

A Field Approach to Spatial Gene Expression in Zebrafish Heart Development

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ABSTRACT

We describe an approach to the analysis of spatial gene expression based on the estimation of gradient fields and their force lines as possible indications of molecular and cellular flow. Zebrafish have been used extensively in recent years in order to study heart formation due to its transparent embryo. The heart is the first organ that forms and functions in a vertebrate animal [1]. Besides the intrinsic importance of studying the development of the heart in order to gain insights on congenital diseases, it also provides the first signs of asymmetry in an anatomical part, due to the so-called “heart looping”.

The original data used was obtained from a 35 hour post-fertilization wild-type zebrafish embryo that expresses a Green Fluorescent Protein (GFP) marker specific for heart mesoderm myosin light chain 2 [2]. A stack of 324 images of 512x512 pixels in grayscale (8-bit) format was generated using a Multiphoton Confocal Microscope Radiance 2100 (Biorad). The magnification used was 20x. All slices were combined in order to form a three-dimensional volume, which is considered as a scalar field $w = g(x,y,z)$, where w is the intensity value at the position (x,y,z) . It is interesting to note that this volume can be directly visualized with direct volume rendering (DVR) algorithms.

The first step is to estimate the gradient field induced by such scalar field, which has as an important property in that it always points towards the direction of maximum increase of a function. The gradient of the gene expression field is given as follows:

$$\vec{\nabla} g(x, y, z) = \left(\frac{\partial g}{\partial x}(x, y, z), \frac{\partial g}{\partial y}(x, y, z), \frac{\partial g}{\partial z}(x, y, z) \right)$$

The partial derivatives $\partial g/\partial x$, $\partial g/\partial y$ and $\partial g/\partial z$ were computed using a property of Fourier transform known as derivative theorem [3,4]. Gaussian smoothing was applied to the data in order to minimize spatial quantization noise caused by digital image representations [4].

After the gradient has been computed for every point, it is interesting to determine the paths that a particle would go through starting from any point inside the volume and

moving along the gradient direction, i.e. the lines of force induced by the gradient vector field. In order to compute such paths, we use a standard finite differences approach. Figure 1 shows some of the obtained lines of force, starting from points uniformly distributed through a sphere centered at the heart, and tending to stop at local maxima of the scalar gene expression field. Such lines of force indicate the possible trajectories of molecules or cells moving in the neighborhood of the heart, involved in its formation. We are currently quantifying the concentration and distribution of such lines of force, which will be compared with other developmental stages.

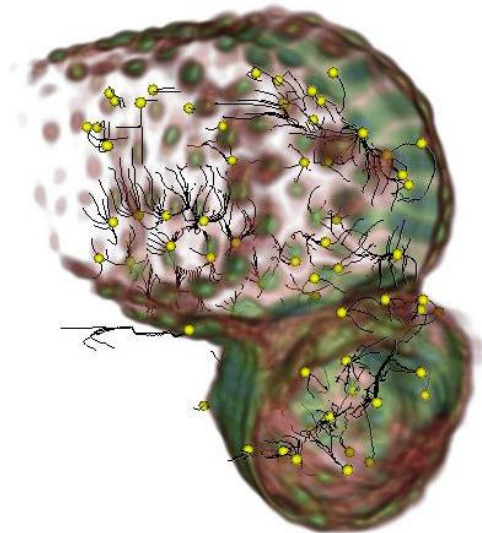


Figure 1 – The lines of force obtained for heart formation considering the expression of heart myosin light chain 2 with a GFP marker.

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