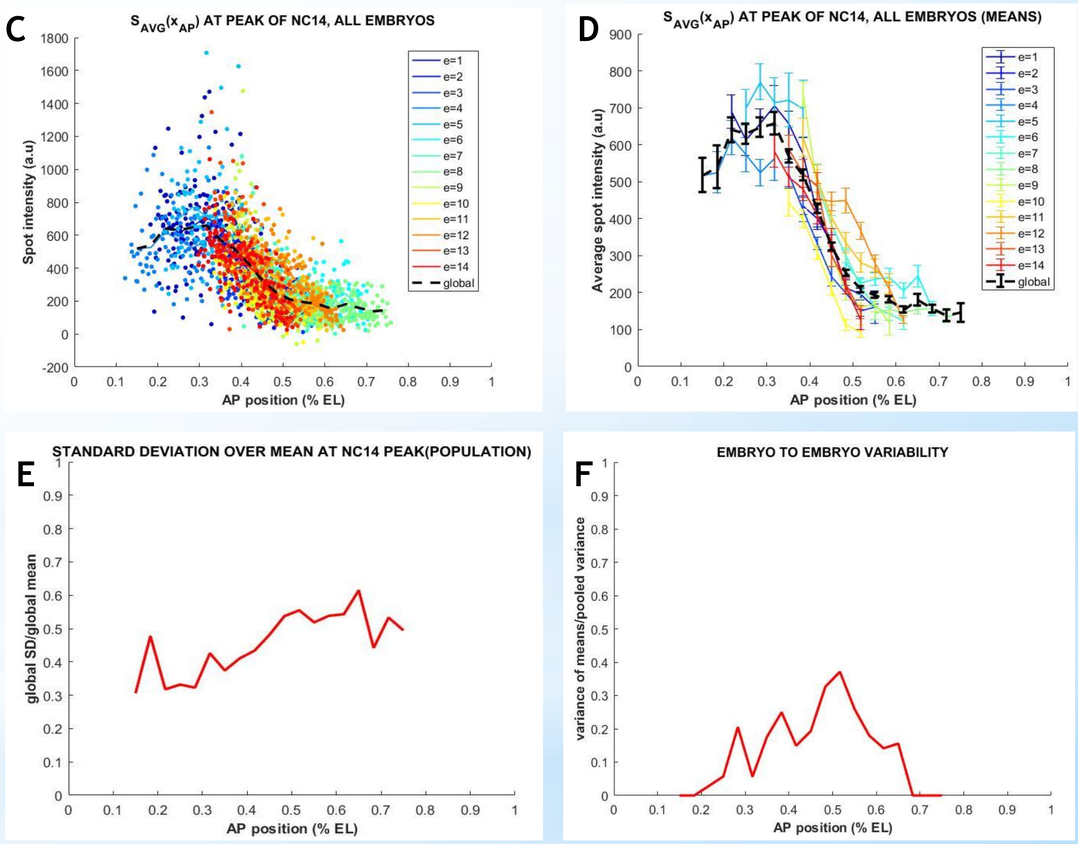
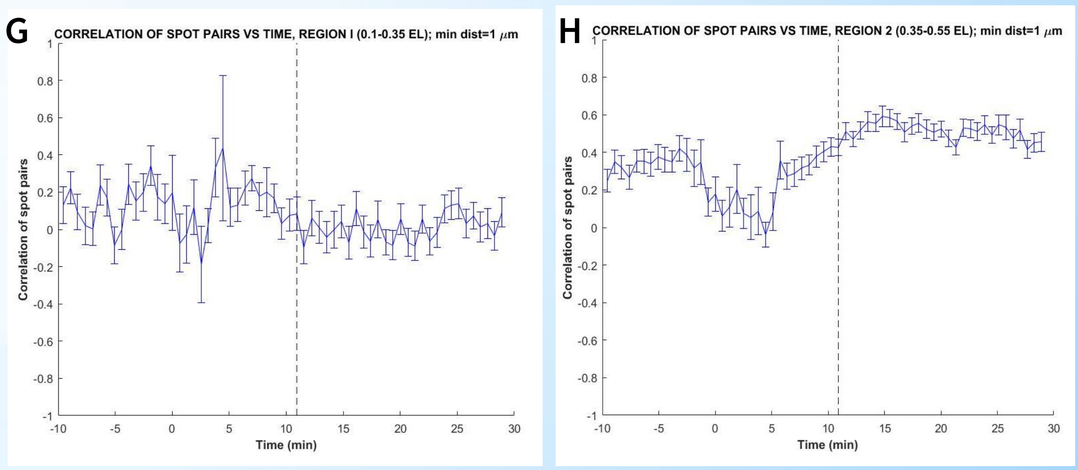


Fig G: Correlation of spot pairs in region (I) vs time, removing pairs closer than 1 micrometer (50% of pairs). Error bars = bootstrapped standard deviation.

Fig H: Correlation of spot pairs in transition region vs time. The increasing correlation could be spurious, possibly due to spatial misalignment.



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