Scalable subcellular resolution mapping of the human brain

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Technical summary:
A detailed understanding of the anatomical and molecular architectures of cells and their system-wide connectivity is essential for interrogating system function and dysfunction. Extensive efforts have been made toward characterizing cells through various omics approaches, and have established invaluable databases yielding new insights. However, we still lack technologies for mapping the connectivity as well as molecular details of individual cells in the human nervous system, at the microscopic scale and human brain-wide scale. This thesis aims to develop various chemical and computational technologies as part of a fully integrated technology platform for simultaneously extracting spatial, molecular, morphological, and connectivity information of individual cells from the same brain at single-fiber resolution. We accomplished this by seamlessly integrating new chemical, mechanical, and computational tools to enable 3D multi-scale proteomic reconstruction of human organ tissues.

To address the challenge of slow and nonuniform fluorescent labeling of tissues in 3D, we developed tissue-hydrogel transformation technologies known as ELAST and mELAST that transform previously weak and sometimes brittle brain tissue into tough, stretchable, and elastic tissue-gel hybrids. In the case of mELAST, the tissues can also be reversibly expanded to enhance transparency and magnification to visualize finer structures. Through repeated thinning via cyclic compression dynamic loading, these tough tissues can be stained significantly faster and more uniformly than passive staining of typically preserved samples. Furthermore, their toughness allows them to be de-stained and re-stained many times to enable highly multi-plexed spatial omics.

To understand the antibody transport mechanisms and potential improvements to compression staining protocols, we developed a computational model for solute transport during high-strain dynamic loading of elastic tissue-gels. This fundamental study showed that while the thinning was the main transport mechanism, increases in convective flow within the tissue also could have significant effects on staining uniformity. Using the model, we also identified the best ways to modulate material properties, reaction kinetics, and cyclic compression loading schedule for enhanced staining uniformity, while also identifying theoretical methods to significantly improve transport over typical protocols by increasing convection.

Because our human brain mapping pipeline still requires tissue slicing due to chemical and optical limitations, we developed a computational pipeline termed UNSLICE to reconstruct the 3D connectivity of neural fibers across multiple brain slabs at the macroscopic (whole brain) and microscopic scales (single axons). By using blood vessels, astrocytic and other neuronal fibers, as well as axons, single fiber resolution reconstruction of sliced tissues can be achieved using UNSLICE, enabling tracing and downstream connectivity analysis. Using the combined technology platform, we analyzed a case study of Alzheimer's Disease (AD) pathology at multiple scales from overall

cytoarchitecture to individual synapses. Finally, we demonstrated, for the first time, the feasibility of scalable neural connectivity mapping in the human brains, establishing a path for probing brain connectivity and its alterations in diseases.

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