

The human brain exhibits a highly structured three-dimensional organization characterized by complex neuronal networks whose connectivity governs both physiological function and central nervous system pathophysiology. Current experimental models present major limitations: conventional 2D cultures fail to reproduce 3D architecture and physiologically relevant cell–cell interactions, whereas neurospheres and brain slices provide limited structural control and accessibility. Despite advances in microfluidics enabling axonal guidance and cellular compartmentalization, precise control of neuronal architecture in three dimensions remains a major challenge.

This project proposes an innovative approach based on acoustophoresis and acoustic levitation (AL), relying on Acoustic Radiation Force (ARF) generated within resonant microfluidic cavities. This technology enables contactless manipulation of suspended cells and their controlled organization into monolayers or 3D aggregates at acoustic pressure nodes. The main objective is to engineer interconnected neuronal layers and multicellular brain organoids with accelerated maturation.

The first task aims to generate confluent neuronal sheets from murine or human neurons cultured under AL conditions. These sheets will then be stacked by tuning cavity geometry and ultrasound frequency to produce multiple pressure nodes. Neuronal differentiation, survival, axo-dendritic outgrowth, and synaptic connectivity will be assessed using immunocytochemistry and fast calcium imaging. In vivo validation will evaluate the integration of transplanted neuronal sheets into murine brain parenchyma.

The second task focuses on the generation of spatially structured multicellular brain organoids under ARF, integrating neurons, microglia, and endothelial cells. AL is expected to promote accelerated stem cell differentiation and controlled self-organization.

This interdisciplinary PhD project combines acoustofluidics and neurobiology to address the critical need for controlled, physiologically relevant three-dimensional neuronal models.