QUANTITATIVE 3D IMAGING TO ANALYZE DEVELOPMENTAL VARIABILITY IN PREGASTRULA *DROSOPHILA* EMBRYOS

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ABSTRACT

The first 14 mitotic cycles in *Drosophila* result in a single layer of nuclei surrounding a yolk sac in the pregastrula embryo. Despite this morphological simplicity, the transcriptional network controlling pregastrula development is complex and little is know about what aberrations occur or the responses they evoke. To understand the role of biological variability during development, we are producing novel optical imaging techniques to map the relative position and gene expression of every nucleus within the embryo. Total DNA and specific gene products are imaged in 3D using confocal fluorescence microscopy. Innovative algorithms allow automatic segmentation of the DNA-image and produce an enumerated mask defining individual nuclear boundaries. The developmental stage of each embryo is classed by mitotic division number and determined from the total nuclei number; then the morphological mask is used to quantify gene-product on a per nuclei basis. What results is a map of pregastrular development showing the variability of nuclear number, relative nuclear packing density, and the expression pattern of specific gene products. This work illustrates the power of quantitative optical imaging and is an initial step towards uncovering the rules determining how patterns of gene expression are generated.

INTRODUCTION

The morphology and development of an animal is mediated by transcription factors that bind to and regulate the differential expression of genes. Usually several factors cooperate to activate any specific gene and the abundance of transcription factors themselves is determined by the expression of other genes. As a consequence, transcription factors form a complicated spatial and temporal network of interaction as they orchestrate the development of an organism. The aim of this work is to create a new imaging and image analysis technology that will enable the study of entire transcriptional networks. The work is in response to the need for novel approaches to understand how complex patterns of gene expression arise. We have focused on early *Drosophila* embryogenesis because the genomic sequence of *Drosophila melanogaster* is known [Adams], an entire *Drosophila* embryo can be imaged with single cell resolution and the early *Drosophila* transcriptional network is controlled by a relatively small number of between 80 and 100 sequence specific factors, most of which have been well characterized.

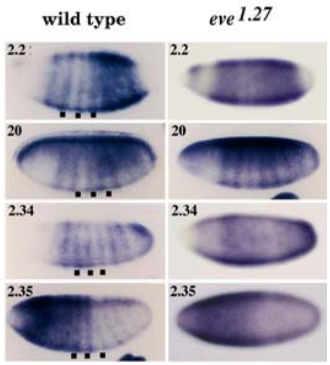

Figure 1

The mRNA expression pattern of 4 randomly selected genes, from clones 2.2, 20, 2.34 and 2.35, just prior to gastrulation in wild type and *eve* minus embryos [Liang]. Such patterns emphasize the necessity of quantifying the spatial distribution of gene expression.

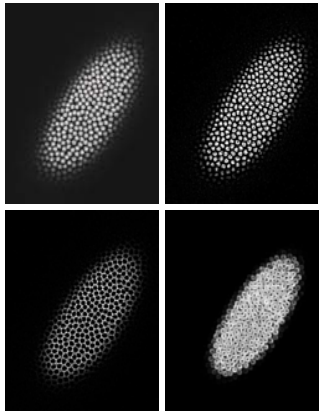

Figure 2

To calculate the position and extent of individual nuclear volumes, a local threshold algorithm analyzes the DNA-stain image (Top Left). The algorithm determines areas in the gray scale image which are brighter than their surrounds (Top Right), darker than their surrounds (Bottom Left), and calculates the local standard deviation (Bottom Right). In each case a single slice from a 3D image is shown.

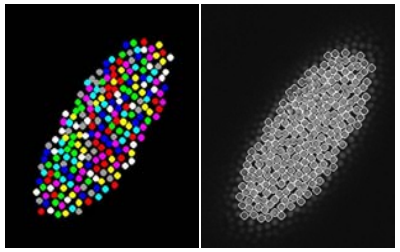

Figure 3

Following the local brightness threshold, a template matching algorithm stamps a non-overlapping, enumerated template of approximate nuclear volume centered on the nuclei. The accuracy of the segmentation is demonstrated here by overlaying an outline of the segmentation mask over the original grey level image (right). In each case only a single slice from a 3D image is shown.

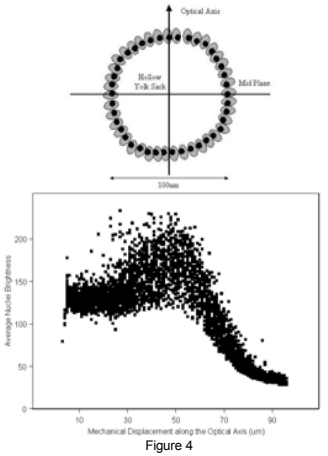

Figure 4

In pregastrula *Drosophila* the first 14 mitotic divisions result in a single layer of nuclei surrounding a yolk sack. The upper cartoon is a cross section through an embryo and demonstrates its geometry relative to the optical axis of the microscope. The lower graph is a plot of the average brightness of every nucleus within an embryo stained for DNA, plotted against the average position of the nuclei along the optical axis.

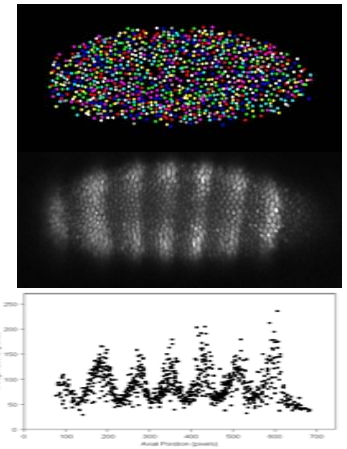

Figure 5

The segmentation mask (top) and corresponding gene-product image of Texas-Red stained EVE protein (middle) are combined to calculate the relative amount of EVE expression per nuclei within an entire embryo. The relative amount of EVE expression from this image is plotted against the average relative anterior/posterior position of the nuclei within the embryo (bottom).

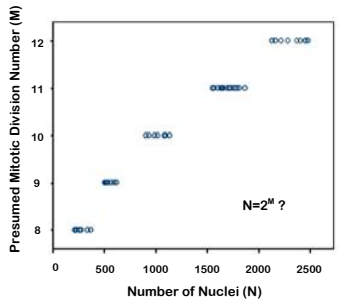

Figure 6

For this plot, images of 55 embryo at various growth stages were segmented and analyzed for total nuclear number. The results showed that individual embryo could be grouped according to total nuclear number. Thus, we have plotted the total number of nuclei (N) (abscissa), from the 55 embryos, against the presumed mitotic division number (M) (ordinate). M was calculated from the relation $N=2^M$ and by assuming that N follows this power law for at least the first 8 mitotic divisions. The segmentation accuracy was checked by eye by overlaying the segmentation mask with the grey scale image (see: Fig.3). For enumeration purposes, the accuracy was nearly 100%.

Conclusions:

- We are developing novel quantitative optical imaging techniques to map gene expression levels at cellular and sub-cellular resolution within an entire organism.
- Pregastrula *Drosophila* embryos have been chosen because they allow high resolution 3D optical imaging, and the components of the transcription network controlling their gene expression are well characterized.
- Our technique allows the position, extent and the total number of nuclei within an embryo to be determined with great accuracy.
- The total number of nuclei (N) indicates the mitotic division cycle of the embryo, even though variation in N was evident at each mitotic cycle.
- The map of nuclear position and extent allows quantification of gene expression at cellular resolution.
- Our goal is to define the transcriptional network of pregastrula Drosophila.

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Acknowledgements:

This work has been supported by the Director, Office of Energy Research, Office of Health and Environmental Research of the U.S. Department of Energy under contract number DE-AC03-76SF00098, and NIH-R01 grant GM42397