METHODOLOGY TO RAPIDLY MAP AND QUANTIFY WHOLE-BRAIN MICROVASCULATURE IN 3D

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The role of microvasculature in the development of cerebral disorders remains ambiguous despite recent implications in ~45% of dementia cases (including Alzheimer's) and ~20% of strokes.[1] Our goal is to develop 3D, high resolution, whole-brain maps of the cerebral microvasculature. This will address the knowledge gap surrounding vasculature changes during disease progression and ultimately support the development of innovative treatment paradigms. This effort is complementary to the BRAIN Initiative's emphasis on comprehensive neuronal mapping. To better understand the role of vasculature in the onset of cerebral pathologies, we have developed a protocol for capture, conversion and comparison of vascular structure and key characteristics in the intact mouse brain with quotidian programs. We created a novel pipeline for 3D whole-brain modeling using techniques of perfusion for vascular labeling, amendment of the iDISCO+ organ clearing protocol, light sheet microscopy (LSM), data handling and image processing. Our protocol relies on vascular labeling via retro-orbital perfusion of fluorescent Lectin-Dylight 649 (Vector Labs), which we have observed to label vasculature in a more comprehensive fashion than other dyes (i.e., lectin-FITC, DiI). It takes up to two days to achieve whole-brain clearing; whereas the iDISCO+ protocol requires the use of secondary antibodies and a timeline of weeks. In lieu of expensive software packages, such as the Filament Tracer feature in Imaris, we trace the vasculature using freeware packages that can be used for 3D reconstruction and manipulation from most personal computers (Figure 1B). Current work involves integration of our data with the Allen Brain Atlas, to merge our vascular computational data sets to an averaged frame of reference map for use by other groups. We anticipate that this approach can be used to study the relationship between microvascular structure and function with cerebral pathology and to fit mathematical models of hypoxia predictive of ischemic conditions in the brain.

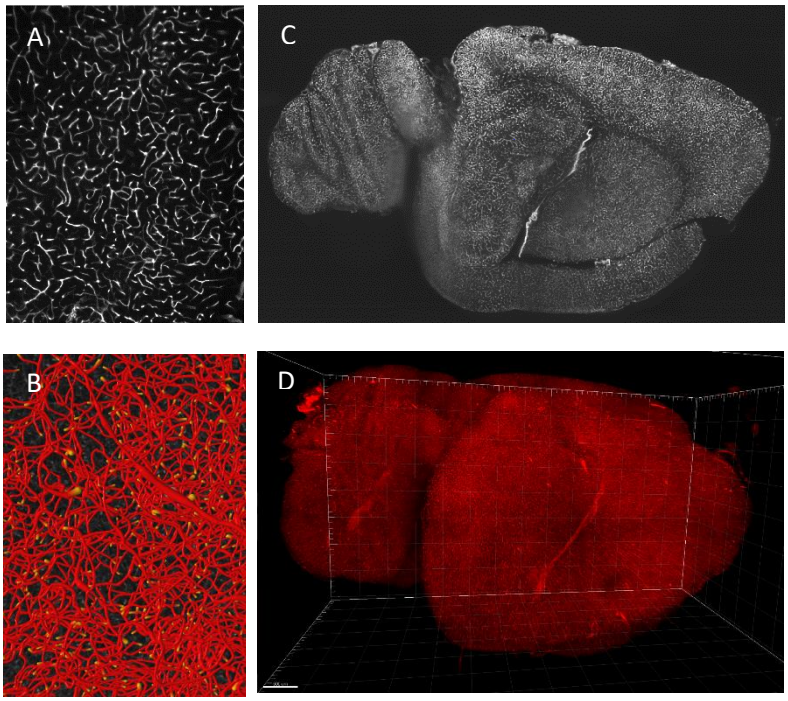

Figure 1: Visualization and quantification of whole mouse brain vasculature using LSM: (A) Micron-resolution microvasculature structure in a single imaged plane (B) Computational tracings of one LSM 3D tile, translation to data for quantification (C) One plane of stitched tiles (D) 3D rendering of stitched whole brain vasculature

[1] Y. Shi and J. M. Wardlaw, "Update on cerebral small vessel disease: a dynamic whole-brain disease," Stroke Vasc. Neurol., vol. 1, no. 3, pp. 83–92, Sep. 2016.