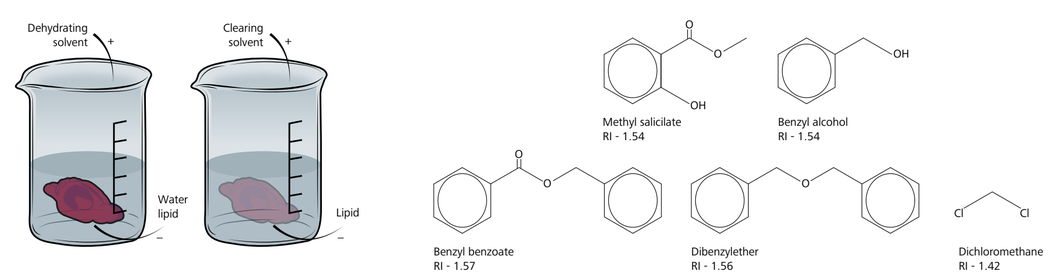
Clearing Methods in Microscopy

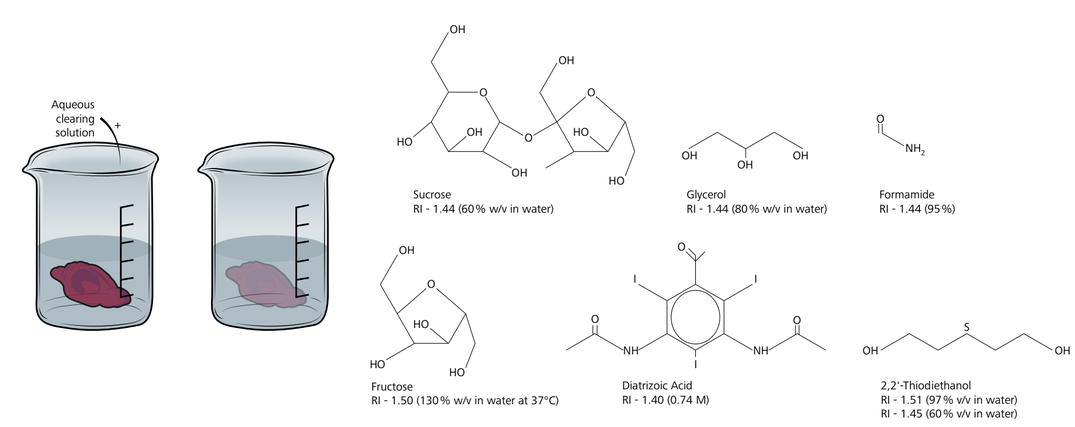
Technology	Method	Final R.I.	Main Agents	Time to Clear	Immuno-staining	Alterations in Tissue Morphology	Fluorophore Stability	Detergent Used	Lipid Preserved	Electro-phoresis	Hydrogel Embedding	Clearing Solution Perfused	Toxic	Reference	Objective to be used	Light-sheet	Single- and Multi-Photon
Simple Immersion																	
	Succrose	1.44	succrose	1 day	+	shrinkage	+	Triton (2 %)	–	–	–	–	–	Tsai et al., 2009b	Clr Plan-NEOFLUAR 20x / 1.0 Corr nd = 1.45 or Lightsheet Z.1 Detection Optic 5x / 0.16 nd = 1.45	+	+
	Focus Clear	1.43 – 1.47	diatrizoic acid (hypaque)	several days	+	–	+	Tween 20	+	–	–	–	–	Chiang et al., 2002	Clr Plan-NEOFLUAR 20x / 1.0 Corr nd = 1.45 or Lightsheet Z.1 Detection Optic 5x / 0.16 nd = 1.45	+++	++
	ClearT	1.44	formamide	2 – 3 days	+	–	–	–	+	–	–	–	+	Kuwajima et al., 2013	"Lightsheet Z.1 Detection Optic 5x / 0.16 nd = 1.45 Confocal: LCI Multi-Immersion objectives should be working with coverslips"	+ (only air objective)	+
	ClearT2	1.44	formamide / PEG	2 – 3 days	+	–	+	–	+	–	–	–	+	Kuwajima et al., 2013	"Lightsheet Z.1 Detection Optic 5x / 0.16 nd = 1.45 Confocal: LCI Multi-Immersion objectives should be working with coverslips"	+ (only air objective)	+
	SeeDB	1.48 – 1.50	fructose / thioglycerol	several days	–	–	+	–	+	–	–	–	–	Ke et al., 2013	"Lightsheet Z.1 Detection Optic 5x / 0.16 nd = 1.45 Confocal: LCI Multi-Immersion objectives should be working with coverslips"	+	++
	FRUIT	1.48	fructose / thioglycerol / urea	days	–	minimal expansion	+	–	+	–	–	+	–	Hou et al., 2015	Clr Plan-NEOFLUAR 20x / 1.0 Corr nd = 1.45 or Lightsheet Z.1 Detection Optic 5x / 0.16 nd = 1.45	++	++
	TDE	1.42	2,2´-thiodiethanol	days – weeks	+	–	+	8 % SDS (optional)	–	optional	optional	–	–	Costantini et al.,2015; Aoyagi et al., 2015; Staudt et al., 2007	Clr Plan-NEOFLUAR 20x / 1.0 Corr nd = 1.45 or Lightsheet Z.1 Detection Optic 5x / 0.16 nd = 1.45	++	++
Hyperhydration																	
	ScaleS	1.44	urea / sorbitol	days	+	–	++	Triton X-100 (0.2 %)	+	–	–	–	–	Hama et al., 2015	Clr Plan-NEOFLUAR 20x / 1.0 Corr nd = 1.45 or Lightsheet Z.1 Detection Optic 5x / 0.16 nd = 1.45	+++	+++
	Scale A2	1.38	4M urea, 10 % glycerol	2 weeks	–	expansion	++	Triton X-100 (0.1 %)	–	–	–	–	–	Hama et al., 2011	Clr Plan-APOCHROMAT 20x / 1.0 Corr nd = 1.38	+++	+++
	Scale U2	1.38	4M urea, 30 % glycerol	months	–	–	++	Triton X-100 (0.1 %)	–	–	–	–	–	Hama et al., 2012	Clr Plan-APOCHROMAT 20x / 1.0 Corr nd = 1.38	+++	+++
	CUBIC	CUBIC1, 1.38; CUBIC2, 1.48	4M urea, 50 % succrose	1 – 2 weeks	+	expansion	+	Triton X-100 (50 %)	–	–	–	–	–	Susald et al., 2014	Clr Plan-APOCHROMAT 20x / 1.0 Corr nd = 1.38 or Clr Plan-NEOFLUAR 20x / 1.0 Corr nd = 1.45 or Lightsheet Z.1 Detection Optic 5x / 0.16 nd = 1.45	+++	+++
	Whole-Body-CUBIC (perfusion)	1.45	4M urea	2 weeks	+	expansion	+	Triton X-100 (10 %)	–	–	–	+	–	Tainaka et al., 2014	Clr Plan-APOCHROMAT 20x / 1.0 Corr nd = 1.38	+++	+++
Hydrogel Embedding																	
	CLARITY	1.45	SDS, boric acid, FocusClear / 80 % glycerol	2 – 4 weeks	+	slight expansion	++	SDS (8 %)	–	+	+	–	–	Chung et al., 2013	Clr Plan-NEOFLUAR 20x / 1.0 Corr nd = 1.45 or Lightsheet Z.1 Detection Optic 5x / 0.16 nd = 1.45	+++	+++
	PACT	1.38 – 1.48	Histodenz, SDS	2 – 4 weeks	+	expansion	+	SDS (8 %)	–	–	+	–	–	Yang et al., 2014	Clr Plan-APOCHROMAT 20x / 1.0 Corr nd = 1.38 or Clr Plan-NEOFLUAR 20x / 1.0 Corr nd = 1.45 or Lightsheet Z.1 Detection Optic 5x / 0.16 nd = 1.45	+++	++
	PARS	1.38 – 1.48	Histodenz, SDS	1 – 2 weeks	+	–	+	SDS (8 %)	–	+	+	+	–	Yang et al., 2014	Clr Plan-APOCHROMAT 20x / 1.0 Corr nd = 1.38 or Clr Plan-NEOFLUAR 20x / 1.0 Corr nd = 1.45 or Lightsheet Z.1 Detection Optic 5x / 0.16 nd = 1.45	+++	++
Solvent Based																	
	Spalteholz	1.55	Benzyl benzoat / Methyl salicilate	months	–	shrinkage	– –	–	–	–	–	–	+	Spalteholz 1914	LS: Simax glass enclosed samples only (see Whitepaper); MP: Tests for dipping objective resistance towards chemical compounds have to be done	(+)	(+)
	BABB	1.55	Benzyl alcohol / Benzyl benzoat	3 days	+	shrinkage	–	–	–	–	–	–	+	Dodt et al., 2007	LS: Simax glass enclosed samples only (see Whitepaper); MP: Tests for dipping objective resistance towards chemical compounds have to be done	(+)	(++)
	3DISCO	1.56	Dichloromethane / Dibenzylether	3 days	– (limited)	shrinkage	–	–	–	–	–	–	+ (DBE)	Ertürk et al., 2012a, 2012b	LS: Simax glass enclosed samples only (see Whitepaper); MP: Tests for dipping objective resistance towards chemical compounds have to be done	(+)	(++)
	iDISCO	1.56	Dichloromethane / Dibenzylether	3 days	+	shrinkage	–	–	–	–	–	–	+ (DBE)	Renier et al., 2014	LS: Simax glass enclosed samples only (see Whitepaper); MP: Tests for dipping objective resistance towards chemical compounds have to be done	(+)	(++)

( ) = glass enclosed

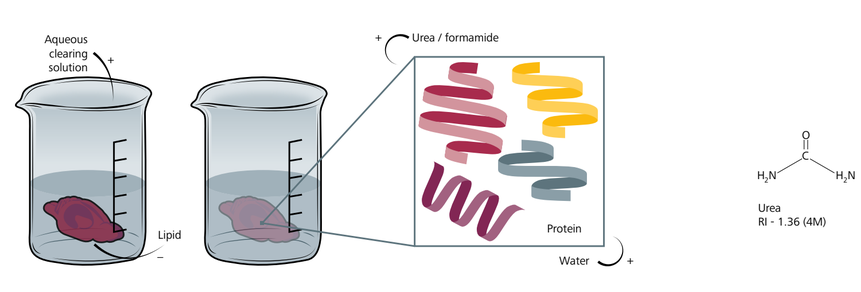
A Solvent based clearing



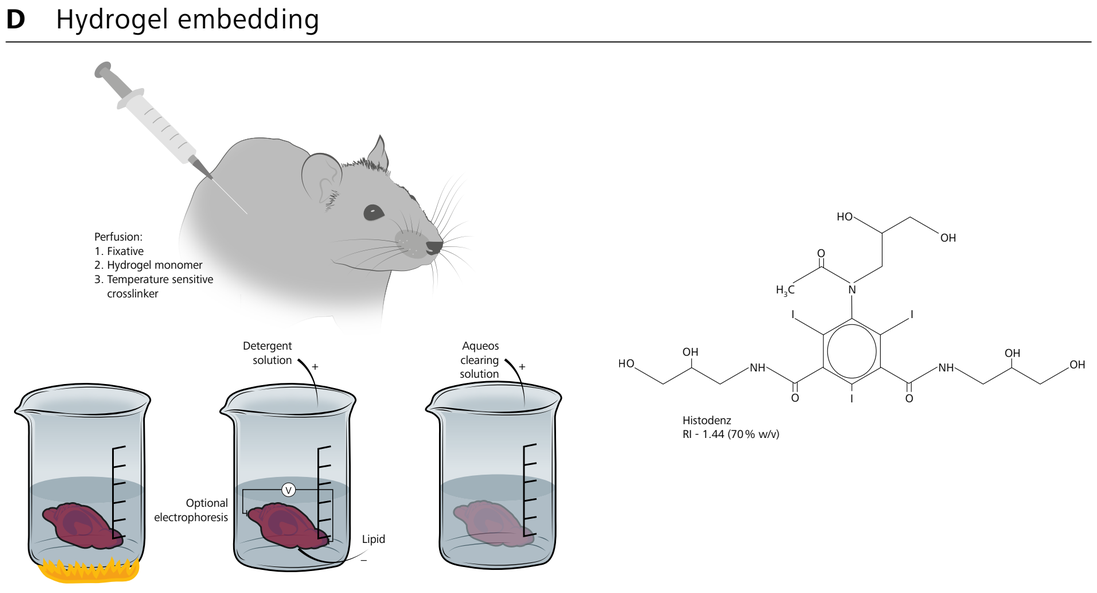
B Simple immersion



C Hyperhydration



D Hydrogel embedding



Reference:
Cell, Volume 162, Issue 2, 16 July 2015, Pages 246–257, Clarifying Tissue Clearing, Douglas S. Richardson[1,2,*] and Jeff W. Lichtman[1,2,3,*]
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