Quantitative Imaging Describes Morphogenetic Nuclear Movements Prior to Gastrulation

BDTNP
Berkeley Drosophila Transcription Network Project

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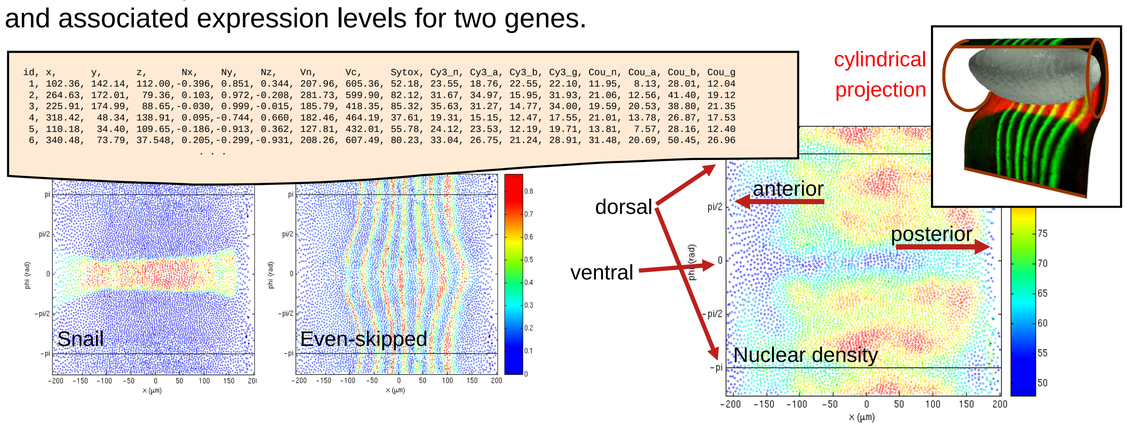
Introduction

This poster describes the systematic movements that the blastoderm nuclei of a *Drosophila Melanogaster* embryo make during stage 5. The nuclear density pattern, as computed from 3D images of fixed, whole embryos, shows a marked change over time. Using living histone 2A - GFP embryos we confirm this process. Only a small portion of the surface of the live embryos can be imaged. By taking data from 23 embryos at random, but known, orientations together we compose a nuclear density map at both the beginning and end of stage 5 that agrees very well with the results from fixed embryos. The same temporal sequences were used to obtain a full-embryo map of the flow of nuclei.

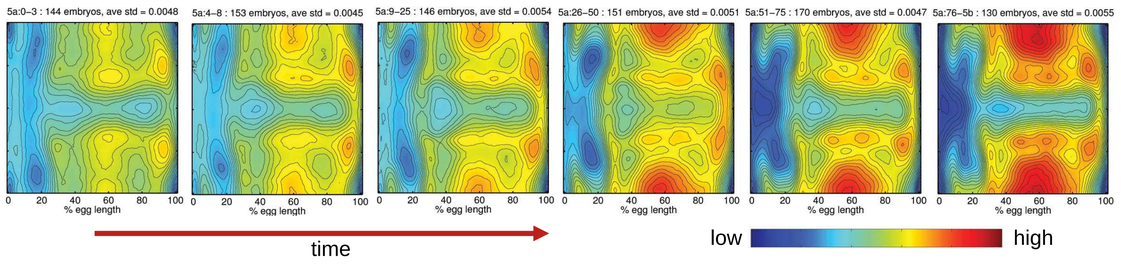
Imaging of Fixed, Whole *Drosophila* Embryos

As part of the Berkeley Drosophila Transcription Network Project we are creating a three-dimensional atlas of gene expression and embryo morphology at cellular resolution (see also #97, #135 and #423B). For this purpose, we are imaging whole embryos with enough resolution to distinguish individual nuclei. A fully automated set of algorithms then converts these images into a much smaller and more useful representation, which we call “point cloud”. This representation contains the 3D coordinates of each nucleus in the blastoderm, and associated expression levels for two genes.



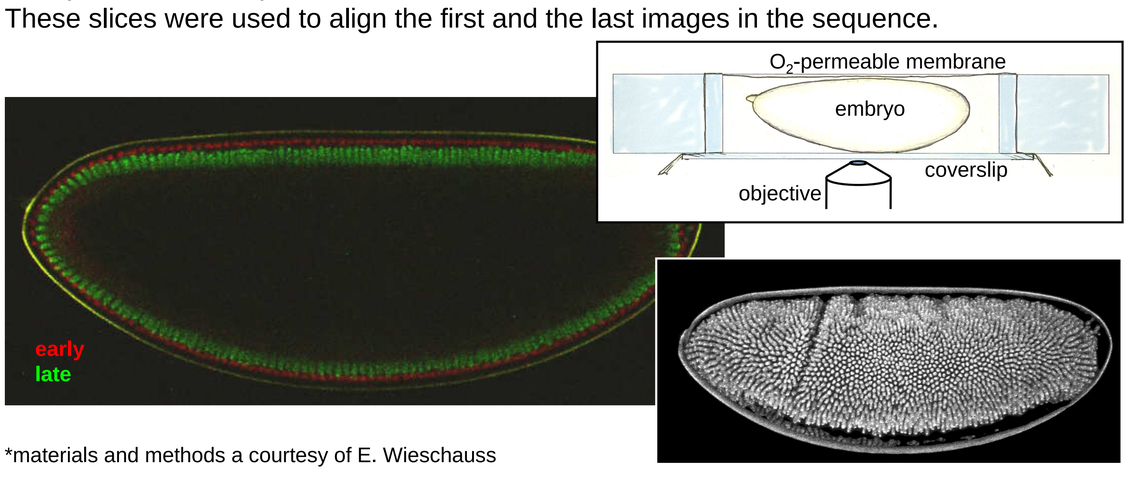
The Nuclear Density Maps from Fixed Embryos

One novel observation we have made is that there is a systematic change in nuclear packing densities during stage 5 (interphase cycle 14) when fixed embryos of different ages are compared (see #135 and #423B). Shown here are local density maps averaged over ~150 individuals in each time cohort. Embryos are classified into time cohorts by the level of invagination of the cellular membrane on the ventral side of the embryo. Standard deviation of each graph is about 20% of the lowest measured density



Imaging of Live Histone2A-GFP Embryos

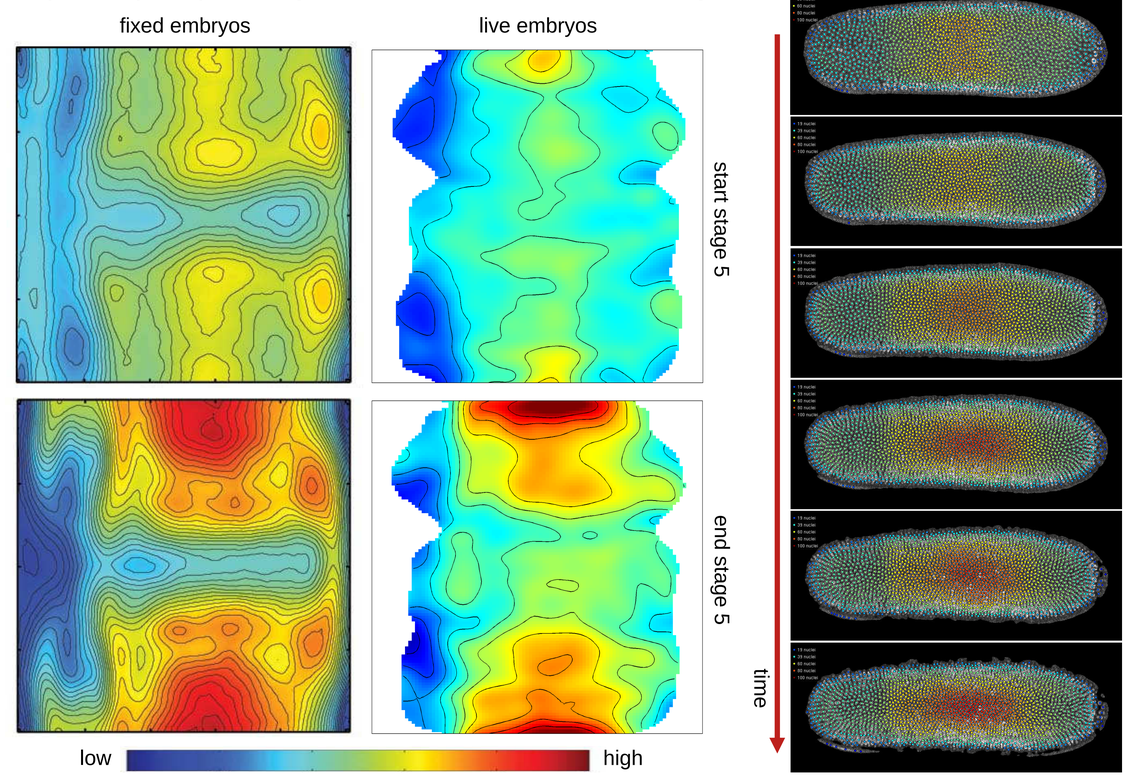
We imaged 23 living histone 2A - GFP embryos mounted in halocarbon oil under air-permeable membrane* and the nuclei in the top 1/3 of the embryo were imaged in a confocal microscope, every 2 to 5 minutes, starting just before cleavage cycle 13 and up to first signs of gastrulation. We also recorded an additional stack well into gastrulation, to determine de d/v orientation of the embryo, and two slices through the middle of the embryo, as close as possible to the start and end of the time interval we were interested in. These slices were used to align the first and the last images in the sequence.



*materials and methods a courtesy of E. Wieschauss

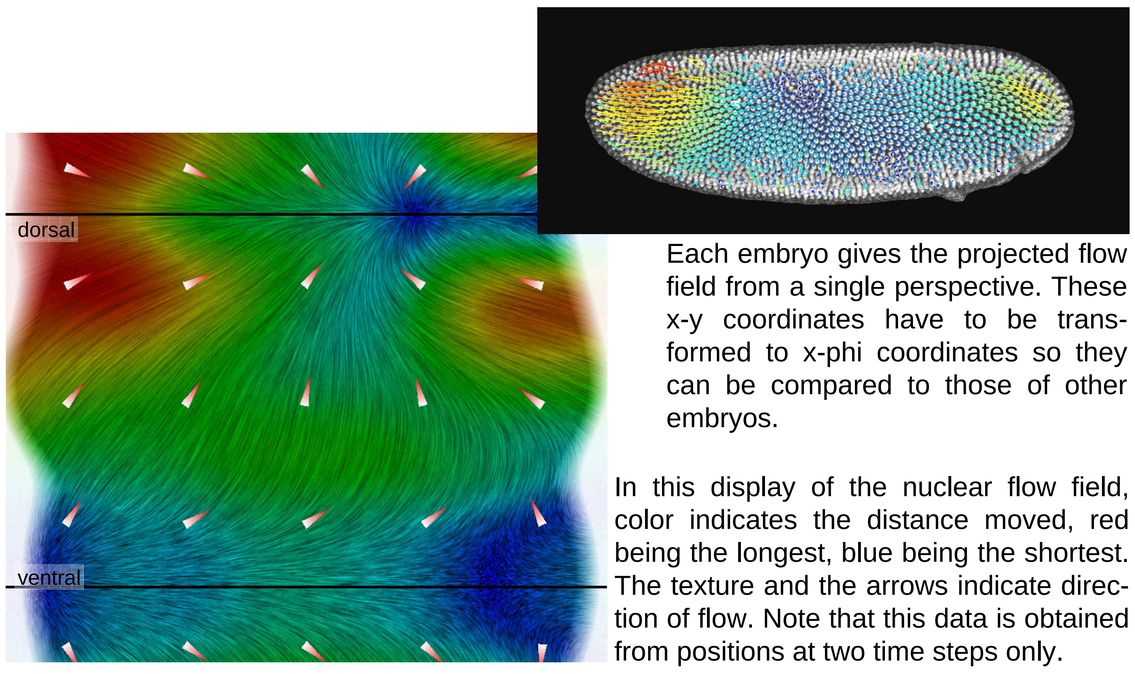
Composing Nuclear Density Maps

Detecting GFP-stained nuclei in the 3D confocal stacks of live embryos is not possible due to the poor signal to noise ratio. Hence we collapsed the stacks into high-quality 2D images, in which a small portion of the embryo surface could be analyzed. We measured the local density in a similar way as in our fixed embryos, and placed the data in the appropriate location of an unrolled embryo. Overlapping data was averaged. This yields a local density map of most of the embryo surface (anterior and posterior ends could not be analyzed). This was done with the first and last image taken within stage 5. The two resulting density maps compare positively with the results from fixed embryos.



Composing Nuclear Flow Maps

In those same 23 time sequences we tracked all individual nuclei. The relative location of one nucleus at the beginning and at the end of the sequence were registered using the slices through the middle of the embryo. This yields an absolute distance and direction each nucleus moves during stage 5. Again mapping these arrows onto an unrolled view and averaging where the data is duplicated, we have created a nuclear flow field for most of the embryo surface. Assuming left-right symmetry better accuracy is obtained.



Each embryo gives the projected flow field from a single perspective. These x-y coordinates have to be transformed to x-phi coordinates so they can be compared to those of other embryos.

In this display of the nuclear flow field, color indicates the distance moved, red being the longest, blue being the shortest. The texture and the arrows indicate direction of flow. Note that this data is obtained from positions at two time steps only.

Conclusions

The movement of nuclei during embryonic stage 5 shown here occurs way before gastrulation. During this stage, evolution of morphogenetic patterns is often studied with respect to distances along the a/p axis. As discussed by Keränen et. al. (423B), this type of study needs to take nuclear movement into account. What is observed as a change in the expression pattern is actually a combination of cell flow and expression flow. That is, both the expression domains change with respect to the nuclei, and the nuclei move with respect to the spatial coordinate axes.