IMAGING AND VISUALIZATION

A map for fly explorers

Virtual composite images generate a map of gene expression in *Drosophila* embryos.

The mapmakers of this century work more often than not at the molecular scale. Unlike the topography of the earth, the peaks and valleys in gene expression during animal development still remain uncharted.

At the Berkeley Drosophila Transcription Network project, the goal is to understand transcriptional regulation in the fruit fly. Array-based studies can map transcription factor binding to the genome, but in multicellular organisms gene regulation may vary from one cell to the next, and array-based studies do not capture this complexity.

"What you want is to look at the interactions of many genes at once," explains Charless Fowlkes of the University of California at Irvine. "If we could stain for a thousand different genes with a thousand different colors, then we could observe the output of transcriptional regulation in each cell. But that's not possible with present techniques." Therefore, to understand spatial patterning of transcription, it is necessary to image genes separately, in thousands of embryos, and then put the data together in a 'virtual embryo'. Fowlkes and colleagues, in work done in the laboratories of Jitendra Malik, Michael Eisen and Mark Biggin at the University of California at Berkeley, now report the results of these efforts (Fowlkes *et al.*, 2008).

The researchers imaged mRNA in fixed embryos by two-photon microscopy, in six temporal cohorts, over the 50-minute period before gastrulation. Not only is this stage practical to image and model, as no cell divisions occur, it is also an important developmental window. During this period, a set of key transcription factors turns on thousands of downstream target genes, which are involved in specifying cell fate and laying down the body plan of the organism.

They generated six morphological templates, one for each time cohort and then automatically aligned the expression pattern of a given gene to the relevant template. In a coarse alignment, they scaled all embryos to be the same overall length and expressed the location of a gene's expression as a percentage of that length. However, this did not eliminate all variability, so they did an additional fine registration, using as markers the boundaries of expression of *ftz* or *eve*. "What we do is to allow a non-rigid deformation of the embryo," says Fowlkes. "We don't allow an arbitrary motion; it's not as if it's a liquid, but it's treated as a soft material that you can warp a little, and we can control how soft it is mathematically, in the algorithm."

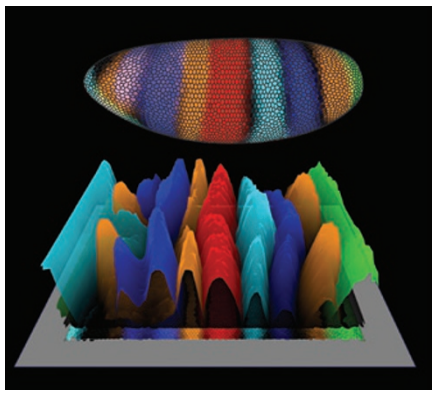

Figure 1 | Visualizing gene expression in the VirtualEmbryo. A 3D model of the blastoderm surface (top) and cylindrical projection (bottom). Reprinted with permission from Elsevier.

The scientists found that for fine-registered embryos the variability in the expression signals of two genes was as low as the variability within a single embryo co-stained for the same two genes. As Fowlkes puts it, "We think we've got rid of the geometrical sources of variability. And it's interesting because when we started out we didn't know if it would even make sense to try and put all [these] data into one common template."

It is clear from this work, however, that virtual multiplexing is indeed possible for the fly embryo. The composite of these results yields the so-called VirtualEmbryo (**Fig. 1**), which in the present work describes the average expression pattern for 95 genes over time. Fowlkes and colleagues then used this spatiotemporal information to help predict which transcription factors regulate which target genes. Using a relatively simple model, they captured many known regulatory interactions and predicted several new ones.

As this work proceeds, the VirtualEmbryo as well as the raw data for each gene, referred to as the gene expression atlas, are available for those who may want to chart their own course.

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RESEARCH PAPERS

Fowlkes, C.C. *et al.* A quantitative spatiotemporal atlas of gene expression in the *Drosophila* blastoderm. *Cell* **133**, 364–374 (2008).