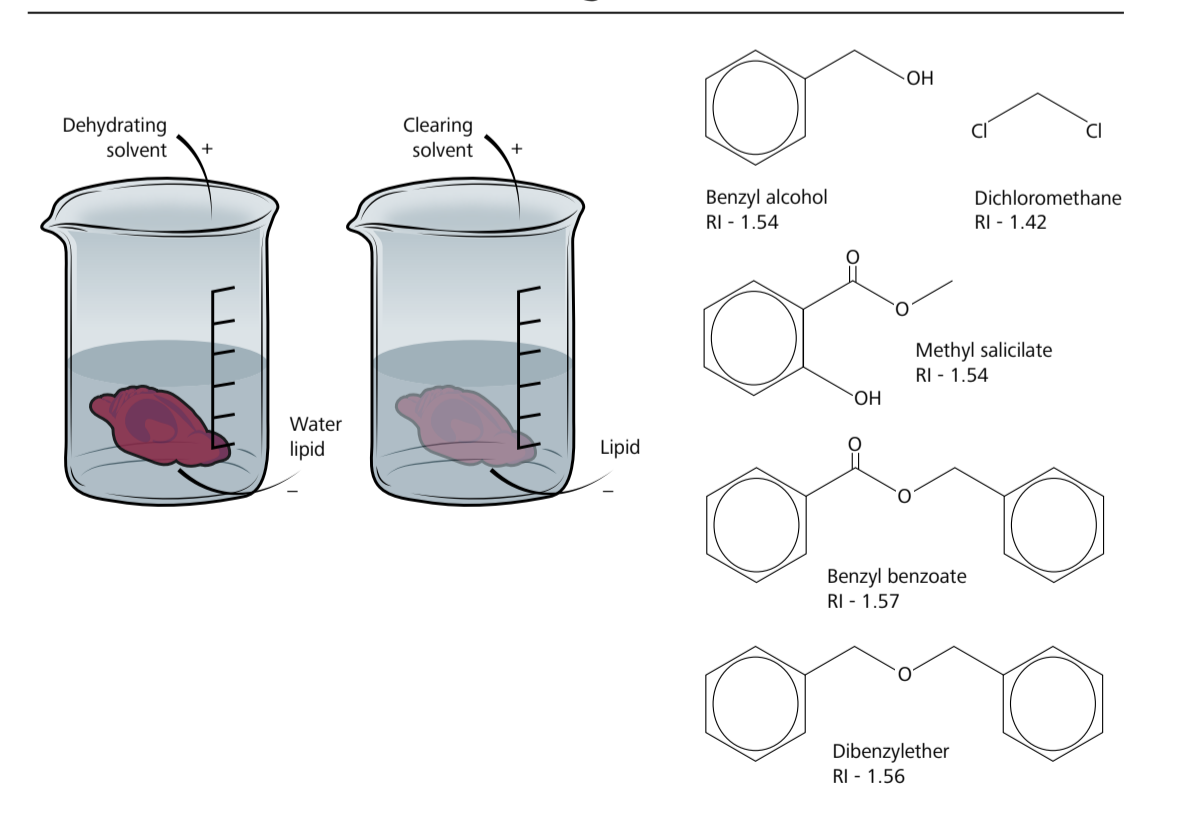


# Clearing Methods in Microscopy

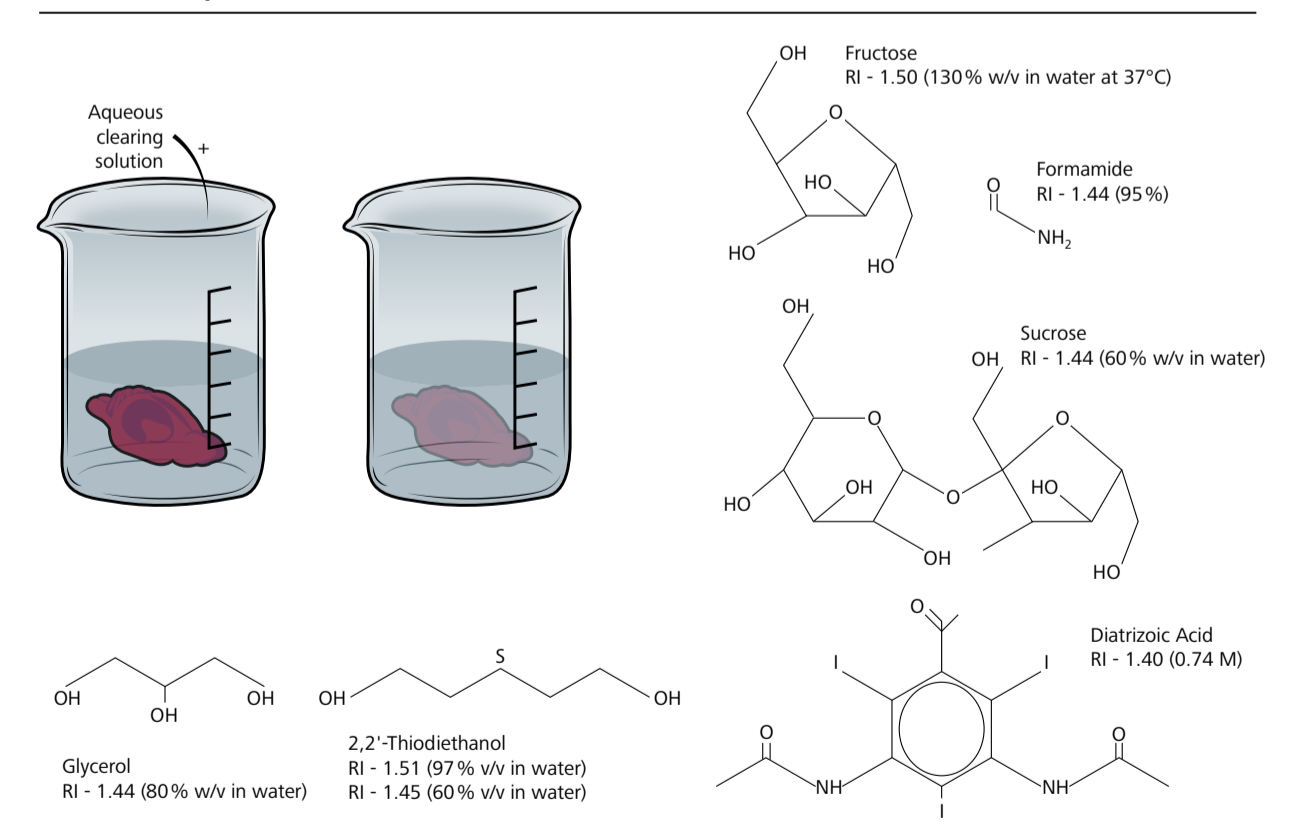
Technology	Method	Final R.I.	Main Agents	Time to Clear	Immuno-staining	Alterations in Tissue Morphology	Fluoro-phore Stability	Detergent Used	Lipid Preserved	Electro-phoresis	Hydrogel Embedding	Clearing Solution Perfused	Toxic	Reference	Objective to be used	Light-sheet	Single-Photon
Simple Immersion	Sucrose	1.44	sucrose	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	+++	++
	Clear T	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"	+	+
	Clear T2	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"	+	+
	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, 1.43	25 wt% Urea 25 wt% Quadrol (N, N, N', N'-Tetrakis (2-hydroxypropyl) ethylene-diamine)	1 – 2 weeks	+	expansion	+ ~ ++	Triton X-100 (10–15 wt%)*	-	-	-	-	-	Susaki 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	+++	+++
		CUBIC2, 1.48	25 wt% Urea 25 wt% Triethanolamine 50 wt% Sucrose	1 – 2 weeks	+	expansion	+ ~ ++	Triton X-100 (10–15 wt%)*	-	-	-	-	-	Susaki 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)	1.43	12.5 wt% Urea 12.5 wt% Quadrol (N, N, N', N'-Tetrakis (2-hydroxypropyl) ethylene-diamine)	2 weeks	+	expansion	+	Triton X-100 (7.5 wt% for perfusion, 15 wt% for immersion)	-	-	-	+	-	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 salicylate	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	-	-	-	-	-	-	+	Dotd 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	-	-	-	-	-	-	+	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	-	-	-	-	-	-	+	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 \*Modified recipe in Susaki & Ueda, Cell Chemical Biology 2016 and http://cubic.riken.jp 10 wt% Urea 5 wt% Quadrol 10 wt% TritonX-100

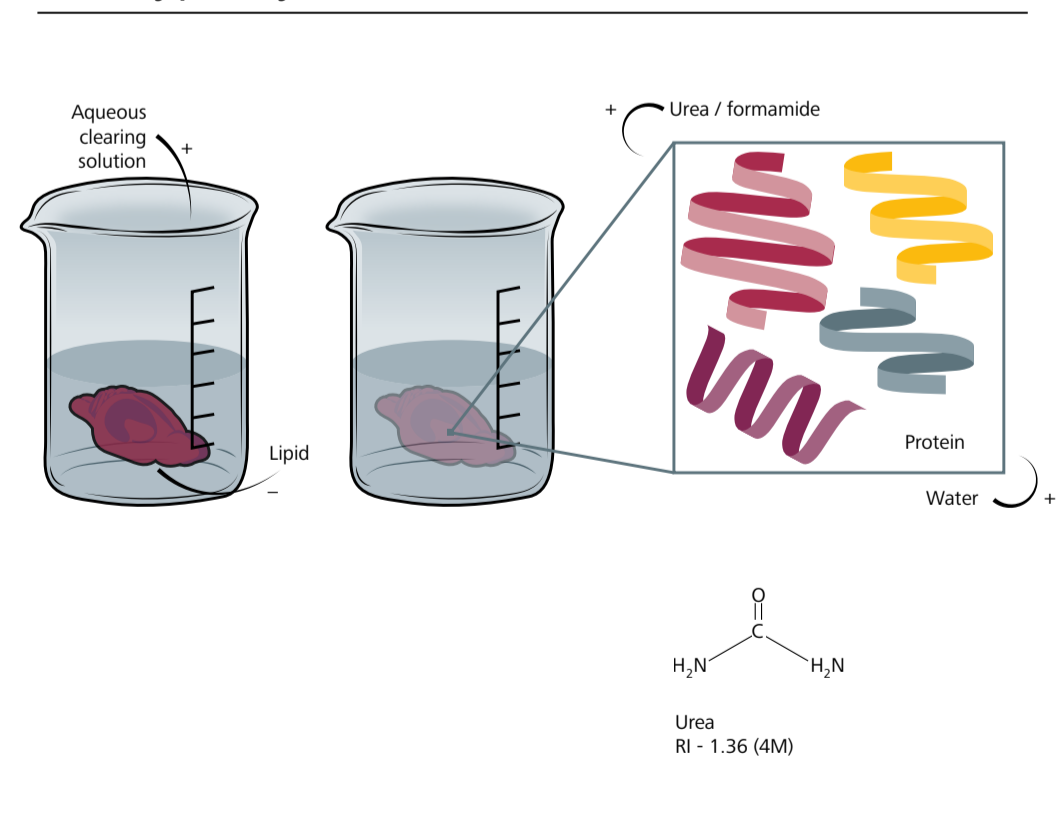
## A Solvent based clearing



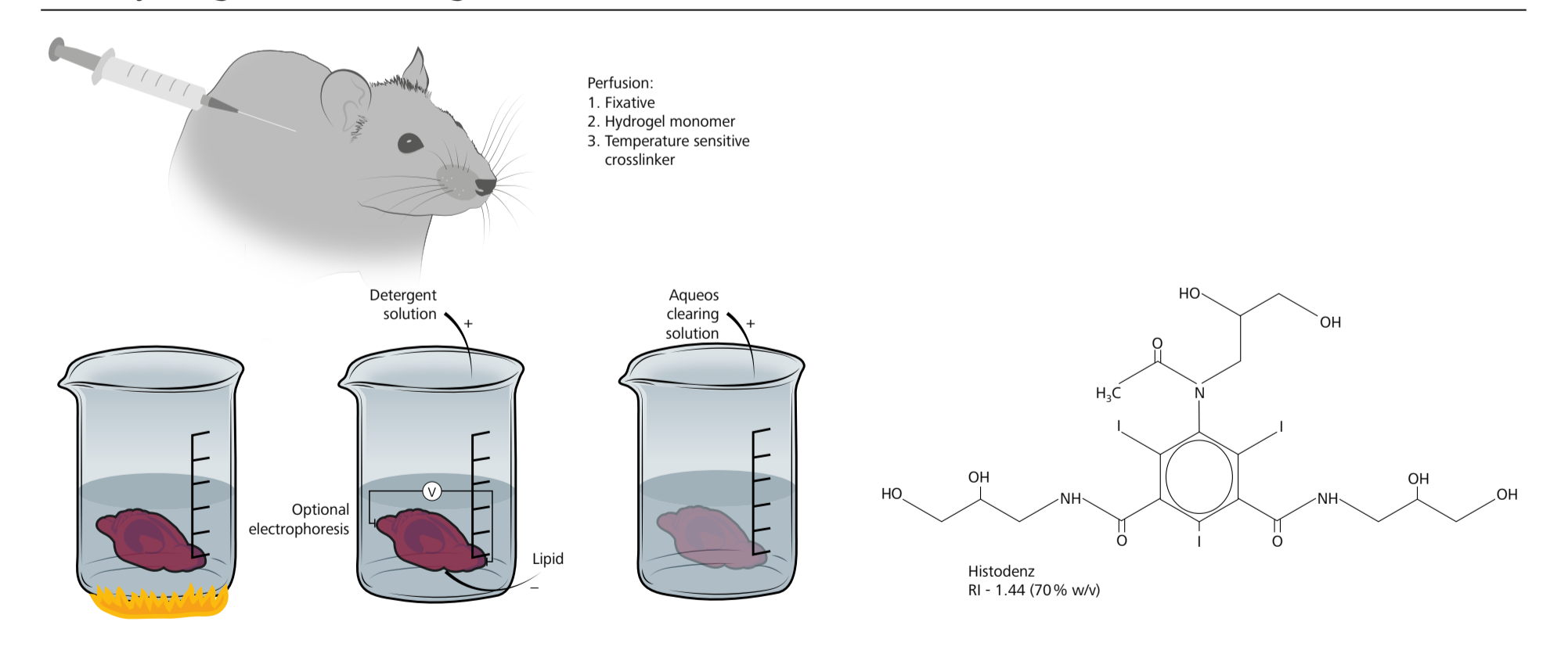
## B Simple immersion



## C Hyperhydration



## D Hydrogel embedding



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