LABORATORY OF BIOLOGICAL DIVERSITY

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Organogenesis is accomplished by a series of deformations of the planar cell sheet into a three-dimensional shape during embryogenesis. This drastic structural change is an integrated result of individual cell behaviors in response to spatio-temporally controlled mechanisms.

To better understand the programs underlying organ formation, it is required to analyze individual cells' morphology and dynamics quantitatively. However, due to the massive images generated by 4D microscopy and their ambiguity, this made it difficult to perform these analyses.

To unveil organogenesis from the point of view of distinct cell behaviors, we are developing application software that is capable of describing cell dynamics out of 4D time-lapse imaging data sets by employing image processing techniques.

I. 4D cell segmentation/tracking system

Epithelial morphogenesis in the developing embryo is considered to be an essential model for collective cell migrations. Drastic cell rearrangements lead drastic structural changes to build elaborate organs such as the tubular network of *Drosophila* trachea. We are developing a software pipeline, which automatically recognizes individual cell shapes out of 3D space and tracks them through time. This system extracts cell boundaries and reconstitutes cell shapes as a skeletonized chain of voxels spanning 3D space. This abstract form of cell visualization makes it possible to describe morphometric quantities and kinetics of cells in single-cell resolution (Figure 1). These morphometric quantities allow us to perform comparative studies on shapes and behaviors precisely among several experimental conditions, to gain a better understanding of the genetic programs underlying organogenesis. We are now applying this system to several experimental models to determine the practicality of the system.

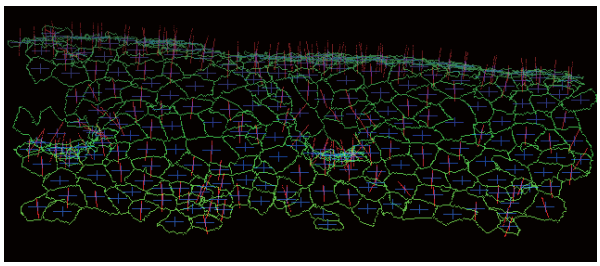

Figure 1. Visualized apical cell surface of *Drosophila* embryonic epidermal cells. A time-lapse confocal microscopic data set of a fly embryo expressing E-cadherin-GFP was subjected to our automatic cell shape recognition system. Cell boundaries (green), center of gravity (blue) and normal vector (magenta) are indicated for each cell.

II. Local image feature tracking for tissue deformation analysis

Besides cell boundary extraction, we also developed a derived algorithm for particle image velocimetry (PIV). This system is designed to measure tissue deformation even though the imaging constraints do not allow identification of individual cells out of images. This implementation detects structural characteristics, such as uneven fluorescence distributed over the specimen and tracks these patterns along a time-series. Despite that the tissue was labeled with non-targeted cytoplasmic GFP, this tracking software successfully outlined developmental dynamics of *Xenopus* neuroectoderm (Figure 2).

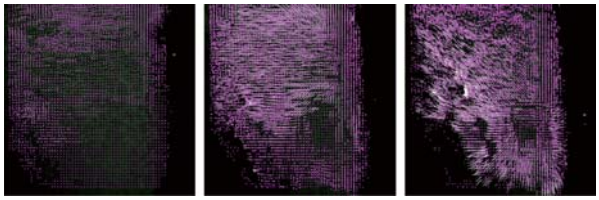

Figure 2. Collective cell migration of *Xenopus* neuroectodermal cells visualized as optical flow along a time-series. A modified PIV method successfully tracks uneven subcellular distribution of GFP signals over time. Dr. M. Suzuki (Prof. Ueno's laboratory at NIBB) performed the microscopy.

III. Software for manual image quantification

Biologically significant image features are not always significant to computational algorithms due to their structural instability. This kind of difficulty requires human eye inspection for feature extractions. A GUI (Graphical User Interface) application we developed can easily visualize 4D imaging data and has made manual feature annotations easy (Figure 3). This application is freely available at our website (https://is.cnsi.nins.jp/).

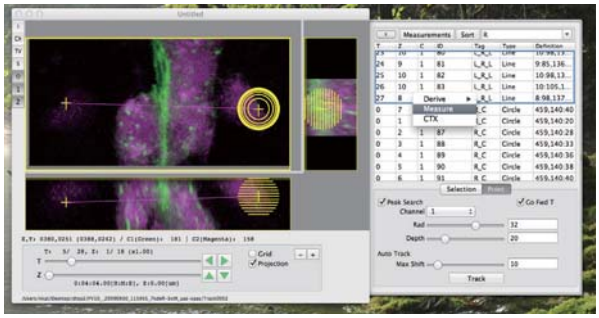

Figure 3. A lightweight/native 4D stack viewer equipped with functions for manual feature extraction.