Scaling Up 3D Imaging, Analysis, and Culture of Complex Brain Models

by

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Technical Summary

The brain is the most complex human organ, containing components from the nanometer scale to the centimeter scale. However, many experimental techniques in neuroscience have been optimized for small brain models. This thesis summarizes a body of work aimed at scaling up 3D imaging, analysis, and tissue culture techniques for large-scale brain models. We present a technique termed SWITCH that inhibits probe binding to allow for diffusion without the formation of a reaction front. To improve imaging resolution, we present a tissue expansion technique called MAP that physically magnifies tissue samples for super-resolution imaging with conventional fluorescence microscopes. Using these tools to achieve volumetric imaging of large-scale brain models generates petabyte-scale data, for which we present horizontally scalable image processing pipelines for analysis of intact mouse brains, marmoset brain samples, and cerebral organoids. The mouse brain pipeline allows region-based statistical analysis of protein expression and cell counts. An efficient single-cell non-rigid coregistration algorithm for multiplexed volumetric fluorescence imaging based on matching corresponding nuclei between imaging rounds is presented. A multiscale phenotyping pipeline allows single-cell, cytoarchitectural, and morphological analyses to be combined into a hyperdimensional statistical analysis of cerebral organoids. We use this pipeline to show phenotypic changes due to neurodevelopment, Zika virus infection, and changes in organoid culture protocols. Current cerebral organoid cultures are limited by nutrient transport since they lack a vascular system. To address this issue in vitro, we fabricated synthetic vasculature by two-photon photopolymerization of polyethylene glycol-based resins. Printed micro-vessels were less than 100 μm in outer diameter, permeable to biomolecules through engineered pore structures, and biocompatible. Perfusion of vascularized cerebral organoids cultured for 30 days resulted in the expected neuronal differentiation as well as integration of the vascular network. Future studies can use and build on these technical advances to further our understanding of the brain through the use of large-scale brain models.

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