Automated Segmentation and Tracking of Neural Stem Cells in Unstained Brightfield Microscopy Images

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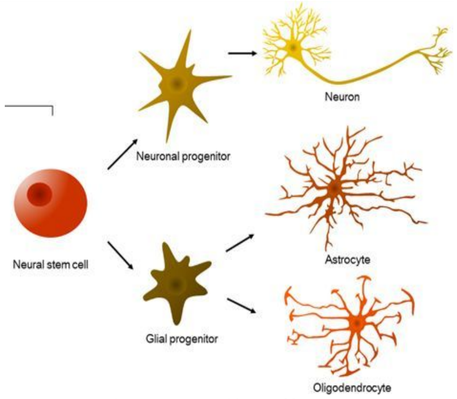
Abstract

There is an incomplete understanding of how neural stem cells respond to key signaling proteins. The Sohn Lab has developed new microfabrication technology that enables us to study the large scale effects of these signaling proteins on stem cells. Using this technology, hundreds of experiments were performed in different microenvironments to test the influence of FGF2 and Ephrin B2 [2]. These experiments produce too much data to process by hand, so the lab is in need of automated analysis techniques. In this project we develop tools for automatic segmentation and tracking of cells in unstained brightfield microscopy images. We trained a convolutional neural network with a U-Net architecture [1] to perform fully automatic segmentation. We implemented automated cell tracking by finding the center of mass for segmented regions and calculating paths of least energy through video frames. With these tools, we hope to uncover how neural stem cells make fate decisions.

Background

Neural Stem Cells

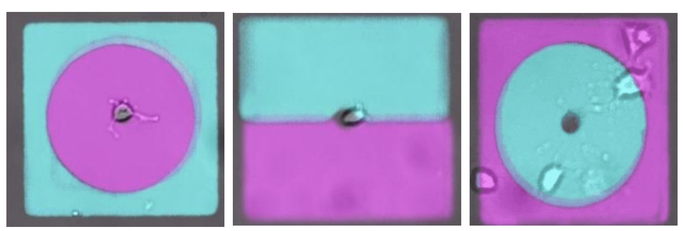
- Differentiate into a number of important cells
- Fate decisions influenced by signaling proteins such as FGF2 and Ephrin B2
- Understanding these influences would advance regenerative medicine and could lead to cures for Alzheimer's



Neural Stem Cell Differentiations
Image adapted from [3]

Data Processing

- Micro-niches patterned with various configurations of FGF2 and Ephrin B2 were created with Sohn Lab's DNA-directed patterning
- Hundreds of experiments run with a neural stem cells placed at the center of each protein pattern and a time lapse captured for 4 days
- Too much data to process by hand so we must automate cell segmentation and tracking in unstained brightfield images



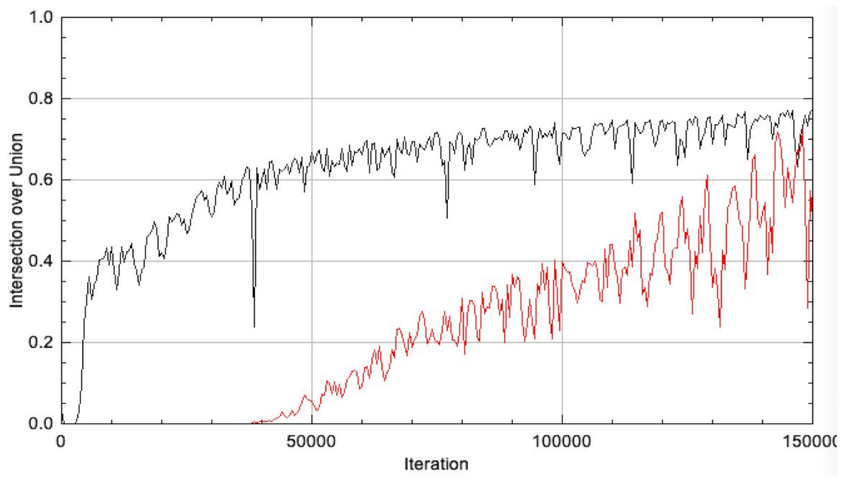
Micro-niche Protein Patterns

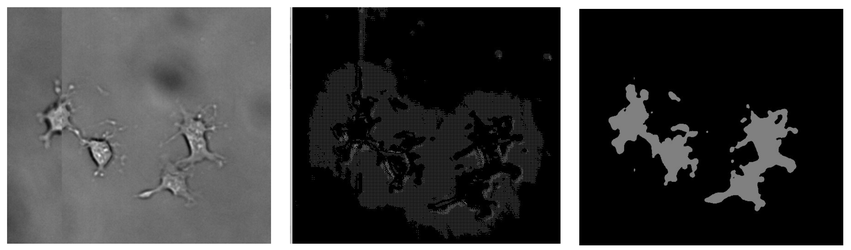
FGF2
Ephrin B2

Methods

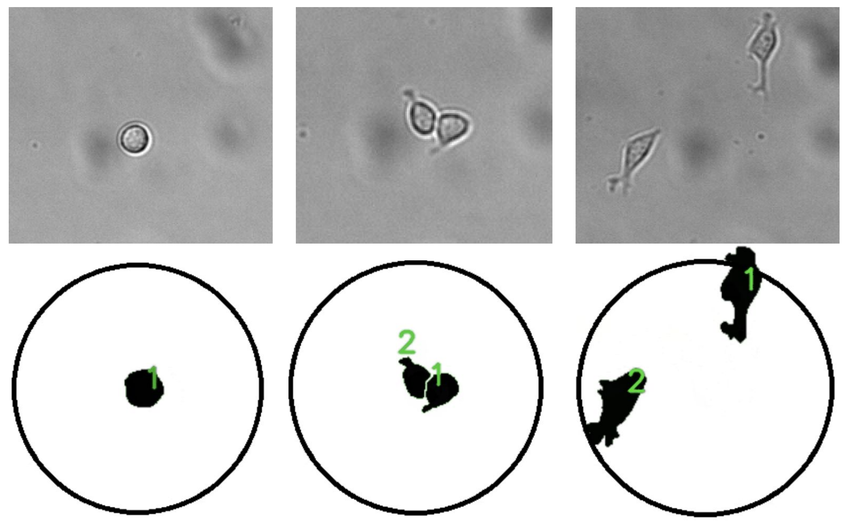
We performed automated segmentation on ImageJ, using a CNN with a U-Net architecture. This required the U-Net plugin and initial network weights, both from the University of Freiburg [1]. 200 images were hand annotated using Fiji's ROI Selector and used for fine tuning. Post processing was done with OpenCV on Python 3.. To track cells, we calculated the path that minimized the collective distance traveled by cell centroids from one frame to the next. Cell centroids were calculated as the average pixel location of a segmented region.

Results





(Top) CNN Training Progression.
(Left) Original (Middle) Partial Segmentation (Right) Segmented



Tracking Segmented Cell's Through Subset of Frames

Discussion

- Segmentation is robust to variation in shape, out of focus regions, and extraneous image artifacts
- Algorithms work for tracking and can handle cell divisions
- Delineating the boundaries of cells while post-processing segmented images is prone to error

Future Steps

To improve cell segmentation, we are experimenting with different CNN architectures and parameter combinations to get cleaner results. For tracking, we are working on improving the algorithm to handle edge cases and ensure accuracy even during cell divisions and cell deaths. Ultimerally, we hope to generate cell lineage maps for every time-lapse.

Cell Lineage Map

- Cell's history leading to its final state
- Critical information on ancestors
- Record time on FGF2 and Ephrin B2 to determine effect on fate decisions

Final Cell States
Image adapted from [2]

Acknowledgements

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References

[1] Olaf Ronneberger, et al. "U-Net: Convolutional Networks for Biomedical Image Segmentation." (2015).
[2] Scheideler, Olivia & Yang, Chun & Kozminsky, Molly & Mosher, Kira & Falcón-Banchs, Roberto & Ciminelli, Emma & Bremer, Andrew & Chern, Sabrina & Schaffer, David & Sohn, Lydia. (2020). Recapitulating complex biological signaling environments using a multiplexed, DNA-patterning approach. Science Advances. 6. eaay5696. 10.1126/sciadv.aay5696.
[3] Tang, Y., Yu, P. & Cheng, L. Current progress in the derivation and therapeutic application of neural stem cells. *Cell Death Dis* 8, e3108 (2017). https://doi.org/10.1038/cddis.2017.504