Imaging and analysis of 3D cellular resolution brain structure using tissue clearing

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Overview

Brain structural differences between cases and controls have been assessed for years using structural and diffusion-weighted MRI to identify brain regions with differential volume, thickness, or fiber orientations. Similarly, genetic associations have been conducted to human brain structure, as measured through structural and diffusion-weighted MRI, identifying hundreds of genomic loci associated with interindividual differences in these gross brain structural measurements. Though these studies have been successful in identifying specific gross structural features associated with brain disorders or genetic variation, they do not have sufficient resolution or cell type specificity to be able to determine the cellular basis underlying a gross brain structural change. Recent advances in tissue clearing and light sheet microscopy are enabling cellular resolution imaging of brain structure in model systems such as mouse brains and human brain organoids, and are now being extended to post-mortem human brains. This symposium will discuss methods for tissue clearing and antibody labeling of human brains, novel microscopy techniques to rapidly image tissue cleared brains at cellular resolution, methods to analyze these large scale images, and application of tissue clearing to measure differences between diffusion weighted and tissue-cleared axonal fiber orientations.

Lecture 1: *Comparing MRI Microstructure Maps and Tissue Clearing 3D Histology in the Same Human Brain Specimens*
Jennifer McNab Presenter

MRI is a powerful non-invasive, whole-brain imaging technique but relies on indirect measures such as magnetic relaxation and water diffusion to infer features of tissue microstructure. In contrast, histology can provide direct visualization of tissue microstructure components but is limited to small sections and/or thin slices of tissue and cross-sectional studies. One way to bridge the gap between MRI and histology is to perform MRI and histology in the same brain tissue specimens. The histological images can help validate or improve the interpretation of the MRI microstructure maps and the MRI can provide whole-brain perspective and a more direct link to in vivo measurements. Recent advances in tissue clearing that enable histological staining and microscopy of intact tissue cuboids (i.e. 3D histology) are particularly advantageous for capturing tissue microstructure features that extend in 3D, such as neuronal fibers, vasculature and the layering pattern of the folded cortex. This presentation will discuss methodological approaches to performing direct comparisons between MRI microstructure maps and tissue clearing 3D histology in the same human brain specimens and outline potential clinical and neuroscientific applications.

Lecture 2: *HuB.Clear: A Simple, Adaptable, and Scalable Clearing Method for Human Brain for Molecular and Cellular Profiling over Large Volumes*
Zhuhao Wu Presenter

The rapid advancements in tissue clearing approaches have greatly expedited systematic investigation of organ-wide cellular compositions and interactions with advanced imaging techniques. Our group has developed several iterations of iDISCO-family tissue clearing protocols (iDISCO, iDISCO+, and AdipoClear) to enable whole mount labeling, imaging, and automated analysis of large intact organs including adult mouse brain. To extend our approach to human brain, we have optimized a new protocol, HuB.Clear, that ensures consistent labeling and clearing of large intact human brain slabs. More importantly, the well-preserved tissue morphology by HuB.Clear will facilitate automatic data registration and analysis across multiple imaging modalities to enable brain-wide anatomical and patho-histological profiling at different scales.

Lecture 3: *Human brain Optimized Light-Sheet (HOLiS) microscopy for cell-type resolved mapping of entire human brains*
Elizabeth Hillman Presenter

We are developing an efficient pipeline for processing, labelling and rapidly imaging entire human brains at cellular resolution. Our approach uses high-speed scanning single-objective light sheet imaging with spectral multiplexing and cell-type encoding. Human brain samples are processed using a clearing and immunostaining approach called HuB.Clear which can generate optically clear, 5 mm thick, whole-mount brain slabs. High-field magnetic resonance imaging (MRI) data acquired on the intact, fixed brain prior to sectioning will provide a vital cross-reference for comparison while also facilitating registration of data to a common coordinate frameworks. HOLiS data will be analyzed to extract cell locations and cell type identities, generating databases that can be compared to standard neuroanatomical atlases, while also permitting careful examination of brain-wide patterns and inter-subject differences.

Lecture 4: *Cellular quantification in tissue-cleared whole-brain mouse images and human cortical organoids*
Jason Stein Presenter

With ~100 million cells in the mouse brain and data sizes of whole-brain images approaching the terabyte scale, advanced image analysis tools are needed to achieve accurate comparisons of cell quantifications. We developed a group of image analysis tools called NuMorph for end-to-end processing to perform cell-type quantification within the mouse cortex after tissue clearing and imaging by a conventional light-sheet microscope. Through manual labeling of nuclei in these images, we are increasing training of deep learning based segmentation algorithms leading to increased accuracy in areas with dense nuclei. We are applying these tools to understand the cellular basis of increased cortical thickness in a Neurofibromatosis Type 1 mouse model. Similarly, we are studying the cellular basis underlying megalencephaly observed in a mouse harboring a heterozygous loss of function mutation in the Chd8 gene, strongly associated with autism risk. Finally, we are also applying these tissue clearing and analysis tools to induced pluripotent stem cell derived cortical organoids in a population of individuals at high risk for autism who exhibited cortical surface area hyper-expansion in early development.