Reconstructing neuronal lineages using whole-brain cell tracking on Midway

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Background

To establish proper connectivity within the brain, neurons migrate from their birthplaces to a set of final locations where they integrate into the surrounding neural circuitry. We are interested in a class of migratory motor neurons called the facial branchiomotor neurons (FBMNs), which control facial expression and jaw movement in mammals and are conserved across vertebrates. FBMNs migrate from their birthplace in the fourth rhombomere (segment) of the hindbrain to the sixth and seventh rhombomeres, where they coalesce into distinct bilateral nuclei. Our studies in zebrafish identified a new mechanism contributing to FBMN migration. The first FBMN to exit rhombomere four acts as a pioneer – it actively explores the signaling environment of the hindbrain and leaves a trailing axon behind it to serve as a migratory scaffold for followers. When the pioneer or its trailing axon is absent, FBMN migration ceases altogether.

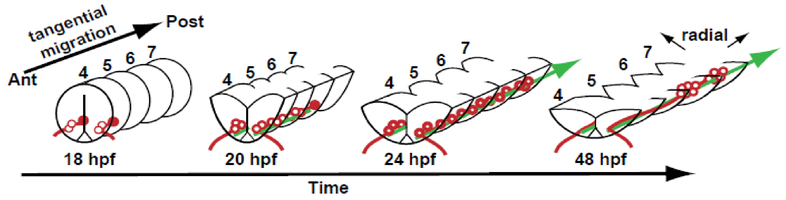


Fig. 1: FBMNs are born in r4 and migrate to r6/7 through the hindbrain neuroepithelium.

Using the single-plane illumination microscopy (SPIM), we can image the rapid cell divisions of FBMN progenitors and the migration of FBMNs themselves. We aim to reconstruct the lineages and movements of the FBMNs to understand how differences between pioneers and followers arise. However, SPIM generates vast amounts of data, with 1-1.5 TB resulting from a single experiment. Manually analyzing this data can take upwards of 20 hours and suffers from practical issues in visualizing 3D reconstructions of image-based data. We have adapted an automated pipeline for data compression, drift correction, and cell tracking (Amat et. al. 2015) for use on Midway, and created new scripts to visualize, manipulate, and edit tracks on any Windows workstation equipped with Imaris, Fiji, and Matlab. This software allows us to generate cell tracks in a more reproducible manner, and can be used by anyone intending to track cell movements in large image-based data sets.

The pioneer neuron and its trailing axon are required for successful FBMN migration

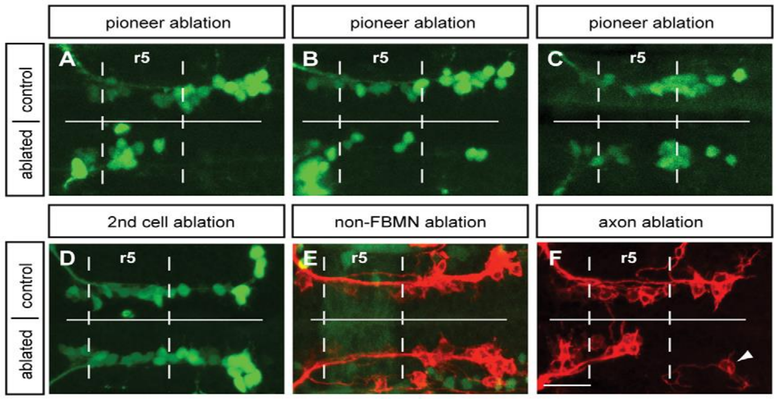


Fig. 2: Ablating the pioneer (A-C) or its trailing axon (F) results in clustering of FBMNs in r4, demonstrating that the pioneer is necessary for successful migration into r6/7. However, ablating the FBMN behind the pioneer does not impact migration (D), nor does ablating the neuroepithelium. Wanner and Prince, 2013.

Do pioneer and follower neurons differ in their developmental lineage?

Imaging with SPIM is rapid and gentle enough to resolve FBMN progenitor divisions

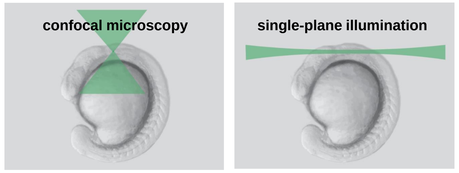


Fig. 3: Comparison of the light paths in confocal and SPIM. SPIM illuminates the sample along the focal plane, reducing background fluorescence and phototoxicity. Camera reads this as a widefield image and acquires all data from the focal plane simultaneously.

A robust nuclear label is needed to track cell movements and divisions

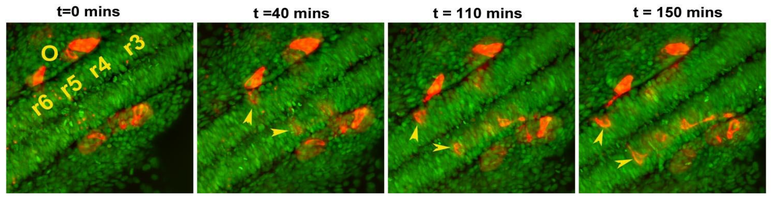
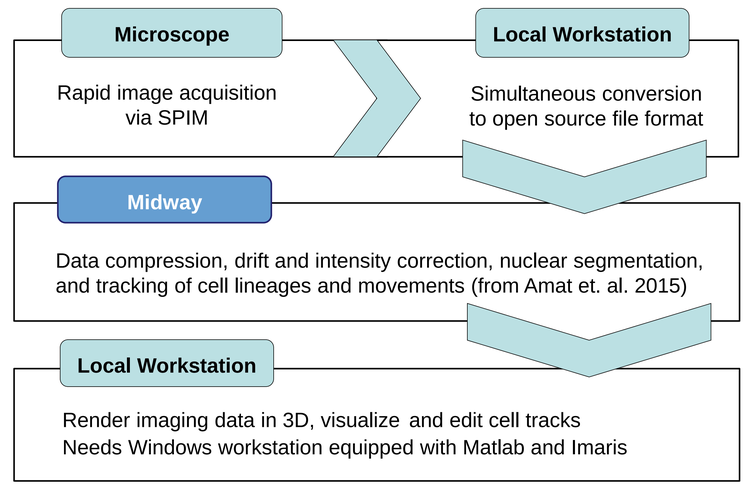


Fig. 4: Maximum intensity projection (MIP) images of a zebrafish embryo expressing transgenes that label all cell nuclei in green and FBMN cell membranes in red.

Automated pipeline has several modules that take data from raw microscope images to whole-embryo tracks



Midway is well-suited to running core components of the tracking pipeline

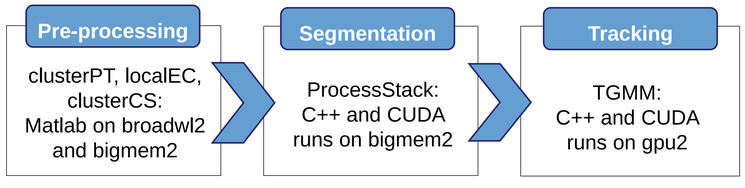


Fig. 5: Automated tracking pipeline adapted from Amat et al, 2015 uses a CUDA-accelerated application, currently built on Midway for gpu2, to track cell movements and reconstruct cell lineages over time. Additionally, the large file sizes generated via SPIM necessitate the use of bigmem2 for more memory-intensive tasks such as nuclear segmentation.

Pipeline generated tracks must be visualized and corrected manually to avoid false negatives

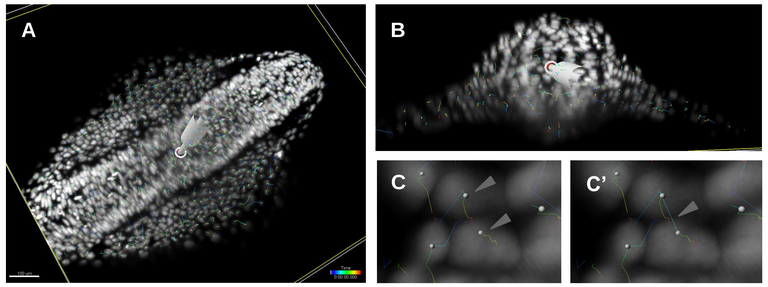


Fig. 6: 3D rendered data in Imaris. 20-slice (A) XY MIP and (B) YZ MIP through the zebrafish hindbrain, showing all cell tracks from the last ten time points. (C) Cell tracking is not 100% accurate, and can generate false negatives like these two daughter nuclei which should be part of the same cell lineage. (C') Matlab scripts can use XYZ coordinates of daughters to concatenate the two lineages and update tracking info, as shown.

Tracking information for individual time points or lineages can be isolated for analysis

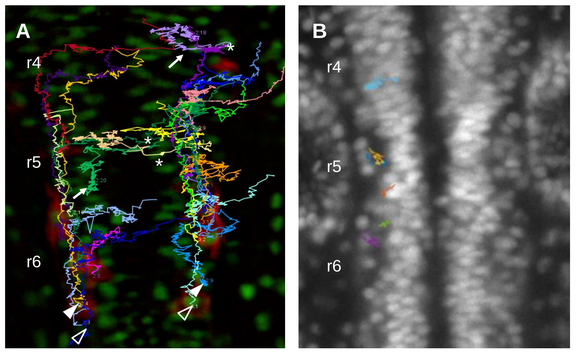


Fig. 7: 20-slice XY MIP of final time point, with tracks overlaid. (A) Tracks generated manually through a FIJI plugin show cell origins in rhombomere 4 of the hindbrain, and demonstrate directed migration of neurons to rhombomere 6. (B) Partial tracks generated using the automated pipeline show similar cell movements on a local (intra-rhombomere) scale. Tracks can be isolated for a group of given time points (50, as shown) or lineages (left-side FBMNs, as shown) using a Matlab script.

Ongoing work: Cleaning up images in pre-processing to enable more accurate cell tracking

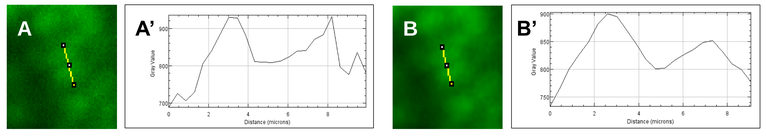


Fig. 8: Nuclear data (A, A') may benefit from slight Gaussian blur (B) to smooth local fluctuations in fluorescence intensity (B') and increase track accuracy. Other methods being tested include regional deconvolution and restriction of GFP signal to local peaks.

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