

A SWITCH/TDE-COMBINED CLEARING METHOD TO RECONSTRUCT THE THREE-DIMENSIONAL CYTOARCHITECTONICS OF HUMAN BRAIN

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Malformations of cortical development (MCD)[1] are heterogeneous disorders frequently associated with epilepsy. The onset of these pathologies is related to alterations of cell proliferation, cell migration, and cortical organization. However, little is known about their pathogenesis. To obtain a deeper characterization of these pathologies three-dimensional reconstruction of the human neural networks at cellular is needed. Commonly, fine slices of samples marked with colorimetric techniques are individually imaged. In this way, space cell organization cannot be analyzed leading to loss of information. The aim of this project is to develop a methodology that allows analyzing the architectural abnormalities in the cortical layering of MCD in three dimensions. In particular, we exploit the possibility of combining high-resolution 3D imaging techniques with clearing methodologies. We successfully integrate the SWITCH immunohistochemistry [2] technique with the TDE clearing method [3] to image pediatric as well as adult human brain tissue with two-photon fluorescence microscopy. Our data demonstrate that the comparison of neurons 3D distribution in the cortex of healthy and affected individuals allows exploring anomalies in the structural organization of the brain, providing novel insights into the pathogenesis of MCD. In conclusion, this new approach enables to characterize large human brain specimens with high-resolution, giving the possibility to expand the histopathological studies to the third dimension.

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