TDE: A NEW VERSATILE METHOD FOR BRAIN CLEARING

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Light scattering inside biological tissue is a limitation for large volumes imaging with microscopic resolution. Based on refractive index matching, different approaches have been developed to reduce scattering in fixed tissue. High refractive index organic solvents [1] and water-based optical clearing agents, such as SeeDB [2] and CUBIC [3] have been used for optical clearing of entire mouse brain. Although these methods guarantee high transparency and preservation of the fluorescence, though present other non-negligible limitations. Tissue transformation addressed by CLARITY [4] allows high transparency, whole brain immunolabeling and structural and molecular preservation. This method however requires a highly expensive refractive index matching solution limiting practical applicability to large volumes. In this work we investigate the effectiveness of a water-soluble clearing agent, the 2,2'-thiodiethanol (TDE) [5] to clear mouse and human brain. TDE does not quench the fluorescence signal, is compatible with immunostaining and does not introduce any deformation at sub-cellular level. The not viscous nature of the TDE make it a suitable agent to perform brain slicing during serial two-photon (STP) tomography. In fact, by improving penetration depth it reduces tissue slicing, decreasing the acquisition time and cutting artefacts. TDE can also be used as a refractive index medium for CLARITY. Thanks to the possibility of fine adjustment of the refractive index is possible to analyze whole mouse brain samples with both two-photon fluorescence microscopy and light sheet microscopy. The potential of this method has been explored by imaging blocks of dysplastic human brain transformed with CLARITY, immunostained and cleared with the TDE. This clearing approach significantly expands the application of single and two-photon imaging, providing a new useful method for quantitative morphological analysis of structure in mouse and human brain.