3D Living Neural Networks: Analysis of Developing Neural Stem Cells

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Introduction

Introduction to Neural Networks

- Neural Stem Cell (NSC) communication is essential for self-organization of the nervous system.
- Network analysis used to study functional connectivity in mature neuron, but not developing cell networks
- We investigate developing cell networks inspired from graph theory. Results of this method, called **Living Neural Networks**,

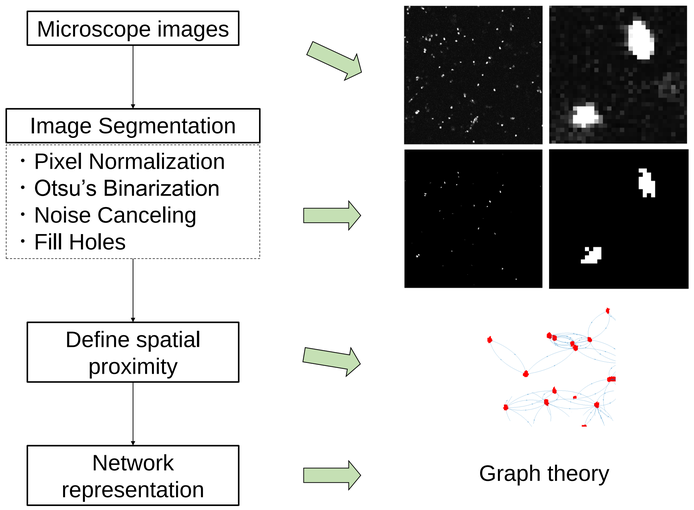
Investigation of 3-D Network Modeling

- Living Neural Networks among immature stem cells use network-wide signaling to evolve hierarchical communication[1,2]
- Hypothesized that 2D observations of neuron formation using Living Neural Networks could be extended to 3D models
- Studied, imaged, and characterized self-assembly of neural stem cells over 72 hours using confocal imaging of neural circuit development

Methods

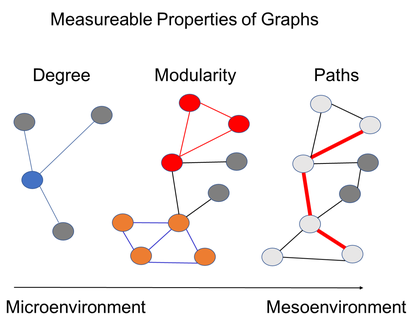
Cell Culture: ReNcell VM immortalized human neural stem cells (Millipore) were cultured in medium containing basic fibroblast growth factor (bFGF) and epidermal growth factor (EGF). To induce differentiation, cells were cultured in medium lacking growth factors.

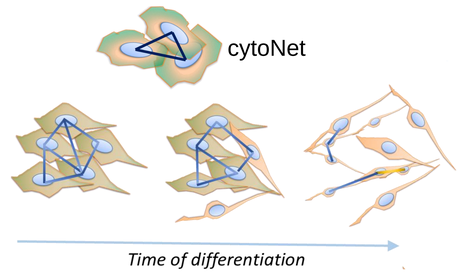
Live Imaging and Analysis: Images of differentiating cultures were captured using a Nikon Eclipse Ti-E conforcal microscope and processed using MATLAB R2014b (Mathworks®).



Graph Theory Applied to Cells

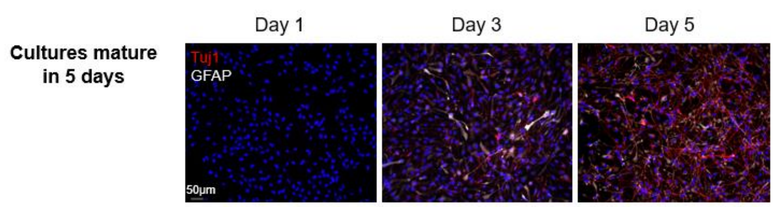
Graphs are mathematical frameworks constructed of nodes or vertices that represent objects of interest, and edges between the nodes that define relationships between the objects. In the Living Neural Networks method for the spatial graphs, nodes are the soma of cells, and edges are draw when cells are within an average diameter of each other. Once a graph is constructed, metrics of three main types (measures of degree, modularity or paths) are obtained to characterize the graph properties.

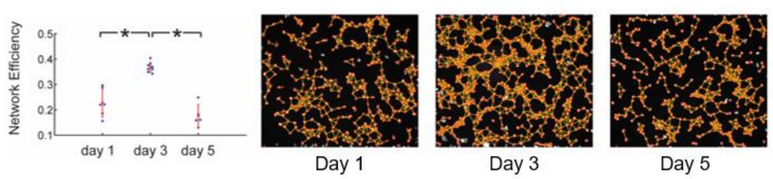




cytoNet (qutubLab.org/cytoNet) is a cloud-based platform to automatically construct a graph from cellular images. However, cytoNet is optimized for 2D, and we extrapolated the program to 3D in our analysis prior to applying it to the confocal images we acquired during stages of NSC differentiation.

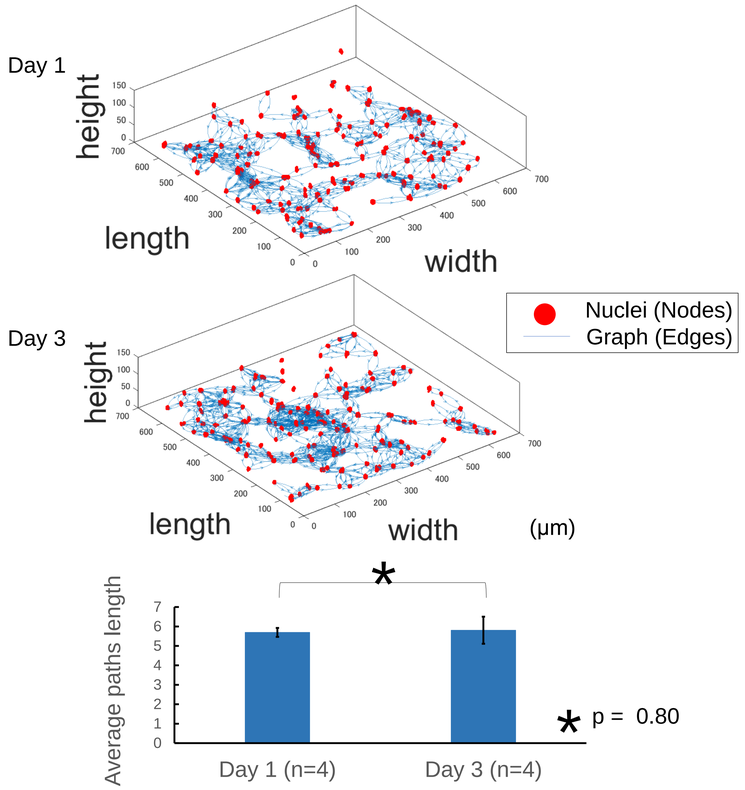
Prior Results: 2D Neural Networks





Mahadevan et al. (2018) showed that spatial network efficiency peaks during the mid-point of neural differentiation in 2D neural networks of ReNcell VM human neural stem cells (Day 3), when immature neurons appear by immunostaining (Tuj1, above). Our hypothesis is that this process occurs similarly in 3D, with a peak in network efficiency as the cells become electrically functional.

3D Nuclei Extraction & Neural Networks



Conclusions & Future Directions

We expanded the Living Neural Networks method to 3D to characterize the process of neural stem cells developing into functional neuronal networks in a more brain-like in vivo environment, through the combination of live imaging and computational graph analysis. In the future, we will analyze how the function of the NSCs changes in relation to the spatial network properties, and whether function is regulated by cell cycle. Additionally, the Living Neural Networks method is being used to investigate neurodegenerative diseases by applying it to NSCs induced from patients' tissue.

Acknowledgements & References

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1. Mahadevan *et al.* cytoNet: Network Analysis of Cell Communities. *bioRxiv* (2018).
2. Mahadevan *et al.* Living Neural Networks: Dynamic Network Analysis of Developing Neural Progenitor Cells. *bioRxiv* (2018).