

# Advanced ultrafast staining method for 3-dimensional volume imaging of biological tissues using focused electromagnetic force

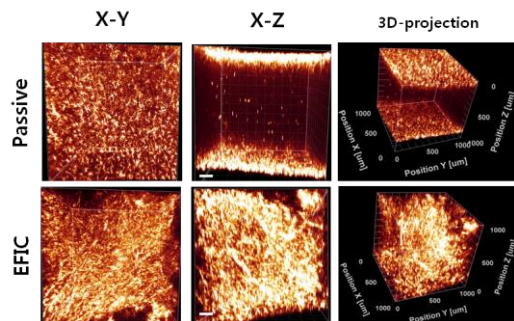
Myeongsu Na<sup>1</sup>, Kitae Kim<sup>1</sup>, Sunghoe Chang<sup>1,2</sup>

<sup>1</sup>Department of Physiology and Biomedical Sciences, <sup>2</sup>Neuroscience Research Institute, Medical Research Center, Seoul National University College of Medicine, 103 Daehak-ro, Jongno-gu, 03080 Seoul, South Korea, E-mail: [sunghoe@snu.ac.kr](mailto:sunghoe@snu.ac.kr)

**Key words:** Tissue clearing, Deep tissue labeling, 3D-imaging, Immunohistochemistry, Electric field focusing

Recent surges of three-dimensional tissue imaging provided an anatomical and phenotypic maps to understand structure-function relationships at cellular, circuit and organ scale. Various optical clearing methods have emerged, which usually require post-labeling of targets with staining probes such as antibodies. Post-labeling, however, still depends mostly on the passive diffusion, suffers from inefficiency, slowness and uneven staining. To overcome these limitations, we developed a staining method using the electric field focus force generated by the magnet, termed EFIC (Electro-magnetic Focused ImmunohistoChemistry). The result show that EFIC achieves fast and efficient penetration of staining probes into tissue over entire depth. We found that EFIC is fully effective to stain not only tissues from brain and other organs

but also from post-mortem human brain. Indeed, EFIC allows quantitative and comparative analysis of tau aggregation and vascular structure between normal and pathological tissue, thus showing its potential as a tool for three-dimensional diagnostic analysis. In summary, EFIC represent as versatile and reliable staining platform for analyzing molecular signatures in thick 3D tissues and for diagnostic purpose.



**Figure 1. EFIC vs. passive staining of anti-GFAP antibody in 4 hours**

[1] K. Chung, K. Deisseroth, CLARITY for mapping the nervous system. *Nat Methods* **10**, 508-513 (2013).

[2] S. Y. Kim, J. H. Cho, E. Murray, N. Bakh, H. Choi, K. Ohn, L. Ruelas, A. Hubbert, M. McCue, S. L. Vassallo, P. J. Keller, K. Chung, Stochastic electrotransport selectively enhances the transport of highly electromobile molecules. *Proc Natl Acad Sci U S A* **112**, E6274-6283 (2015).

[3] E. Lee, J. Choi, Y. Jo, J. Y. Kim, Y. J. Jang, H. M. Lee, S. Y. Kim, H. J. Lee, K. Cho, N. Jung, E. M. Hur, S. J. Jeong, C. Moon, Y. Choe, I. J. Rhyu, H. Kim, W. Sun, ACT-PRESTO: Rapid and consistent tissue clearing and labeling method for 3-dimensional (3D) imaging. *Sci Rep* **6**, 18631 (2016).