



Spatial Gene Expression Quantification in Changing Morphologies

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Summary

The purpose of this thesis, entitled “Spatial gene expression quantification in changing morphologies”, is threefold. First, a spatial gene expression quantification method for the starlet sea anemone *Nematostella vectensis* is described. Second, new insights are provided by applying this method on collected gene expression microscopy images and analyzing the resulting numerical profiles. Third, the feasibility of quantifying spatial gene expression for other animals is discussed.

Our method for quantifying *N. vectensis* gene expression pictures consists of three basic steps. The first step is a time series of graphical embryo outlines derived from microscopy pictures of *N. vectensis* embryos. The changing shape is visualized in detail by fluorescent markers attached to the cell membranes. These embryo micrographs are converted into digital templates. In the second step, the cell layer is extracted from images that display the spatial distribution of gene products. If the gene expression pattern is cylindrically symmetric about the main body axis, then a graphical outline is adjusted to the observed cell layer outline. Otherwise a geometric transformation is performed before the cell layer is indicated. After the third step, a one- or two-dimensional numerical profile is obtained. The software program divides the cell layer into segments and plots the average color values of the pixels in each segment. Regions where the selected color profile visibly deviates from the actual expression pattern are manually indicated and corrected; the result is a 1D gene expression profile. For transformed images, the color profiles are measured for multiple cross sections and collected into a 2D profile.

Additional knowledge has been obtained from studying a set of genes involved in gut formation and from another study of all genes in a database for *N. vectensis* gene expression. Spatial gene expression images of gut formation genes have been collected from publications in scientific journals. The 1D numerical profiles extracted from these expression images have been systematically compared to classify the genes based on similarity of their spatial expression patterns. Four genes with dissimilar patterns have been selected for computational simulations. The parameter estimations of a mathematical gene interaction model resulted in a preliminary gene regulation network for *N. vectensis* gut formation.

One-dimensional numerical profiles have been extracted from all suitable *N. vectensis* images in the Kahi Kai gene expression database. These profiles have been compared separately for each distinct stage of development. The genes were found to be clustered in groups that display expression in different spatial domains. A statistical analysis showed that the domain clusters are persistent during early development. This result suggests that very little mixing occurs between cells and that a localized tissue region in a later stage can be mapped to a region in an earlier stage.

To quantify gene expression in other animals, high-resolution images are required to generate accurate shape templates. Moreover, spatial gene expression images are needed to produce numerical expression profiles for comparison among various genes and for input data in computational simulations.

Microscopy techniques to obtain both types of images are already applied to many model animals.

Therefore, gene expression quantification is expected to be used with other animals besides *Nematostella* and to increase our general understanding of regulatory interactions.