Automated delineation of cells and nuclei and quantification of gene expression in 3D images of whole *Drosophila* blastoderm embryos

BDTNP
Berkeley Drosophila Transcription Network Project

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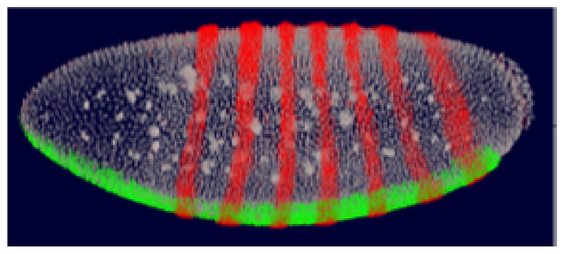
Introduction

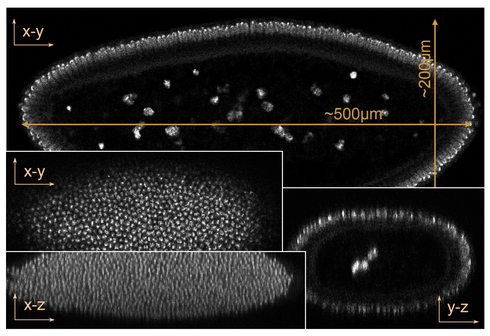
This poster describes image analysis algorithms that automatically identify the location and extent of all individual nuclei and their associated cytoplasm in 3D images of whole embryos. The embryos are fluorescently labeled for the genes of interest and total DNA. For each detected nucleus, the associated expression levels in the nucleus and surrounding cytoplasm are measured. These methods yield a list of all nuclei, containing their 3D coordinates, morphological features and relative gene expression. Here we show some of the new findings obtained from this data. Keränen (poster #356B) and Fowlkes (poster #350B) show some other results.

Imaging of Whole *Drosophila* Embryos

- 2-photon fluorescent confocal microscopy
- 1 laser line (750nm) excites 3 dyes
- 20x lens
- Embryo size: ~500µm by 200µm
- Pixel size: 0.45x0.45x1.5µm
- Stained for DNA and 2 different mRNAs



ftz (red), *sna* (green), DNA (white)



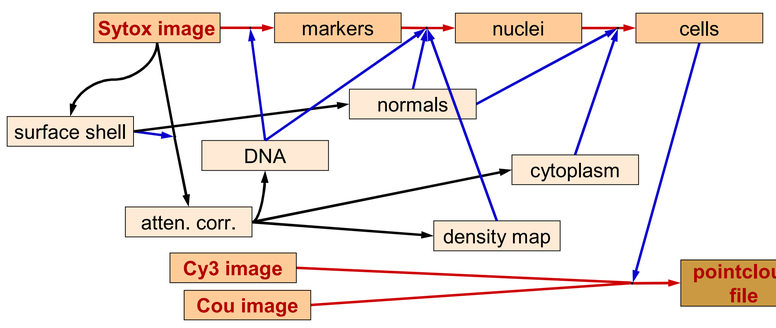
Imaging problems:

–Penetration loss
–Z-resolution just sufficient
–DNA density not uniform across nucleus
–Yolk DNA, pole cells

One image contains the whole embryo with enough resolution to segment individual nuclei: **about 0.5 Gb of data**

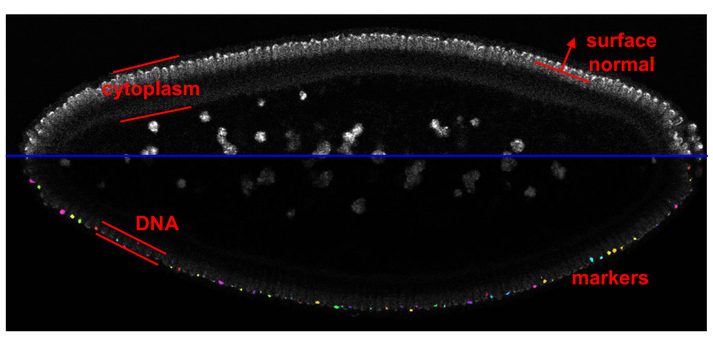
The Segmentation Algorithm

To overcome the difficulties mentioned above, novel image segmentation and analysis algorithms were developed. These are specifically tailored to detect the monolayer of nuclei that form the *Drosophila* blastoderm. Local maxima of the smoothed Sytox image (which shows total DNA) are used as markers. Because many nuclei will contain two markers instead of one, a pruning algorithm is required. The markers are then extended to fill the nuclei, which in turn are extended to fill the cells. All of these processes require knowledge of the blastoderm shape. A novel 3D rendering tool was developed to examine the intermediate results of this algorithm (see poster #374B by Weber).



Outline of the segmentation algorithm:
boxes are data sets, red and black arrows indicate where each one is derived from, blue arrows indicate use of a data set to steer a step

The elements detected by the algorithm, as named in the graph above



A pointcloud file contains position, volumes and expression levels in each detected nucleus, measured separately for the nuclear, apical and basal regions of the cell.

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id, x,      y,      z,      Nx,     Ny,     Nz,     Vn,     Vc,     Sytox, Cy3_n, Cy3_a, Cy3_b, Cy3_g, Cou_n, Cou_a, Cou_b, Cou_g
 1, 102.36, 142.14, 112.00,-0.396, 0.851, 0.344, 207.96, 605.36, 52.18, 23.55, 18.76, 22.55, 22.10, 11.95,  8.13, 28.01, 12.04
 2, 264.63, 172.01,  79.36, 0.103, 0.972,-0.208, 281.73, 599.90, 82.12, 31.67, 34.97, 15.95, 31.93, 21.06, 12.56, 41.40, 19.12
 3, 225.91, 174.99,  88.65,-0.030, 0.999,-0.015, 185.79, 418.35, 85.32, 35.63, 31.27, 14.77, 34.00, 19.59, 20.53, 38.80, 21.35
 4, 318.42,  48.34, 138.91, 0.095,-0.744, 0.660, 182.46, 464.19, 37.61, 19.31, 15.15, 12.47, 17.55, 21.01, 13.78, 26.87, 17.53
 5, 110.18,  34.40, 109.65,-0.186,-0.913, 0.362, 127.81, 432.01, 55.78, 24.12, 23.53, 12.19, 19.71, 13.81,  7.57, 28.16, 12.40
 6, 340.48,  73.79, 37.548, 0.205,-0.299,-0.931, 208.26, 607.49, 80.23, 33.04, 26.75, 21.24, 28.91, 31.48, 20.69, 50.45, 26.96
                          . . .
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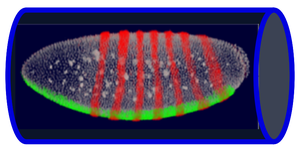
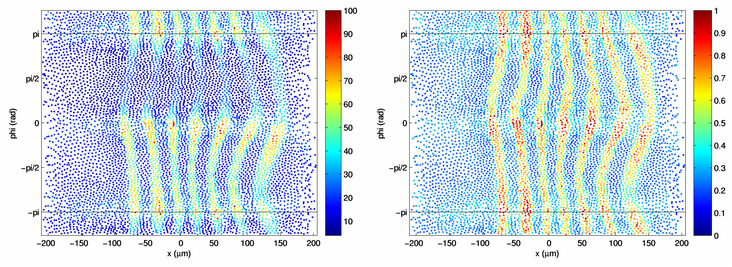
One pointcloud file contains data and expression levels for each nucleus/cell in the embryo: **about 1 Mb of data**

Attenuation Correction

We correct for intensity attenuation (penetration loss) of the detected signals by dividing by the measured intensity of the DNA fluorescence. This is done on a per-cell basis, from the result of the segmentation procedure. This is only an approximate correction, since each color attenuates slightly differently.

Mercator projection: projecting nuclei onto cylinder aligned with AP axis of embryo



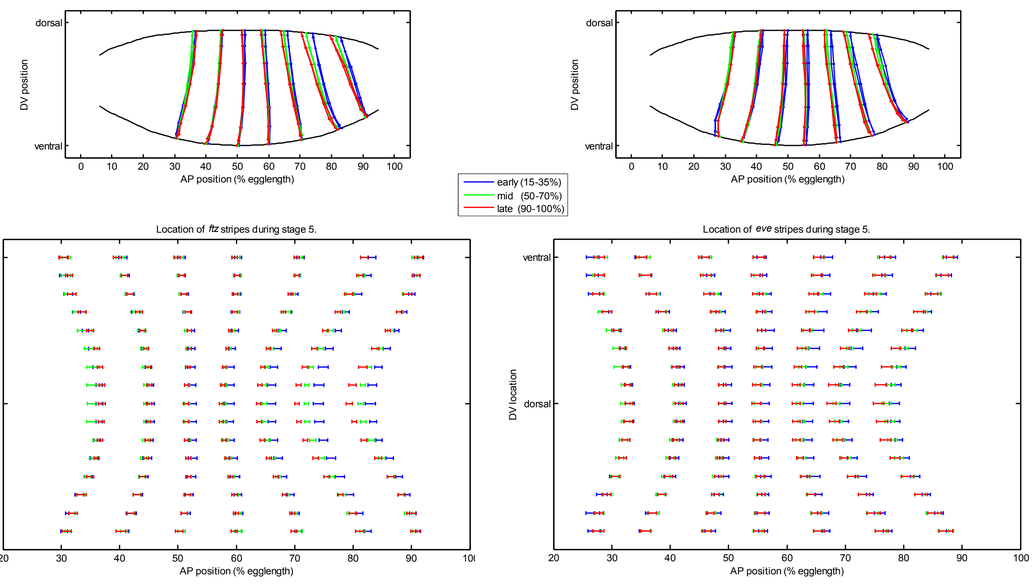


Measured *ftz* signal level

Estimated *ftz* expression

Movement of *ftz* and *eve* stripes

Fushi-tarazu and Even-skipped stripes move dorsally during stage 5, but not ventrally. We projected the location of these stripes on the AP axis and averaged the results over three time-cohorts: 15% to 35%, 50% to 70%, and 90% to 100%. These percentages indicate the extent of invagination of the cell membranes, which is a distinguishable morphological marker for subdividing stage 5.



Nuclear Density

We have found that nuclear density changes considerably during stage 5 (prior to gastrulation). In the mid-dorsal section density increases steadily, whereas in the anterior-ventral region it decreases. The colored plots on the bottom show these measurements for 16 AP stripes around the embryo. We will next explore the relationship between changes in gene expression stripe position and changes in cell location relative to the overall geometry of the embryo.