CUBIC-HistoVIsion: a pipeline for three-dimensional whole-organ/bodystaining and imaging with single-cell resolution based on chemical properties of tissue gel

Summary
Recent development of various tissue clearing and three-dimensional (3D) methods enabled the comprehensive observation of whole organ/body with cellular resolution or more. Several studies tried to integrate whole-mount staining into the clearing-imaging scheme. However, due to the difficulty in efficient penetration of stains and antibodies, they have only been applied in loose embryonic tissues or with a limited number of antibodies/stains for adult rodent tissues. To logically identify critical parameters for the efficient penetration, we began by investigating material chemistry of fixed and delipidated biological tissue. Then, we performed a surrogate assay with an artificial material similar to tissue in order to widely examine multiple chemical parameters for efficient staining. The identified parameters were integrated as a general 3D staining protocol, with which we have confirmed ~30 chemicals and antibodies used in whole adult mouse brain staining and imaging with single-cell resolution. The developed "CUBIC-HistoVIsion" pipeline for 3D histology and volumetric imaging provides opportunities for multi-channel imaging of functional and structural molecules of whole adult mouse organs as well as primate organs, thus will be widely applied to life science and medical researches in future.