Extracting structural connectomes from living neurons in culture by combining digital holographic microscopy with deep-learning methods

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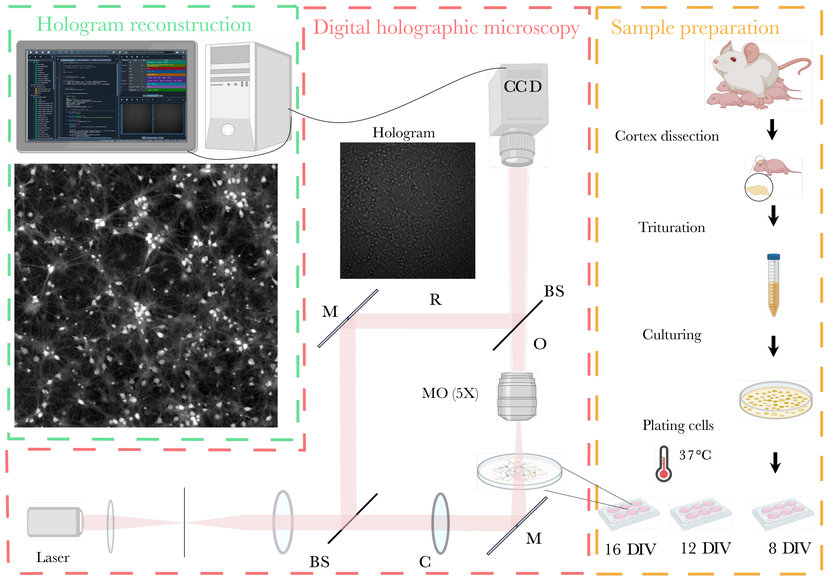
Introduction

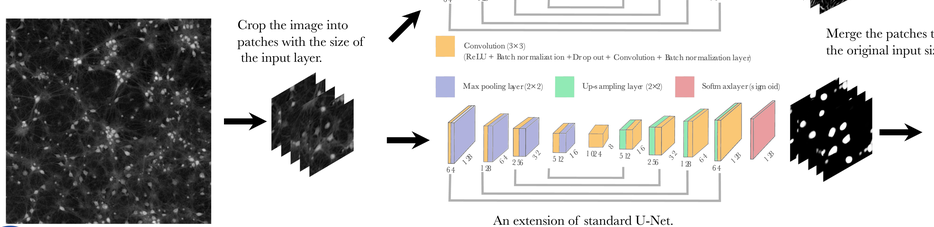
By providing quantitative phase images (QPIs), digital holographic microscopy (DHM) has emerged as a powerful non-invasive imaging technique for studying living cells. Many appealing applications require the ability to study a large number of cells, especially when screening compounds or studying the structural networks of neurons in culture. Therefore, we developed a new computational framework capable of performing fast, automatic, and accurate quantitative analysis, thereby allowing systematic analysis at the network level.

Methods

DHM returns holograms, which encode data regarding the morphology and structure of living cells within interference fringes. A reconstruction pipeline was employed to retrieve the phase image of the object from the recorded hologram. The algorithm enabled the conversion of each DHM hologram plane into a QPI, providing a visualization of the neuronal processes and morphology of the living cells. To investigate the neuronal processes of cortical neurons in rats at different days in vitro (DIV), we used QPI for different field of views (FOVs) of cell cultures. Imaging was performed on over 100 coverslips with varying DIV, ranging from 7 to 20 DIV, to demonstrate the growth of cells and neuronal processes on the coverslips over time[1].



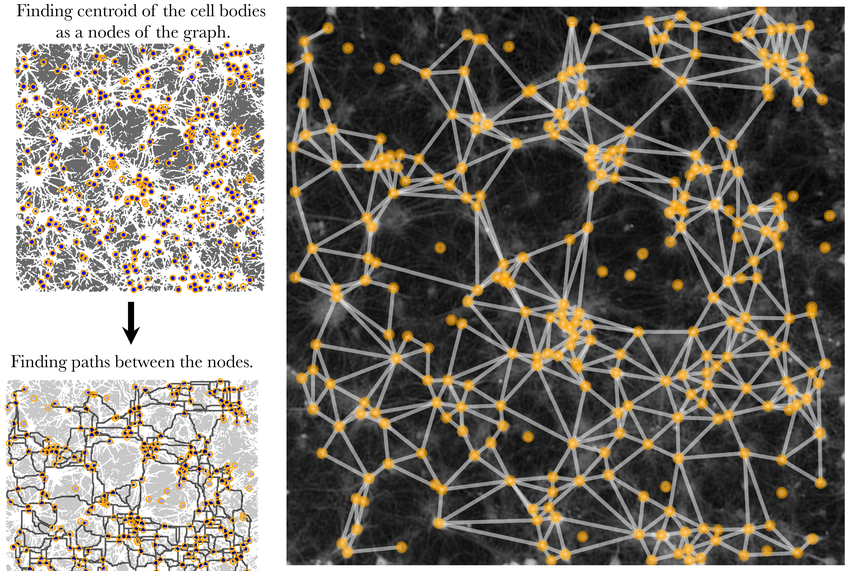
We implemented two U-Net architectures with a depth of 4, trained on manually segmented images of cell bodies and cell connections, to segment the structures of interest and convert them into mask images. Each down-sampling block in the encoding path consists of four 3x3 convolutional layers with ReLU activation and batch normalization. Similarly, the decoding path has four up-sampling blocks with ReLU activation and batch normalization. The final layer utilizes a sigmoid activation function to map 64 feature channels to a single channel, resulting in a grayscale probability map image[2].



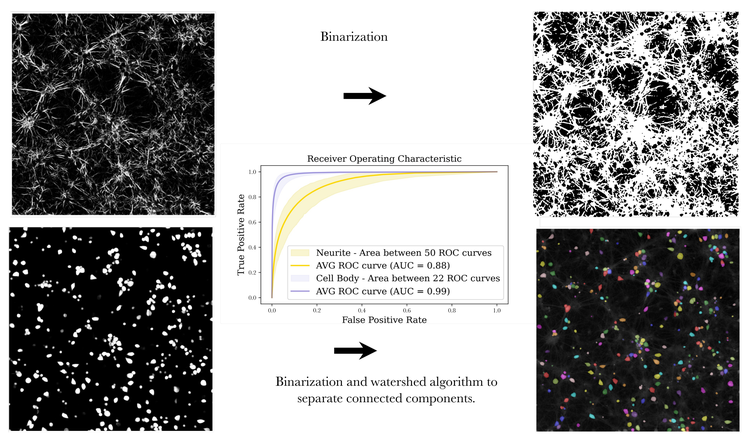
An extension of standard U-Net.

Results

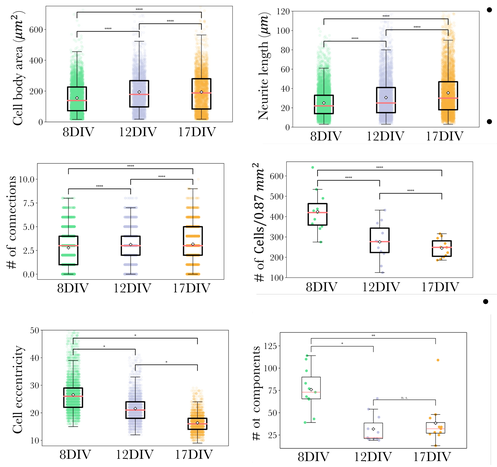
To construct the structural connectome, we have developed another pipeline. This pipeline begins by binarizing the probability maps obtained from the U-Net models. It utilizes two masks to represent cells and neurites. These masks undergo additional processing to create a maze-like structure, where cell centroids serve as sources and paths are formed by the neurite and cell body masks. By employing a basic pathfinding algorithm, we are able to determine the shortest paths between the sources. These paths are then used to define the edges of the structural connectome, with the shortest path between two sources representing the connection between cells.



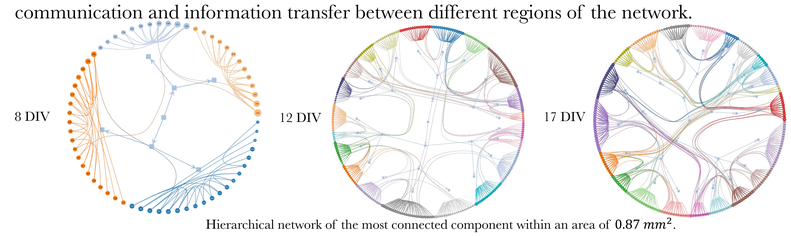
By representing the neuronal network as a graph, with cell bodies as vertices and neurites as edges, we can apply various graph-theoretical measures to gain insights into the network's organization. These measures allow us to assess important statistical properties, such as degree distribution, clustering coefficient, path length, and centrality measures[3].



Results



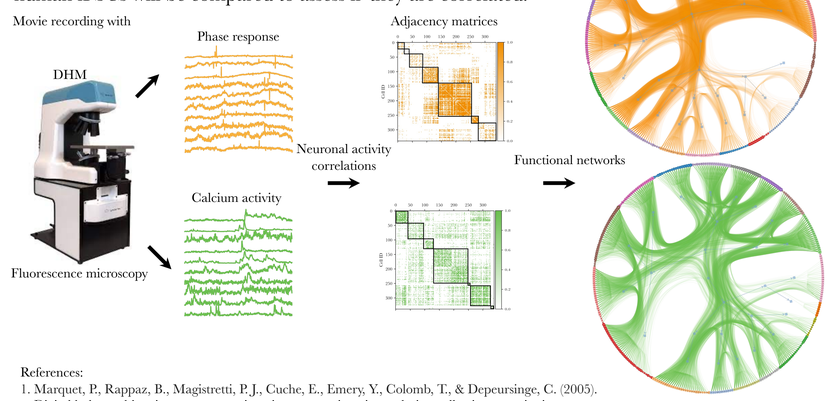
- These two measures show that, when cells grow in culture, both the area of cell bodies and the length of neurites increase significantly.
- During the growth of cells in culture, there is a significant increase in the number of cell connections. This trend is accompanied by the distancing of cells on coverslips, a decrease in cell density within an area of $0.87\ mm^2$.
- Eccentricity quantifies how far apart a cell is from any other cell in the network. The eccentricity of cells decreases over time. This reduction signifies a closer average distance between cells, leading to a more compactly connected network.
- As the neuronal network matures, the interconnections between distinct clusters become more pronounced, highlighting a higher level of organization and hierarchy. The increasing connectivity between clusters enhances the overall integration of the neuronal network, facilitating efficient communication and information transfer between different regions of the network.



Hierarchical network of the most connected component within an area of $0.87\ mm^2$.

Prospective avenue

- Comparing the structural graph measures of human induced pluripotent stem cell (IPSc) cultures derived from patients with brain disorders with healthy controls. The graph measures could signify differences between the two groups.
- Functional networks should also be compared with the structural one.
- The time-series of phase responses and calcium responses of human iPSCs will be compared to assess if they are correlated.



References:

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