

APPLICATION OF A NEW SIMPLE TISSUE CLEAR USING AQUEOUS MATCHING SOLUTION FOR BRAIN SLICE

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Biological tissues are intrinsically opacity and consist of three dimensional structure; for this reason, scientists have always tried to extend tissue imaging to thick specimens. Fortunately, many optical clearing techniques compatible with fluorescent proteins have been developed in the past few years and we can take deep imaging such as mouse whole brain. The optical clearing techniques can clarify to 4 families based on the main physical mechanism: organic solvents, aqueous solutions with high refractive index(RI), protein hyperhydration, and tissue transformation with hydrogel embedding. These methods have individual advantages for experimental purpose, but they still have many restrictions such as tissue volume changes, fluorescent bleaching and image distortion by high viscosity of RI solution. Here, considering time consumption and experimental convenience, we try to focus on simple immersion method with aqueous solution. We suggest that a new clear matching (CM) solution base on aqueous solution with a high RI for semi thickness brain slice tissue. CM solution can be rapidly cleared within 1.5 hour to 1mm brain slice. Besides, few expansion of the brain slice and could see fine structure(spine morphology) of neuron. We performed comparative analysis between commercialized RI matching solution and our CM solution. CM solution has relatively low viscosity which closed to water, well preserved fluorescent proteins for one month later. CM solution can be maintained as the solution state while exposed to air for a long time. This property opens possibility to take imaging with large volume sample using confocal microscopes for a long time. Thus, CM solution is applicable for deep imaging of various tissue and can achieve high-resolution fluorescence imaging of thick tissues for long time without other clear step.

[1] Douglas S. Richardson et al. "Clarifying Tissue Clearing", *Cell*, volume 162 Issue 2 Pages 246-257(2015)

[2] Xinpei Zhu et al. "Ultrafast optical clearing method for three-dimensional imaging with cellular resolution", *PNAS*, 116 (23) 11480-11489(2019)