

Materials List for:

Targeted Labeling of Neurons in a Specific Functional Micro-domain of the Neocortex by Combining Intrinsic Signal and Two-photon Imaging

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Materials

Name	Company	Catalog Number	Comments
1. Life support/experiment prep			
Isoflurane	Webster Vet	NDC 57319-474-05	
Isoflurane vaporizer	Midmark	VIP 3000	
Feedback regulated heating blanket	Harvard Apparatus	50-7079F	
ECG monitor	Digicare Biomedical	LifeWindow Lite	
EEG amplifier	A-M Systems	1800	
EEG display monitor	Hewlett Packard	78304A	
End tidal CO ₂ monitor	Respironics	Novamatrix Capnoguard 1265	Optimize ventilation
Carbide drill burrs for drilling bone	Henry Schein	fine (0.5 mm tip) and coarse (1.25 mm tip)	
Cement for headplate/chamber	Dentsply	675571, 675572	
Black Powder Tempera Paint	Sargent Art Inc.	22-7185	Add to cement to improve light shielding and reduce reflections
Agarose - Type III-A	Sigma	A9793	For minimizing pulsations during intrinsic signal and two-photon imaging
Coverglass: 5 or 8 mm diameter, 0.17 mm thickness	World Precision Instruments	502040, 502041	For minimizing pulsations during imaging, the coverglass may be cut as needed
Brudon curettes	George Tiemann	105-715-0, 105-715-3	Cleaning skull surface
Bone wax	Ethicon	W31G	Quickly stop bleeding
Cotton Tipped Applicator	Electron Microscopy Sciences	72308-05	Clean and dry bone surface
Dumont #5CO Forceps	Fine Science Tools	11295-20	Grab individual layers of dura or pia
Vannas Spring Scissors	Fine Science Tools	15000-03	Cut dura
Gelfoam	Pfizer	09-0396-05	To stop bleeding on the dura
Absorption spears	Fine Science Tools	18105-01	Ultra-fast and lint-free wicking of CSF
Blackout material	Thorlabs	BK5	Shield craniotomy
2. Dye preparation / injection			
Dimethyl Sulphoxide (DMSO)	Sigma	D2650	
Pluronic	Sigma	P2443	
Oregon Green 488 Bapta-1 AM	Invitrogen	O6807	Calcium indicator
Alexa Fluor 594	Invitrogen	A10438	
Centrifugal filter (0.45 µm pore size)	Millipore	UFC30HV00	To remove impurities before injection

Glass pipette puller	Sutter Instruments	P97	
Borosilicate glass filamented capillary (1.5 mm outer diameter)	World Precision Instruments	1B150F-4	Dye ejection pipette
Microloader	Eppendorf	5242 956 003	For loading dye into pipette
Micromanipulator	Sutter Instruments	MP-285	To position pipette
Pressure pulse controller	Parker Hannifin	PicoSpritzer III	For pressure injection of the dye
Single-cell electroporator	Molecular Devices	Axoporation 800A	For electroporation of the dye
3. Intrinsic imaging			
4x Objective (0.13 NA, 17 mm WD)	Olympus	UPLFLN4X	
Intrinsic hardware / software	Optical Imaging Inc.	Imager 3001 / VDAQ	VDAQ software is used for episodic imaging
CCD Camera	Adimec	Adimec-1000	
Light source power supply	KEPCO	ATE 15-15M	
Light source	Optical Imaging Inc.	HAL 100	Light intensity at the cortical surface is 3-5 mW
Green filter (for vascular image)	Optical Imaging Inc.	$\lambda = 546$ nm (bandpass 30 nm)	For reference image of surface vasculature
Red filter (for intrinsic signal)	Optical Imaging Inc.	$\lambda = 630$ nm (bandpass 30 nm)	To collect intrinsic signals
Heat filter	Optical Imaging Inc.	KG-1	
4. Two-photon rig/imaging			
Two-photon microscope and software	Prairie Technologies		See Shen <i>et al.</i> 2012 for light path, filters and laser power
Ti:Sapphire laser	Spectra-Physics	Mai Tai XF	
20x (0.5 NA; 3.5 mm WD)	Olympus	UMPLFLN20X	0.5 NA objective is used only for aligning pipette over the craniotomy (not for two photon imaging)
20x (1.0 NA; 2.0 mm WD)	Olympus	XLUMPLFLN20X	
40x (0.8 NA; 3.3 mm WD)	Olympus	LUMPLFLN40X/IR	
Air table	Newport	ST-200	Isolates preparation from external vibrations
xy stage	Mike's Machine Co. (Attleboro, MA)		Experimental subject and Sutter micromanipulator placed on xy stage
Recipes			
Artificial Cerebro-Spinal Fluid	NaCl (135 mM), KCl (5.4 mM), MgCl ₂ (1.0 mM), CaCl ₂ (1.8 mM), HEPES (5 mM), pH 7.4		
Pipette Solution ¹⁴	NaCl (150 mM), KCl (2.5 mM), HEPES (10 mM), pH 7.4		