Experimental Determination of Intrinsic *Drosophila* Embryo Coordinates by Evolutionary Computation

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Abstract. Early fruit fly embryo development begins with the formation of a chemical blueprint that guides cellular movements and the development of organs and tissues. This blueprint sets the intrinsic spatial coordinates of the embryo. The coordinates are curvilinear from the start, becoming more curvilinear as cells start coherent movements several hours into development. This dynamic aspect of the curvature is an important characteristic of early embryogenesis: characterizing it is crucial for quantitative analysis and dynamic modeling of development. This presents a number of methodological problems for the elastic deformation of 3D and 4D data from confocal microscopy, to standardize images and follow temporal changes. The parameter searches for these deformations present hard optimization problems. Here we describe our evolutionary computation approaches to these problems. We outline some of the immediate applications of these techniques to crucial problems in *Drosophila* developmental biology.

1 Introduction

The completion of many genomic projects in the last decade has given rise to a new scientific objective, that of functional genomics - the next step towards the ultimate goal of a detailed understanding of how genome works [1].One of the critical questions in development is how the correct set of genes is expressed in each cell in order to form differentiated tissues. Research in *Drosophila* is reaching a stage where the expression of multiple genes can be followed dynamically in early embryogenesis at single cell resolution, in order to begin to understand the regulation underlying spatial patterning [2,3]. For instance, the BDTNP project [2] has currently mapped the expression of about 100 genes in each of about six thousand nuclei in early stage embryos; but these are initial steps of a very challenging project to trace as many related

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genes in individual development as possible, for as long and in as much detail as possible.

In *Drosophila*, the impressive experimental progress comes with unique data challenges. For instance, major challenges arise in mapping gene expression in early *Drosophila* development. The information comes from confocal microscopy scans [4], which present unique challenges for preprocessing, processing and analyzing sets and stacks of images. In this publication we will concentrate on computationally hard optimization problems in multidimensional confocal imaging of *Drosophila* embryos.

Data from large numbers of embryos must be combined to create data atlases from multiple genes and at multiple stages of development. Single embryos (fixed & stained) can be imaged for a few (usually three) segmentation genes. Therefore, data sets integrated from multiple embryos, stained for the variety of segmentation genes and over the entire patterning period, are necessary for gaining a complete picture of developmental dynamics. Images from individual embryos must be standardized to create such integrated data sets. Numerous sources of variability between images present challenges for data processing. These sources are both experimental and intrinsic to the biochemistry and biophysics of the developing embryos. Processing techniques which can separate experimental sources of variability allow for quantitation of the biological variability between embryos.

The standardization of multiple images is in essence a transformation of diverse sets of data into a single coordinate system; it is a general problem in medical and biological imaging. In *Drosophila*, major challenges arise from the different shapes and sizes of embryos, and the intrinsic curvilinearity of the chemical gradients specifying cell type. Intrinsic biological variability affects these factors, as do experimental treatments for data acquisition.

Standardization problems for *Drosophila* embryo images have been approached for 1D (gene expression profiles [5, 6, 7]), 2D (expression surfaces [8, 9]), and even 3D data [2, 10]. These approaches have involved elastic (or non-rigid) deformation of images to a single coordinate system [5, 6], which involve heavy use of computational resources. 3D views of the data are impressive and informative, but many statistical analyses and modeling projects are done in 1D or 2D; methods for reducing dimensionality are needed for data validation of such theoretical projects, and elastic deformation can also be used for this.

We have developed a type of elastic deformation for *Drosophila* analysis, following biometric coordinate transformations [5,6,11,12] first pioneered by D'Arcy Thompson [13], and used this for systematic studies of within- and between-embryo noise in 1D and 2D gene expression data [6, 14]. The approach has been adopted more recently by other teams [15, 16, 17].

In recent years, more and more laboratories are studying large sets of confocal images of early *Drosophila* embryos. Web bases include: FlyEx [18], which we have been involved with; the large-scale 3D BDTNP project (BID) [2]; and FlyFISH [19]. Similar datasets are under study in other labs [20,21,22,23,24]. All workers in this area face image processing challenges in extracting reliable information from confocal data. In this communication, we discuss the challenges presented in these types of datasets, present our approach to some of these fundamental problems, and report on new techniques we are developing, especially for application to new methods of data acquisition and to optimize processing.

2 Data and Nature of the Problems

In the first 4 hours of development, the major axes of the *Drosophila* embryo are established by gradients of gene expression products specifying particular cell fates in precise locations. The major, anterior-posterior (AP), axis is established by the segmentation network, a set of some 15-20 genes that establishes the striped patterns of gene expression which precede the anatomical appearance of the segmented body plan. This system has been intensively studied as a model for the functional genomics of spatial patterning [25, 26]. Figure 1 shows these striped ('pair-rule' gene) patterns. There are also chemical patterning gradients in the dorsal-ventral (DV) axis, orthogonal to the AP system. The intersection of these two systems establishes a coordinate system for the early embryo. Numerous cell types and structures have been shown to differentiate at particular intersection values of the AP and DV axes, for instance: the salivary glands, localized AP by a narrow band of *scr* gene expression and DV by the *dpp* gene [27]; neural cells differentiating at the intersection of achaete-scute gene patterns [28]; or structures developed at the intersection of *wg* and *sog* expression. These positions can be manipulated experimentally, such as by mutation. This intrinsic coordinate system is curvilinear, as seen by the bending of stripes in Fig. 1. The patterns become more curved with time. While patterning can be described in these intrinsic coordinates, standardization of images and subsequent analysis is aided by use of standardized coordinate systems, such as confocal elliptical or Cartesian. This communication presents techniques for transforming the embryo's intrinsic coordinates into a standard one.

2.1 Flattened vs. Intact Embryos

The quantitative data on segmentation genes are generally of two types, each presenting challenges to data analysis. These are 1) from confocal scans of flattened embryos, squeezed under a cover glass (Fig. 1A), and 2) from complete 3D scanning of physically intact embryos [29] (See Fig. 2). Gene expression datasets on flattened embryos are available on the FlyEx (protein) and FlyFISH (mRNA) web bases [30, 31]. (Data is more frequently taken in this way, and newer published data is also available from authors upon request.) 3D reconstructions of intact embryos are available on the BDTNP web base [32, 33].

These two approaches each have their advantages and disadvantages. Scanning of flattened embryos allows for a single focal plane, and is the most common, used in such databases as FlyEx. There are a number of methodological pitfalls with this approach, however, which must be addressed in the processing of such data. The chief problem is from the nearly arbitrary orientation of embryos under the cover glass. As an analogy, the problem is similar to placing a bunch of soft toy Rugby balls on a table and pressing them down with a sheet of glass. The lacing on the balls is analogous to the pair-rule stripes on the embryo. Not only will the laces curve as pressure is applied, different balls will have their laces oriented in different directions. This squeezing problem does not apply to intact embryo 3D reconstructions, so comparison of flattened 2D to intact 3D datasets first requires correction of the effects of the cover glass.

Datasets taken by each method offer different information - e.g. FlyEx has protein data and BDTNP has mRNA data – so it is desirable to be able to map between the flattened and intact data. In addition to correcting for the effects of flattening, this requires finding common landmarks (or ground control points), with the following challenges: 1) for flattened embryos we can observe slightly less than half of the nuclei (half of the cylindrical unwrapping; cf Fig. 1A & B); 2) if we superpose a flattened scan on the cylindrical projection of an intact embryo, the exact position of one image against another will be to some degree arbitrary.

An evident landmark in the 3D images is the dorsal axis of symmetry (see Fig. 1), where the stripes are closest to one another and locally perpendicular to the line of symmetry. The position of this line can be estimated in some 2D images (such as Fig. 1A), aiding alignment, but this is not a general property for all images. Adding to the alignment challenge is the curvature of the stripes. Part of this curvature is due to the intrinsic biological coordinates. But flattened images have an additional (and poorly controlled) curvature imposed from the experimental method.

2.2 Coordinate Transformation

Accounting for experimental effects on pattern and the steps to standardize the intrinsic curvilinear coordinates of embryos can be seen as problems in coordinate transformation. Correcting for experimentally-induced curvature (from embryo flattening) is a first step in data processing. Since intrinsic curvature varies between embryos, this too must be corrected to standardize multiple images. One approach to this standardization is to transform the curvilinear coordinates into a rectilinear Cartesian system. In one of the first works to investigate elastic coordinate transformations with respect to body plans, D'Arcy Thompson [13] made a classic deformation from a sunfish in curvilinear coordinates to a puffer fish in Cartesian coordinates. A similar transformation applies to the problem of standardization via stripe straightening in *Drosophila*. It took some 60 years after Thompson's graphical demonstrations for techniques to be formalized so that such transformations could be automated: in the 'bioorthogonal analysis' of Bookstein [34]; and in Siegel's [35, 36] technique for aligning and comparing homologous sets of landmark-coordinates. Morphometric coordinate transformations have expanded greatly in 30 years [37], for instance being applied in 2D structures such as insect wings [38]. We have developed a number of techniques in this area for application to *Drosophila* image processing [5, 6, 11, 12]. Stripe straightening is a major tool for standardizing images, which can be followed by registration of stripes for integrated data sets. Stripe-straightened data also provides dimensional reduction, producing data for verification of models and statistical analyses focused on 1D AP patterning. In addition, we have used the approach to standardize image intensity within and between images [7].

While stripe straightening focuses on the AP coordinate, there is also curvilinearity in the DV direction, especially for ventral positions. (For the intrinsic coordinates, it is known that DV morphogenetic gradients affect AP organization [see 39].) This two-dimensional curvilinearity is illustrated in the right hand images of Fig. 2. This secondary curvature can become a serious obstacle for automated data processing. Again, this may reduce to a coordinate transformation problem, if the intrinsic AP and DV curvature can be properly captured and transformed into a rectilinear system.

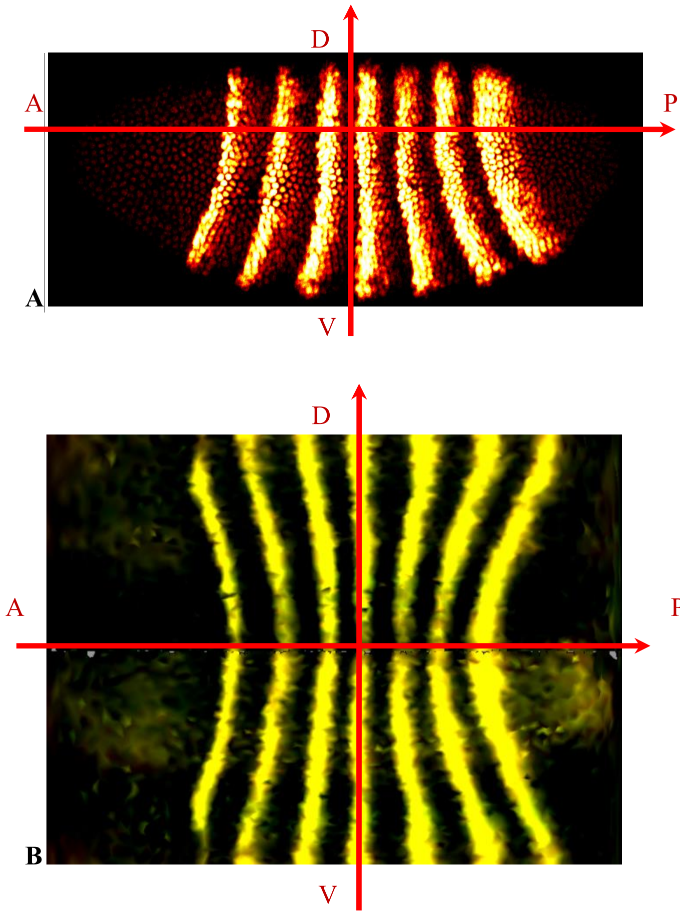

Fig. 1. The challenge of finding landmarks to juxtapose patterns from flattened and intact embryos. The two orthogonal axes of the striped pattern (red: y-axis along straightest stripe; vertical displacement of x-axis chosen to be most orthogonal to other stripes) tend to be invariant between the two approaches. (A) Image of flattened embryo with crescent-like stripes of expression of the pair-rule gene *eve*. (B) Unrolled (cylindrical projection) *eve* pattern for an intact embryo (3D reconstruction), with the same two orthogonal axes.

Intrinsic curvature also increases during development, especially as cells begin to move at the onset of the gastrulation stage. This change in geometry is important to study in its own right, as well as needing quantification for standardization of confocal data. The increasing curvature can be considered as an extension of the elastic deformation between Cartesian and curvilinear coordinates.

Computing such coordinate transformations is challenging: in addition to the wide range of intrinsic biological, experimental, and instrumental/observational sources of variability, there are no defined or standard reference solutions for such computations. Evaluating pattern coincidence between pairs of embryos at single cell resolution (at a

stage when embryos have ~6000 cells) can involve heavy, non-standard computation. Such problems can be well suited to evolutionary optimization; we have tested and developed a number of Genetic Algorithms (GA) approaches for this (see [5,6,11,12] & next section).

3 Techniques

Our coordinate transformations are based on optimization of polynomial maps between coordinate systems.

3.1 Stripe Straightening

The stripe straightening procedure is a transformation of the AP (x) coordinate by a polynomial of the form:

$$x' = Axy^2 + Bx^2y + Cxy^3 + Dx^2y^2 + \dots \qquad (1)$$

where $x = w - w^0$ and $y = -h - h^0$, and w and h are the initial spatial coordinates (AP and DV, respectively). The y-coordinate remains the same, while the x-coordinate is transformed as a function of both coordinates w and h (for details see [5, 6, 11, 12]). The exact form of (1) must be determined (more below), and the parameters w^0, h^0, A, B, C, D, etc. for each image must be found by an optimization technique. We tested a number of methods: GA; simplex; and a hybrid of these [5, 6, 11, 12]. We found GA to be the best for solving problems like (1) (especially for the multi-quadrant fitting discussed below). For GA optimization, we subdivide the image into a series of longitudinal strips. Each strip is subdivided into bins and the mean brightness (local fluorescence level) is calculated for each bin. Each row of means gives a profile of local brightness along each strip. A cost function is computed by pair-wise comparison of all profiles, summing the squared differences between bins. The task of the GA procedure is to minimize the cost function. The smaller the summed differences between strips, the closer the process is to the straightened endpoint. There is a possibility of over-straightening: this can be compensated by applying a penalty to any solution (parameter set) that moves more than one nucleus position past a predefined threshold (having a defined endpoint of straightened stripes helps here), though the penalty can influence search efficiency.

Intuitively, one can think of the straightening process as shrinking the image in such a way that the farther a given nucleus is from the dorsal edge and horizontal midpoint, the farther it must be moved towards the horizontal midpoint. More formally, we assume that the center of a pair-rule stripe follows a curve of constant AP position. The origin of the image coordinate system is at the top left, with image coordinates for width w increasing to the right and height h increasing down. To begin determining the final (straightened) AP and DV coordinates, x' and y' respectively, we note that there is an AP position (near mid-embryo) at which one stripe is vertical for its whole length. The center of this stripe defines x'=0 (the y'-axis). Each pair-rule stripe other than the one at x'=0 is curved; we vertically shift the x' axis so that it intersects each of the stripes at the point where it is vertical. Because of the vertical stripe: 1) the y- and y'-axes coincide; and 2) lines of y' = const are orthogonal

to the y- and y'-axes. The new coordinate system (x',y') has the same orientation and w0, h0 origin as the (x,y) system.

Analysis of the series has allowed us to eliminate all but three terms from the series [5, 6], so now we write an initial model of image transformation as

$$x' = x + Axy^2 + Bx^2y + Cx^3 \tag{2}$$

All of these terms have a clear interpretation. The xy^2 term is the main one: it represents the quadratic DV curvature that increases with distance from the x-axis. The x^2y term gives the residual DV asymmetry and the x^3 term gives the residual AP asymmetry. Finally, expressing (2) in terms of w and h, gives

$$x' = w - w^0 + A(w-w^0)(-h-h^0)^2 + B(w-w^0)^2(-h-h^0) + C(w-w^0)^3 \tag{3}$$

In tests with this initial model, however, we found that in more than half of the cases it was insufficient for straightening stripes. Therefore, we expanded the model empirically, adding 4th order terms.

For performance on confocal images, we found the best polynomial to be

$$A + Bxy + Cxy^2 + Dx^2y + Ex^2y^2 + Fx^3y + Gxy^3 \tag{4}$$

We can understand some these additional fourth order terms as follows: Cx^2y^2 is a correction term for parabolic bending, while Dxy^3 serves to correct DV asymmetry. In general, the situation is typical of a polynomial approximation problem, where one polynomial is best but many others are very good.

We have found some independence in the stripe curvature between head and tail ends of the embryo, perhaps reflecting differences in underlying patterning mechanisms. This affects the straightening process, and we have found improved fitting by independent elastic deformations on the head and tail halves of the image [5, 6]. Test computations indicate independent deformation on the four quadrants of the image may be best, to also account for DV dependencies in stripe curvature. A full optimization can operate, therefore, on 3 quadrants times 7 parameters in eq. (4) for a total of 28 parameters (plus an evaluation of values w^0, h^0).

With sufficient data on DV patterning (currently only available on the BDTNP Web base, [29]), we can also apply an elastic deformation to straighten in DV. We have applied DV straightening after AP straightening, and found a third order polynomial (Cf with (4)) gives good results:

$$x' = h - h^0 + A(h-h^0)(w-w^0)^2 + B(h-h^0)^2(w-w^0) + C(h-h^0)^3 \tag{5}$$

(in terms of w and h). The DV procedure is generally less precise than for the seven-striped AP patterns.

To summarize, stripe-straightening has a number of steps and challenges, including: finding the exact form of the deformation polynomial; finding efficient optimization algorithms for this task; limiting over-deformation; using multi-strip and multi-sector (i.e. quadrant) optimization; and the complicated and variable 3D

geometry, including the squeezing effects of flattened images, which can affect the efficiency of the evolutionary computations.

3.2 Implementation

We have developed a set of computational tools to process 2D data for about ~3,000 (flattened) or ~6,000 nuclei (intact embryo). The tool uses ASCII files for individual embryos in the format of the FlyEx Web base. We also developed a script to convert PointCloud data files from the BDTNP Web base. The main function is the GA search for parameters of the elastic deformation (stripe straightening). The software includes a C++ version for Windows, a Delphi version (Windows), and a Free Pascal version for Linux/Unix. For each input file the software produces two output files: one with the straightened data (in the input data format); and one with the polynomial coefficients for the deformation. The software is available from the authors upon request.

4 Biologically Significant Results and Discussion

The spatial patterns we have presented here are created by genetic regulation, the extremely complex and at best partially understood system of interactions between gene products (and other factors). A number of theoretical models have been developed for the AP patterning system to characterize these interactions. Many of these models are developed in 1D, so the dimensional reduction discussed above, with stripe-straightening, serves as an important tool for data processing to validate models. The quantification of variability arising from the coordinate transformation also sheds light on other biological questions. We present a few examples of the biological application of our work here.

A fundamental question in development is how spatial expression patterns can develop precisely and reliably, despite operating at low concentrations which are associated with high noise. Many investigators are working on quantifying this intrinsic biochemical noise, and studying how it is reduced in order to produce embryonic patterns of the required precision.

The natural variability in stripe curvature between embryos also reflects variability in developmental conditions. Two embryos of the same age class can show large qualitative differences in this respect. Fig. 2 shows the middle (fourth) eve stripe curving to the right in one embryo and to the left in another. Quantification of curvature via the stripe-straightening transformation can allow for a deeper investigation of these effects; for instance studying the correlation between stripe bending and embryonic geometry.

In addition to noise and variability in gene expression, there is significant variability in cellular order. This variability increases as the embryo becomes cellularized and begins the process of gastrulation. This variability is temporal (loss of synchrony) as well as spatial [40]. Progress on the 2D transformation techniques will be especially relevant for analyzing these phenomena.

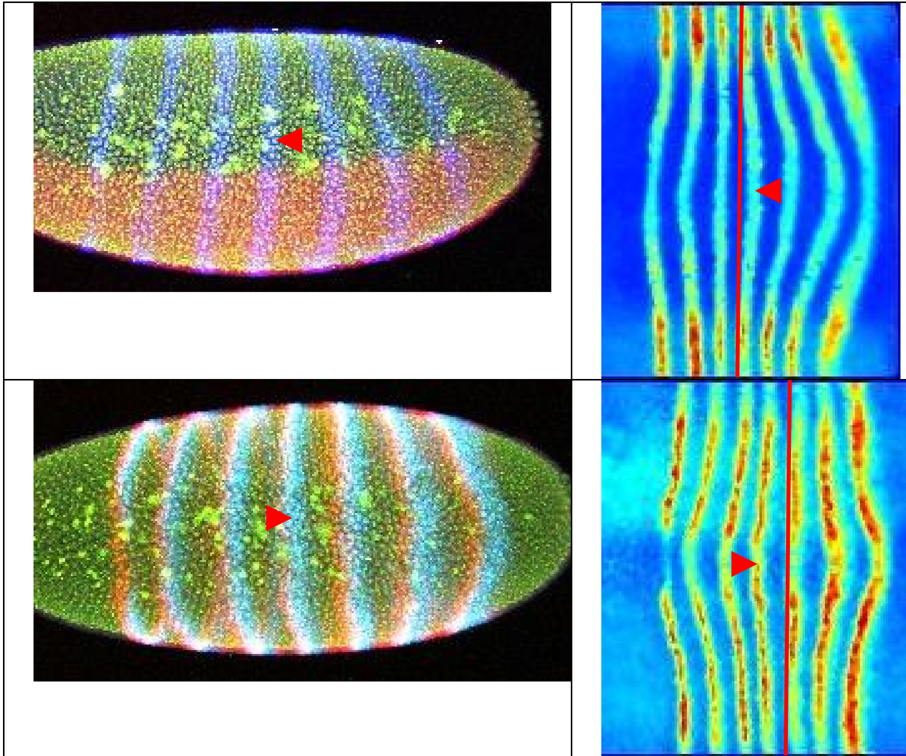

Fig. 2. Variability of intrinsic biological coordinates, as seen in *eve* patterns from two embryos. The fourth stripe (red arrows) can be curved to the left (head end of embryo) or to the right (tail end), red lines are drawn to show the stripe's curvature (BID BDTNP [29] embryos). I.e., the straight stripe forming the y-axis of the coordinate transformations can vary – here we see it at the 3rd stripe in one embryo and the 5th stripe in another.

Finally, the approach described with respect to Fig. 1, to transform between FlyEx and BID BDTNP types of data, allows for a much richer combined dataset: FlyEx contains chiefly protein data, while BID contains mRNA data. And while BDTNP has intact embryos, best for studying geometric effects, the flattened embryos have more accurate and sensitive detection of signal. The two approaches are complementary for many problems, and coordinate transformation between them can be an important tool for such investigations.

5 Challenges and New Developments

Our rotation & elastic deformation approach to 2D data: We are extending the approaches described above to use elastic deformation and rotation to fit 2D data of one embryo to another (flattened embryo data or cylindrical projections of intact embryos). A superposition of one embryo surface to another has several challenges.

Embryos differ in: spatial dimension (either in physical, micrometer, units, or in biological ones of nuclei numbers); nuclear density or total amount of nuclei; and in pattern features (a small but biologically significant factor). The procedure should be able to match embryos by patterns alone, or by patterns and nuclear positions together. There should also be freedom in choosing the spatial coordinates along which to optimize matching. Three operations should be able to match an embryo pair: horizontal and vertical shifting; rotation; and elastic deformation. These appear simple enough, but the high variability of embryo geometry and expression patterns makes the optimization tasks very hard. Some proportion of pairs will be very similar and matching gene patterns will give nearly perfect matches of nuclear positions. The larger proportion of pairs, however, even for coincident patterns, will not have coincident nuclei. This indicates deeper biological questions regarding the correlation between cell order and expression patterns, in addition to being a challenge to data processing.

6 Conclusions

Drosophila confocal image banks are not the only resources to which the approaches described here could be applied. Similar datasets exist for confocal scans of gene expression in other model organisms [1, 10]. We hope that the transformation techniques discussed here can also be applied to such cases.

Quantitative models of gene regulation are an integral part of understanding the mechanisms underlying functional genomics. *Drosophila* currently offers the highest resolution quantitative data available for validating models. This allows models to be tested on: the reduction of molecular noise during gene expression; the effects of cell movements and cell order on the developmental program; and the natural limits of reproducibility for gene expression patterns between embryos (as well as the effects of mutation on these limits). All of these efforts require the highest degree of quality from complex data sets. The techniques presented here have been developed to solve specific problems in the standardization and analysis of the biological data, so that such theoretical approaches can be tested, deepening the understanding of how genomes function in the development of tissues, organs, and individuals.

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References

1. De Boer, B.A., Ruijter, J.M., Voorbraak, F.P.J.M., Moorman, A.F.: More than a decade of developmental gene expression atlases: where are we now? Nucleic Acids Res. 37, 7349–7359 (2009)
2. Fowlkes, C.C., LuengoHendriks, C.L., Keränen, S.V.E., Weber, G.H., et al.: A Quantitative Spatio-temporal Atlas of Gene Expression in the Drosophila Blastoderm. Cell 133, 364–374 (2008)

3. Pereanu, W., Hartenstein, V.: Digital three-dimensional models of Drosophila development. Curr. Opin. Genet. Dev. 14, 382–391 (2004)
4. Pawley, J.B. (ed.): Handbook of Biological Confocal Microscopy, 3rd edn. Springer, Berlin (2006)
5. Spirov, A.V., Kazansky, A.B., Timakin, D.L., Reinitz, J., Kosman, D.: Reconstruction of the dynamics of the Drosophila genes from sets of images sharing a common pattern. Special Issue on Imaging In Bioinformatics. Journal of Real-Time Imaging 8, 507–518 (2002)
6. Spirov, A.V., Holloway, D.: Evolutionary techniques for image processing to construct integrated dataset of early Drosophila embryo genes activity. EURASIP Journal on Applied Signal Processing 8, 824–833 (2003)
7. Surkova, S., Kosman, D., Kozlov, K., Manu, M.E., Samsonova, A.A., Spirov, A., Vanario-Alonso, C.E., Samsonova, M., Reinitz, J.: Characterization of the Drosophila segment determination morphome. Dev. Biol. 313, 844–862 (2008)
8. Kozlov, K., Myasnikova, E., Pisarev, A., Samsonova, M., Reinitz, J.: A method for two-dimensional registration and construction of the two-dimensional atlas of gene expression patterns in situ. Silico Biol. 2, 125–141 (2002)
9. Sorzano, C.O.S., Blagov, M., Thevenaz, P., Myasnikova, E., Samsonova, M., Unser, M.: Algorithm for Spline-Based Elastic Registration in Application to Confocal Images of Gene Expression. Pattern Recognition and Image Analysis 16, 93–96 (2006)
10. Preibisch, S.W., Saalfeld, S., Schindelin, J., Tomancak, P.: Software for bead-based registration of selective plane illumination microscopy data. Nat. Methods 7, 418–419 (2010)
11. Spirov, A.V., Timakin, D.L., Reinitz, J., Kosman, D.: Using of Evolutionary Computations in Image Processing for Quantitative Atlas of Drosophila Genes Expression. In: Proceedings of Third European Workshop on Evolutionary Computation In Image Analysis and Signal Processing, Milan, pp. 374–383 (2001)
12. Spirov, A.V., Timakin, D.L., Reinitz, J., Kosman, D.: Experimental Determination of Drosophila Embryonic Coordinates by Genetic Algorithms, the Simplex Method, and Their Hybrid. In: Oates, M.J., Lanzi, P.L., Li, Y., Cagnoni, S., Corne, D.W., Fogarty, T.C., Poli, R., Smith, G.D. (eds.) EvoIASP 2000, EvoWorkshops 2000, EvoFlight 2000, EvoSCONDI 2000, EvoSTIM 2000, EvoTEL 2000, and EvoROB/EvoRobot 2000. LNCS, vol. 1803, pp. 97–106. Springer, Heidelberg (2000)
13. Thompson, D.W.: On Growth and Form. Dover reprint of 2nd edn., 1942, 1st edn., 1917) (1992)
14. Holloway, D.M., Harrison, L.G., Kosman, D., Vanario-Alonso, C.E., Spirov, A.V.: Analysis of pattern precision shows that Drosophila segmentation develops substantial independence from gradients of maternal gene products. Dev. Dyn. 235, 2949–2960 (2006)
15. Zamparo, L., Perkins, T.J.: Statistical lower bounds on protein copy number from fluorescence expression images. Bioinformatics 25, 2670–2676 (2009)
16. Myasnikova, E., Samsonova, M., Kosman, D., Reinitz, J.: Removal of background signal from in situ data on the expression of segmentation genes in Drosophila. Dev. Genes Evol. 215, 320–326 (2005)
17. Surkova, S., Myasnikova, E., Janssens, H., et al.: Pipeline for acquisition of quantitative data on segmentation gene expression from confocal images. Fly 2, 58–66 (2008)
18. Poustelnikova, E., Pisarev, A., Blagov, M., Samsonova, M., Reinitz, J.: A database for management of gene expression data in situ. Bioinformatics 20, 2212–2221 (2004)
19. [ ]Lecuyer, E., Yoshida, H., Parthasarathy, N., Alm, C.et al: Global analysis of mRNA localization reveals a prominent role in organizing cellular architecture and function. Cell 131 (2007) 174-187

20. Zinzen, R.P., Senger, K., Levine, M., Papatsenko, D.: Computational models for neurogenic gene expression in the Drosophila embryo. Current Biology 16, 1358–1365 (2006)
21. Gregor, T., Bialek, W., de Ruyter van Steveninck, R.R., Tank, D.W., Wieschaus, E.F.: Diffusion and scaling during early embryonic pattern formation. Proc. Natl. Acad. Sci (USA) 102, 18403–18407 (2005)
22. He, F., Wen, Y., Deng, J., Lin, X., Lu, L.J., Jiao, R., Ma, J.: Probing intrinsic properties of a robust morphogen gradient in Drosophila. Dev. Cell 15, 558–567 (2008)
23. Crauk, O., Dostatni, N.: Bicoid determines sharp and precise target gene expression in the Drosophila embryo. Current Biol. 15, 1888–1898 (2005)
24. Arvey, A., Hermann, A., Hsia, C.C., Ie, E., Freund, Y., McGinnis, W.: Minimizing off-target signals in RNA fluorescent in situ hybridization. Nucleic Acids Res. (2010), doi:10.1093/nar/gkq042
25. Akam, M.: The molecular basis for metameric pattern in the Drosophila embryo. Development 101, 1–22 (1987)
26. Lawrence, P.A.: The making of a fly. London, 228 p. Blackwell Scientific (1992)
27. Panzer, S., Weigel, D., Beckendorf, S.K.: Organogenesis in Drosophila melanogaster: embryonic salivary gland determination is controlled by homeotic and dorsoventral patterning genes. Development 114, 49–57 (1992)
28. Skeath, J.B., Panganiban, G.F., Carroll, S.B.: The ventral nervous system defective gene controls proneural gene expression at two distinct steps during neuroblast formation in Drosophila. Development 120, 1517–1524 (1994)
29. `http://bdtnp.lbl.gov/Fly-Net/bioimaging.jsp`
30. `http://urchin.spbcas.ru/flyex/`
31. `http://fly-fish.ccbr.utoronto.ca`
32. Preibisch, S.W., Saalfeld, S., Rohlfing, T., Tomancák, P.: Bead-based mosaicing of single plane illumination microscopy images using geometric local descriptor matching. In: SPIE Medical Imaging Conference, Lake Buena Vista, Fla, pp. 1–10 (2009)
33. LuengoHendriks, C.L.: KeränenS.V.E., Fowlkes, C.C., Simirenko, L.et al.: Three-dimensional morphology and gene expression in the Drosophilablastoderm at cellular resolution I: data acquisition pipeline. Genome Biology 7, R123 (2006), doi:10.1186/gb-2006-7-12-r123
34. Bookstein, F.L.: Principal Warps: Thin-Plate Splines and Decomposition of Deformations. IEEE Trans. Pattern Analysis and Machine Intelligence 11, 567–585 (1989)
35. Siegel, A.F.: Geometric data analysis: An interactive graphics program for shape comparison. In: Launer, R.L., Siegel, A.F. (eds.) Modern Data Analysis, pp. 103–122. Academic Press (1981)
36. Siegel, A.F., Benson, R.H.: A robust comparison of biological shapes. Biometrics 38, 341–350 (1982)
37. Bookstein, F.L.: Morphometric Tools for Landmark Data: Geometry and Biology, p. 435. Cambridge U. Press, Cambridge (1991)
38. Abbasi, R., Mashhadihan, M., Abbasi, M., Kiabi, B.: Geometric morphometric study of populations of the social wasp, Polistesdominulus (Christ, 1791) from Zanjan province, north-west Iran. New Zealand Journal of Zoology 36, 41–46 (2009)
39. Keränen, S.V.E., Fowlkes, C.C., LuengoHendriks, C.L., Sudar, D., Knowles, D.W., Malik, J., Biggin, M.D.: Three-dimensional morphology and gene expression in the Drosophilablastoderm at cellular resolution II: dynamics. Genome Biology 7, R124 (2006), doi:10.1186/gb-2006-7-12-r124
40. Zallen, J.A., Zallen, R.: Cell-pattern disordering during convergent extension in Drosophila. Journal of Physics: Condensed Matter 16, S5073–S5080 (2004)