

Supplementary File 1

<u>Solution</u>	<u>Duration</u>	<u>Temp.</u>		<u>Relevant Supplemental Figures</u>
ACSF	~10 min	4° C	Dissection, acute sectioning	
			↓	
4% PFA + 0.005% GA in 0.175 M PB*, pH 7.4	4 - 16 hr	4° C	First fixation	Figure 1-figure supplement 2 - Optimization of fixation parameters Figure 1-figure supplement 3 - Optimal duration of primary fixation
			↓	
0.05 M glycine in 0.175 M PB, pH 7.4	o/n	RT	Glycine rinse	
			↓	
0.175 M PB, pH 7.4	4 hr	RT	Rinse	
			↓	
0.17 M NaCl + 0.01 M PB + 1° Ab	2 - 7 days	RT	Primary antibody	Figure 1-figure supplement 4 - Effect of serum blocking Figure 1-figure supplement 7 - Antibodies tested
			↓	
0.175 M PB, pH 7.4	o/n	RT	Rinse	
			↓	
0.17 M NaCl + 0.01 M PB + 2° Ab	2 - 7 days	RT	Secondary antibody	Figure 1-figure supplement 5 - Effect of prolonged antibody incubation
			↓	
0.175 M PB, pH 7.4	4 hr - o/n	RT	Rinse	
			↓	
2% PFA in 0.175 M PB, pH 7.4	2 hr	RT	Second fixation	
			↓	
0.05 M glycine in 0.175 M PB, pH 7.4	4 hr	RT	Glycine rinse	
			↓	
0.175 M PB, pH 7.4	4 hr	RT	Rinse	
			↓	
20 → 100% fructose	20 - 48 hr	RT	SeeDB refractive index matching	Figure 1-figure supplement 6- Effect of SeeDB clearing
			↓	
100% wt/vol or 80.2% wt/wt fructose	< 1 day	RT	2P imaging & NIRB	
			↓	
0.175 M PB, pH 7.4	12 hr	RT	Return tissue to PB	
			↓	
0.15 M CB, pH 7.4	2 hr	4° C	Rinse	
			↓	
2% GA in 0.15 M CB, pH 7.4	2 hr	4° C	Third fixation	
			↓	
0.15 M CB, pH 7.4	o/n	4° C	Rinse	
			↓	
	2 days		EM staining/embedding	
			↓	
			EM imaging	