NEW IMAGING PIPELINE FOR WHOLE MOUSE BRAIN VASCULATURE INVESTIGATION WITH LIGHT-SHEET FLUORESCENCE MICROSCOPY

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Cerebral blood vessels are charged with the fundamental task of insuring and regulating blood supply depending on neuronal demand, which is known as "neurovascular coupling" [1]. However, in spite of its essential role, we still miss a complete map of brain vasculature network on a brain wide scale. Moreover, mainstream functional imaging methods like BOLD-fMRI rely on the level of blood oxygenation for indirect measure of neuronal metabolism [2]. A greater understanding of vascular organization would then also provide a better interpretation of these methodologies. Methods based on micro-optical sectioning tomography (MOST) have shown the possibility to label and image the entire mouse brain vascular system with high resolution and contrast [3]. On the other hand, these approaches still provide a moderate throughput (about 1 week to image a single sample) and use a staining protocol designed for transmission imaging rather than fluorescence, preventing simultaneous imaging of blood vessels and fluorescently-labeled neurons. We describe an approach enabling high throughput *ex vivo* whole mouse brain vasculature imaging using light sheet microscopy in combination with CLARITY technique and a specialized blood vessels labeling. The procedure preserve endogenous fluorescence, allowing for simultaneous imaging of vessels and neurons in transgenic animals. Contrary to serial sectioning techniques, the samples is preserved during imaging acquisition, allowing for successive analysis. Noteworthy, the fast whole brain image acquisition of the method proposed foster a proper characterization of morphological variability both in physiological and pathological conditions. We speculate that the high-contrast provided by our staining and imaging protocol will also be beneficial for the development of segmentation algorithms, which should be fully automatic and capable of dealing with datasets exceeding one TeraByte.

References

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