Visualizing the Trajectories and Contexts of Facial Branchiomotor Neuron Pioneers

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Scientific Context

Neuronal migration is essential for proper nerve development. Facial branchiomotor neurons (FBMNs) are located in the vertebrate hindbrain and migrate tangentially to innervate muscles in the jaw and pharynx. Similar to pioneer axons described in insects, each zebrafish pioneer neuron lays down a tract for other FBMNs to follow. Currently, we do not know the developmental stage or precise location at which the pioneer is born, or the mechanisms that control its role in migration. FBMNs are unusual in that they migrate tangentially, not just radially.

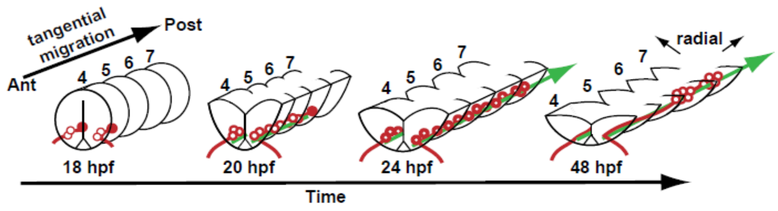

Figure 1: FBMNs are born in rhombomere 4 (r4), migrate to r6/7 through the neuroepithelium of the hindbrain.

Visualization Problem

The visualization system plays a critical role in laying foundation for the discovery of neural migration. We need to be able to effectively:

- Visualize the trajectory of the pioneer neurons.
- Visualize the context and interaction of the pioneer neurons with surrounding environment.
- Extract and convey essential information from 5D data on a 2D display.

Light-sheet Microscopy Imaging

Studying neural development requires imaging a large region over a long period. Confocal imaging is not adequate because phototoxicity from imaging would kill the specimen, photobleaching would reduce signal quality, and the neurons migrate faster than high-quality Z-stacks could be acquired. Light-sheet imaging solves these problems (Figure 2).

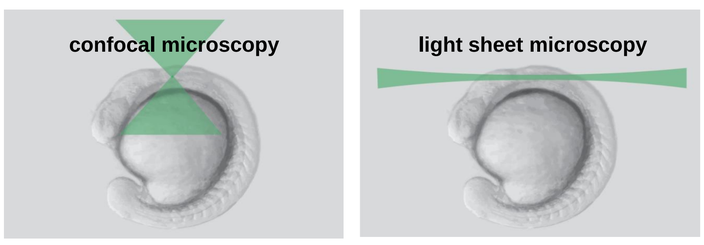

Figure 2: Schematic of the light paths in a confocal and light sheet microscope. Light sheet microscopy illuminates only the focal plane being imaged, reducing the amount of time and light required to acquire that slice.

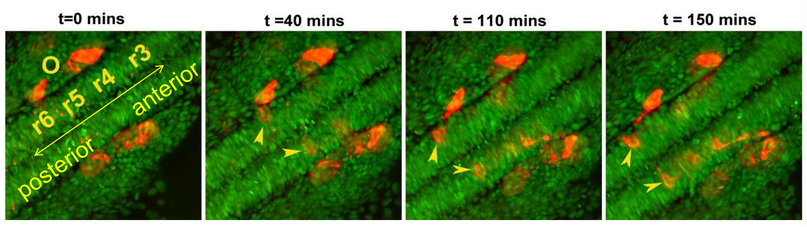

Figure 3: Double transgenic embryos allow for identification of the pioneer and tracking of progenitor nuclei. Transgenes that label (A) all cell nuclei with green fluorescent protein (GFP) and (B) FBMN cell membranes with red fluorescent protein (RFP) facilitate cell tracking.

Visualization System

In work to date, we have developed visualizations of time-steps with annotated pioneer locations, and are now developing interfaces to select and render a time-step for a particular point along the trajectory.

Pioneer neurons were manually tracked with ImageJ plugin MTrackJ [4], but our biology collaborators are not satisfied with its 3D display capabilities besides tedious tracking effort. Therefore, we are also in the process of developing an interactive system for tracking and visualizing data in 3D space.

Processing Pipeline

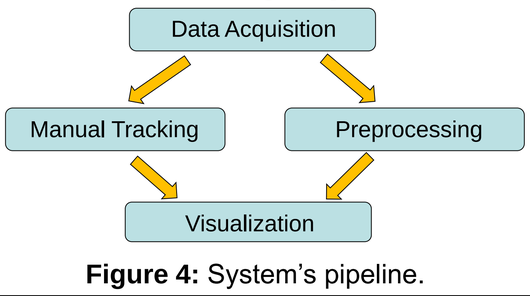

Figure 4: System's pipeline.

Preprocessing

- **Raw Data:** Images were acquired with a ZEISS Lightsheet Z.1 (purchased June 2013). 5D data with 16-bit 2 channels per voxel (RFP for FBMN cell membranes & GFP for all cell nuclei) in 3D space over time. 3D resolution 1280x1280x450. Image size of one time step ~ 3GB. The development is imaged every 2 minutes, over 7 hours. Total size ~ 800GB.
- **Drift Correction:** Embryos can drift within their agarose suspension during imaging. Cross-correlation based registration with summation projections [1] was performed to correct drift motion across time-points (Figure 5).

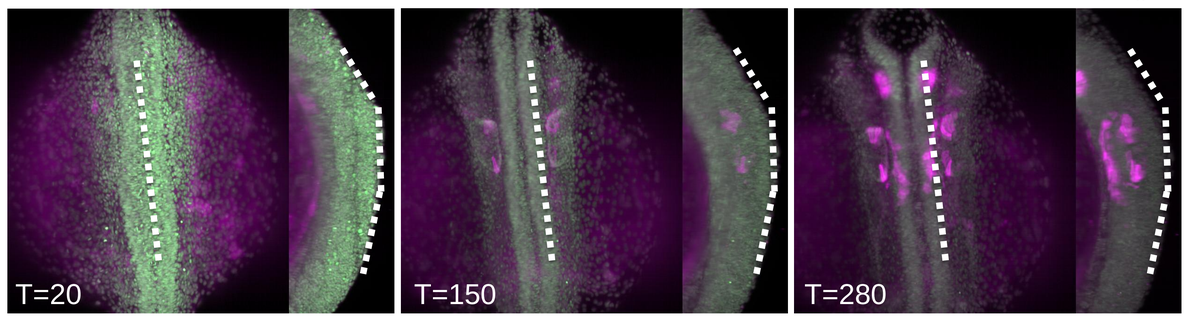

Figure 5: Maximum-Intensity-Projections indicate the extent of specimen drift (dashed white reference lines).

- **ROI-Definition and Resampling:** An anatomically oriented region-of-interest (ROI) is manually defined (Figure 6) to capture the extent of pioneer and other FBMN over all time steps, and images are cropped and resampled to this to simplify subsequent processing.
- **Crosstalk Correction:** A joint histogram of the GFP and RFP signals (Figure 7) reveals how they are correlated, due to GFP emission spectra bleeding into RFP filter. We correct for this to maximize the ability to detect increases in RFP expression at earlier time points.

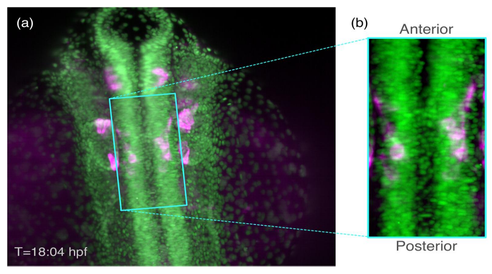

Figure 6: (a) Raw data with ROI. (b) Cropped ROI.

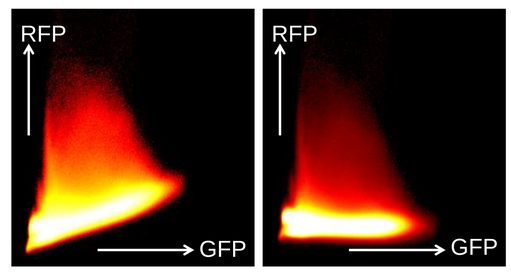

Figure 7: Joint histogram of GFP and RFP signals.

- **Pre-processed Data:** 3D resolution 496x258x190. Image size of one time step ~ 100MB. Total size ~ 27GB.

Visualization Techniques

We explore multiple visualization methods to provide scientists different ways to effectively inspect the data.

- **Two-Channel MIP:** Maximum-Intensity-Projection of two 3D volumes of RFP and GFP channels, and composite them into a single image (Figure 5). Data inspection starts with MIPs through and within slices, to verify specimen health. MIPs also show onset of RFP expression in FBMN membranes, at which time pioneers can be identified as the neurons with most posterior nuclei.
- **RFP-Isosurface-bounded GFP-MIP:** Doing MIP for the whole volume can result in a very cluttered image (Figure 8(a)) and the occlusions of desired information. Therefore, we can limit the area of GFP-MIP inside an RFP-isosurface (Figure 8(b)). It also helps in only visualizing the neurons instead of all cell nuclei. The bounding RFP-isosurface is shaded [3] to provide 3D perspective view of the membrane surface.
- **RFP-Isosurface-bounded Newton-based Maxima Rendering of GFP:** The intensity distribution of the cell nucleus roughly follows a 3D Gaussian, with a local maximum at the nucleus center. We utilize Newton Optimization to approximate the centers and visualize them inside RFP bounding isosurface (Figure 8(c)). This analytical visualization provides much cleaner view for neurons' nuclei, appearing as approximate spheres.

Newton's Method: $\nabla f(x_0 + \Delta x) = 0 \Leftrightarrow \Delta x \approx -(\nabla \otimes \nabla f(x_0))^{-1} \nabla f(x_0)$, which means Δx indicates how far a point is from the maxima. The distance function is used as alpha value for rendering GFP channel.

- **Interactive Trajectory Widget:** Use pioneer trajectory as interactive widget instead of traditional time-line, allowing user to inspect a specific time-step corresponding to clicked point along the trajectory (Figure 8(c,d)).

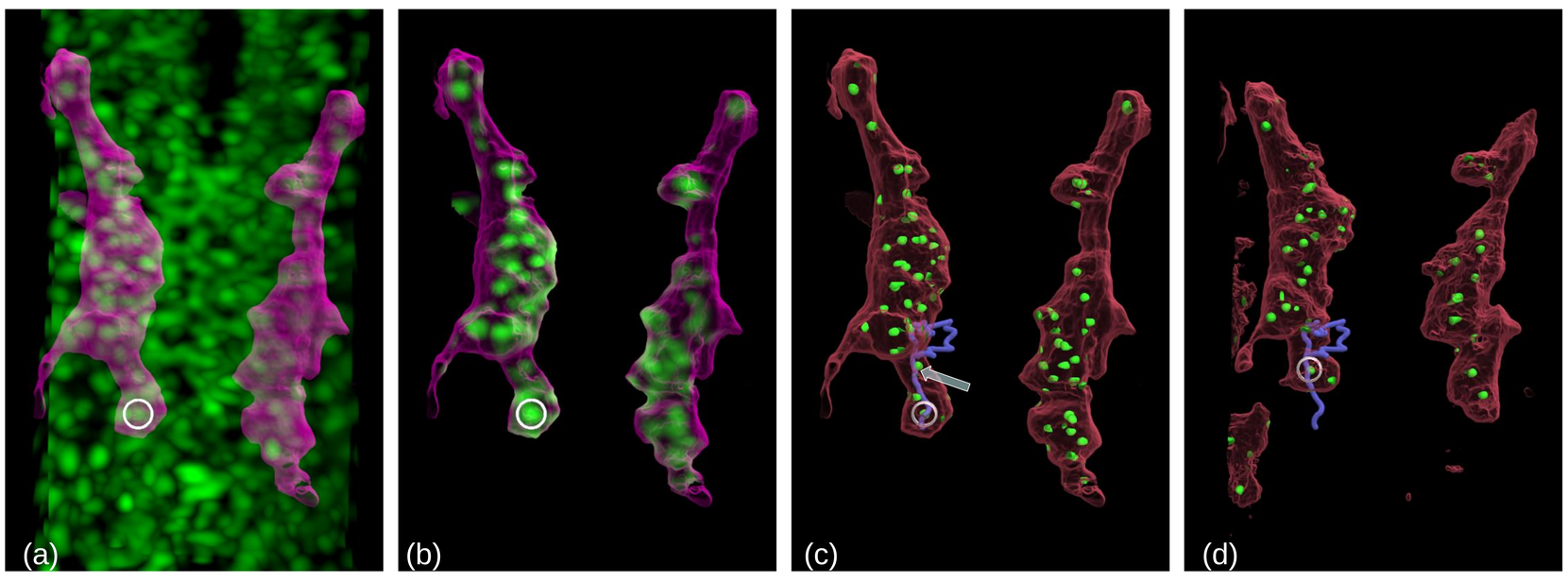

Figure 8: Rendering possibilities for showing pioneer in 3D context. RFP isosurface with GFP MIP that is unconstrained (a) or constrained (b) to the RFP isosurface interior. Newton-based GFP maxima with 3D track rendering (c) offers surface for clicking on track (white arrow) to change rendered timepoint (d).

Future Work

- Build interactive tracking and visualization system in 3D.
- Develop 3D widgets to handle effective user interactions, and precise selection of a point in 3D space.
- Further develop more sophisticated analysis and visualizations particularly useful in 5D microscopy data.

Acknowledgement

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References

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[3] Marc Levoy. *Display of Surfaces from Volume Data*. IEEE Computer Graphics and Applications, 8(3): 29-37, 1988.

[4] http://www.imagescience.org/meijering/software/mtrackj/