Development of novel methods for rapid and efficient labeling of cleared sample and for tissue clearing with minimal sample deformation for optical imaging in nanoscale

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Recent development of the number of different tissue clearing and labeling methods facilitates the three-dimensional imaging of large tissues. Labeling thick tissues with antibody typically relies on slow diffusional process, thus hampering efficient and rapid penetration of macromolecules into deep tissues for labeling. Recent methods that applied centrifugal pressure or stochastic electrotransport have addressed this problem, but they are still laborious and require specialized equipment. Here, we present a novel method for rapid and efficient penetration of antibody into deep biological tissues. This method effectively disperses antibody molecules into a cleared sample, and we could stain large sample efficiently and rapidly (up to 1 mm deep sample within 4 h) with a limited amount of antibody. We successfully applied this method to formalin-fixed postmortem human brain tissues and could stain proteins deep down to 500 μm. We also developed a novel clearing methods to minimize deformation artifacts that are due to the harsh treatment and transient sample swelling during CUBIC or CLARITY process. Our method is optimized to achieve minimal change in sample size and no loss of cellular materials for high-resolution fluorescence imaging. A combination of this method and super-resolution microscopy is now in progress.

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