3D Mapping of Zebrafish Heart Development

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Abstract

Congenital heart defects are the leading birth defects worldwide, accounting for 1-3% of live births. One field of interest in studying congenital heart disease is the development of the heart. However, this organ is highly complex and requires three-dimensional imaging to model its morphological structure. In this project, three-dimensional time-lapse confocal data was obtained from zebrafish embryos, imaged over multiple time points in order to track the development of the heart. Using a cell imaging software we analyzed the 4D data acquired with confocal microscopy, identifying individual cells and then tracking their movement within the two layers of the heart over time. In all, around 80 cells were mapped: 10 cells per quadrant per layer of the cardiovascular model. This data was then generated into videos in order to show the movement of the cells in three-dimensional space. This project demonstrates that overall tissue changes are driven by the complexity of individual cellular changes, as each cell displayed unpredictable behavior which required intuition in order to interpret. Furthermore, this project may lead to future research on the application of machine learning models to speed up this process and analyze a larger amount of datasets.

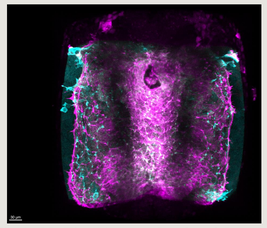

Fig 1: 3D Time Lapse Confocal Data of Ventral (Cyan) and Dorsal (Magenta) cells

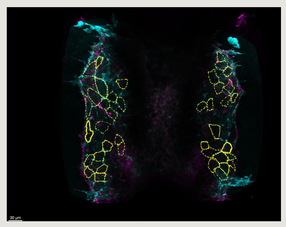

Fig 2: Non-Orthogonal Cellular Segmentation of 3D Time Lapse

Objective

Analyze complex biological tissues using:
I. 3D time lapse confocal fluorescence microscopy data with
II. Non-orthogonal dynamic, structurally guided segmentation

LPM representation (20 hpf)

Background

In early cardiovascular development, the heart progenitors are made up of two cell layers, with the dorsal on top and the ventral on the bottom (*Fig.1*). As the heart develops, each cell undergoes morphogenesis towards the midline. 3D fluorescence microscopy imaging of the heart is a method to better understand the migration of dorsal and ventral layer cells in cardiovascular development. Each cell within the dorsal and ventral layers characterized by actin protein cables along the membrane (*Fig 3*). However, there are several challenges in data analysis due to the complexity of this biological tissue:

1. ***Complex experiment*:** obtaining the data requires immense effort to prepare zebrafish samples and image the zebrafish properly over every time point
2. ***Complexity of dynamics*:** the model features multiple cells moving in 3D space over time
3. ***Complexity of geometry*:** the model is a non-orthogonal, multi-layered image whose shape changes over time (*Fig. 4,5*)
4. ***Complex analysis*:** the analysis of the data requires intuition to interpret the biological images

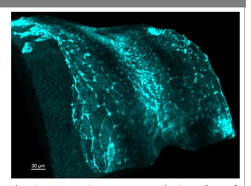

Fig 3: Non-Segmented Confocal Data with Cells bounded by Actin Protein Cables

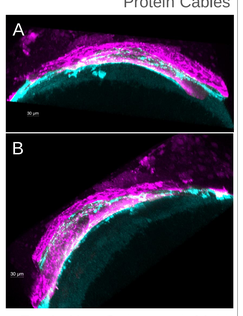

Fig 4 : Non-Orthogonal, Multi-Layered Image with Dorsal (Magenta) and Ventral (Cyan) Layer (a) lateral view (b) diagonal view

Methods

The dataset was acquired using confocal time lapse volumetric imaging of live zebrafish embryos, with multi-channel detection over a series of 169 time points acquired over 7 hours of development. Imaris v.10.0.1 was utilized to analyze the confocal data. We first segmented and then tracked individual cells, discerning between dorsal and ventral layers of the heart.

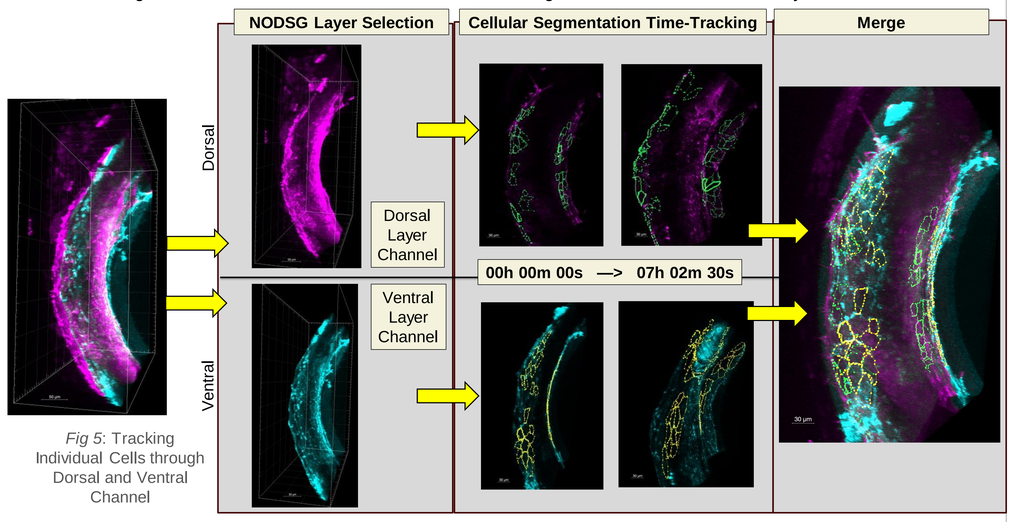

Fig 5: Tracking Individual Cells through Dorsal and Ventral Channel

Results

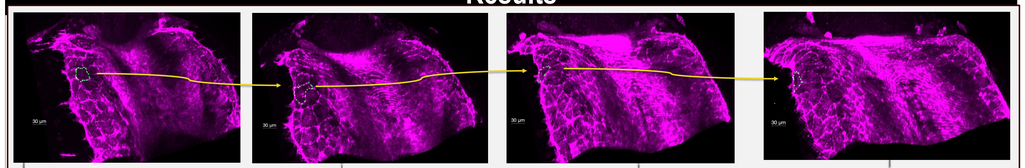

Fig 6: Tracking a Single Dorsal Cell

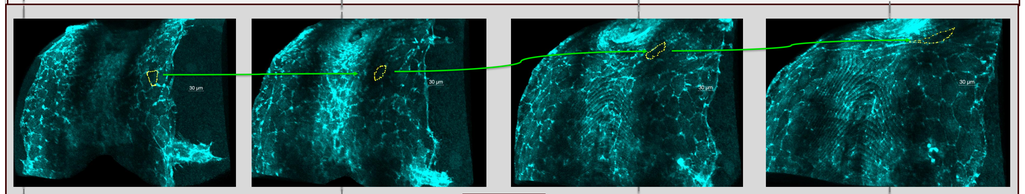

Fig 7: Tracking a Single Ventral Cell

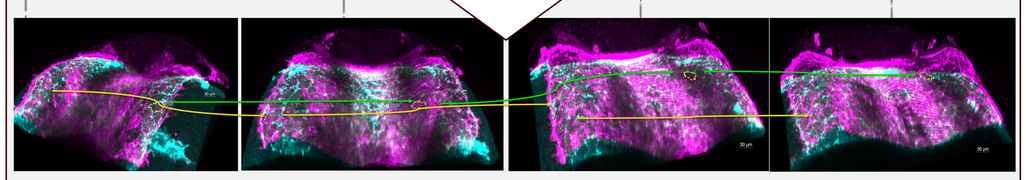

Fig 8: Merged Tracking for two cells: one Dorsal (green border, yellow arrow) and one Ventral (yellow border, green arrow)

Summary

- At several points, individual cells would divide, and the daughter cells would migrate in different directions towards the midline.
- Dorsal and ventral cells behave in different ways: within this sample, the dorsal cells migrate outwards, while ventral cells generally migrate upwards.
- Analysis of confocal fluorescent microscopy data is challenging and requires human intuition to interpret individual cellular behavior.
- Interpreting the location of each cell in three-dimensional space at each time point necessitates viewing the cell from several angles to determine which layer the cell lies on.
- Cardiovascular tissue changes are driven by the complexity of each individual cellular change.
- Individual movements of the cells spurred the overall geometric change in the non-orthogonal, multi-layered model. Figures 11 and 12 show the initial and final shape of the 3D data model, taken from the same angle and scale.

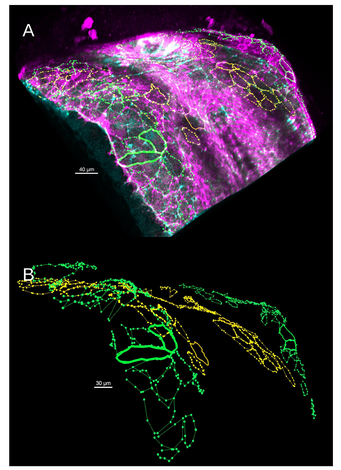

Fig 9: 80+ cells at final time point 07h 02m 30s (a) dorsal (green) and ventral (yellow) cells with layer showing (b) dorsal (green) and ventral (yellow) cells without layer showing

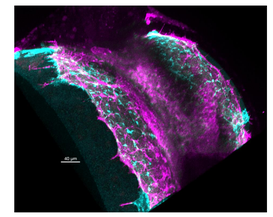

Fig 10: 3D View of Initial Non-Orthogonal Image

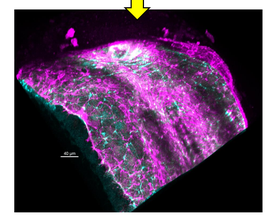

Fig 11: 3D View of Final Non-Orthogonal Image

Future Directions

In furthering our understanding of cellular motility during cardiac development, it is essential to expand the sample size of zebrafish for imaging and subsequent 3D modeling analysis. A larger dataset may elucidate patterns in cardiovascular cellular migration, particularly in contrasting the dorsal and ventral layers of the heart. This approach also offers insights into genetic mutations impairing early heart development, aiding our understanding of gene-mediated congenital heart defects. The integration of Artificial Intelligence (AI) in this endeavor holds significant potential as it can shorten the experimental timeline to these insights. Utilizing machine learning algorithms trained on comprehensive datasets, like the one proposed, AI could accelerate the process of individual cell mapping, thus improving project efficiency.

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