Table S1. Methodological Considerations of Major Clearing Protocols of the Last Decade, Related to Figures 1, 4, S1, and S5

Technique	Clearing time for whole-brain	Complete transparency	Fluorescent quenching	Tissues validated	Significant contribution to field	Drawback
BABB, THF, DBE**(Becker et al., 2012; Dodt et al., 2007)**	hours-days	Yes, but tissue shrinkage	Yes (Ertürk et al., 2012a; Ke et al., 2013)	Rodent brain, spinal cord, peripheral tissues	Among first clearing reagents	Harsh reagents (Ke et al., 2013)
ClearT2**(Kuwajima et al., 2013)**	days	No	No-partial (Ke et al., 2013)	Rodent brain and embryo	Less quenching than BABB; novel reagents	Immunlabeling only through 120 μm
Scale (A2, U2) **(Hama et al., 2011)**	weeks-months (slowest)	Yes, but tissue swelling (Chung et al., 2013; Ke et al., 2013; Kuwajima et al., 2013)	No-minimal (Ke et al., 2013; Kuwajima et al., 2013)	Mouse brain, embryo (Hama et al., 2011)	Transparency without quenching; IHC/F	Slow; tissue deformation; potential protein loss with clearing (Ke et al., 2013)
3DISCO **(Ertürk et al., 2012a; Ertürk and Bradke, 2013)**	< week	Yes	No, but signal decay w/in days (Ertürk et al., 2012a; Ertürk and Bradke, 2013)	Peripheral/central organs, embryos, tumors (Ertürk and Bradke, 2013); Central (Erturk et al., 2012b) and peripheral (Jung et al., 2014) nerves	Balance between rapidity and quality of cleared tissue; imaging protocol	Requires immediate sample imaging; IHC-very limited
CLARITY (Chung and Deisseroth, 2013; Chung et al., 2013; Kim et al., 2013)	10 days	Yes	No	Rodent, human and non-human primate brains, spinal cord, zebrafish (Zhang et al., 2014)	Hydrogel-embedding; best tissue quality when performed correctly; IHC/F	ETC difficult, customized equipment, expensive (Chung et al., 2013)
Advanced CLARITY (Tomer et al., 2014; Zhang et al., 2014)	3 weeks	Yes	No	Whole mouse brain	No ETC – passive thermal CLARITY, COLM, CLARITY objectives, rapid imaging protocol	Requires COLM set-up
SeeDB **(Ke et al., 2013; Ke and Imai, 2014)**	days (fastest)	No	No	Young rodent brains (Ke et al., 2013)	No tissue deformation, fast	Tissue browning, incomplete clearing,
CUBIC **(Susaki et al., 2014)**	2 weeks	Mostly-Yes	No	Rodent and non-human primate brain	CUBIC informatics, optimized Sca*l*e (Susaki et al., 2014)	Brain only; potential protein loss during clearing

PACT, PARS	days-weeks	Yes	No	All major rodent organs; whole-body clearing	optimized/simplified CLARITY; permits long-term tissue storage; IHC/F	Slower than 3DISCO

IHC: Compatible with immunohistochemistry
IHC/F: Compatible with immunohistochemistry, immunofluorescent labeling; validated for (> 0.5 mm) depth of antibody penetration
~~IHC~~: IHC-incompatible, IHC-unverified, or strong restrictions, such as only compatible with lipophilic tissue dyes, or poor antibody penetration (<< 0.5 mm)
COLM: CLARITY-optimized light sheet microscopy; CLARITY objectives possess a several-millimeter working distance, which permits whole-brain or thick slice imaging. The COLM set-up grants rapid sample imaging and thus improves the throughput of whole-brain analysis. However, regardless of the imaging methodology followed: e.g. such as those provided in COLM, 3DISCO and CUBIC protocols, whole-brain and large sample imaging requires a specialized, expensive microscopy set-up.