

Materials List for:

# Real-time Iontophoresis with Tetramethylammonium to Quantify Volume Fraction and Tortuosity of Brain Extracellular Space

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## Materials

Name	Company	Catalog Number	Comments
A/D and D/A converter	National Instruments Corporation	NI USB-6221 DAQ	The NI USB-6221 is still sold as a 'Legacy' device by NI. They recommend using NI USB-6341 X Series DAQs for new installations, however we have not tested the newer units. We describe the use of the NI USB-6221 with MATLAB and Windows 7 (32-bit). Alternatives: the much older PCI-MIO-16E-4 A/D converter (Used under Windows XP or older OS only) with BNC-2090 BNC connector panel and SH68-68-EP cable. As noted in the Wanda Manual, an experimental MATLAB program to use Axon Binary Files is available.
agarose	Lonza	NuSieve GTG Agarose #50081	to prepare dilute agarose gel for RTI measurements
amplifier for ISM	Dagan	Model IX2-700 Dual Intracellular Preamplifier	ion and reference voltage amplifier with N=0.1 (for reference barrel) and N=0.001 (for ion barrel) headstages
biological compound microscope (with 4x and 10x objective)			for chipping the microelectrode tips and inspecting microelectrodes; various suppliers, e.g. AmScope
borosilicate theta capillary glass tubing	Harvard Apparatus	Warner Instruments model TG200-4; order #64-0811	double-barreled glass tubing for ion-selective microelectrodes and iontophoretic microelectrodes; O.D. 2.0 mm, I.D. 1.4 mm, septum 0.2 mm, length 10 cm
brush	Winsor & Newton	University Series 233, size 0	round soft handle brush, available from Amazon
bunsen burner	Fisher		
camera for visualizing micropipettes	Olympus	OLY-150	requires monitor, IR filter on substage illuminator is optional
chart recorder			to record continuously voltages on ion-selective microelectrode during calibration in tetramethylammonium standards and during RTI experiment; e.g.

			Kipp & Zonen type BD112 dual-channel chart recorder, available refurbished
chlorotrimethylsilane, puriss., > 99%	Sigma-Aldrich	catalog # 92360	for silanization; CAUTION: flammable, acute toxicity (oral, dermal, inhalation), skin corrosion, eye damage, reacts violently with water, see Sigma-Aldrich Safety Information for full description
Commercial Software	The MathWorks	MATLAB, Data acquisition toolbox	for data acquisition and analysis using Wanda and Walter programs. Note that an academic license is available.
eye protective goggles	Fisher		
fixed-stage compound microscope	Olympus	BX51WI	can use other compound microscopes with fixed stages
forceps	Fine Science Tools	#11251-10	to chip glass capillary; Dumond #5, preferably used and no longer needed for fine work
fume hood			for silanization and filling the tip of ion-selective barrel with liquid ion exchanger; various suppliers, e.g. Captair with appropriate filter sold by Erlab
glass microscope slide	Fisher	#12-550A	to chip microelectrode tips
heater/stirrer	Fisher	Corning PC-420D	to prepare dilute agarose gel and stir solutions
iontophoretic unit	Dagan	ION-100 and PS-100	ION-100 is a single channel iontophoresis unit +/- 130 V compliance; PS-100 is an external power supply; alternatives: e.g. Axoprobe-1A made by Axon Instruments (now Molecular Devices), out of production, check for availability of refurbished units (eBay and other sites)
liquid ion exchanger (LIX) for tetramethylammonium	World Precision Instruments	IE190 Potassium Ion Exchanger	Note: this is equivalent to the original Corning potassium exchanger 477317 based on tetraphenylborate - do not confuse with neutral carrier potassium exchanger originating from the laboratory of Dr. Simon, ETH, Zurich, which does not sense tetramethylammonium, and is sold by Fluka. You can also make liquid ion exchanger for tetramethylammonium yourself: 3% by weight potassium tetrakis = (p-chlorophenyl) borate dissolved in 2,3-dimethylnitrobenzene. Buy chemicals from Fluka (now part of Sigma). See Oehme and Simon (1976) Anal. Chim. Acta 86: 21-25; CAUTION: The toxicological properties of this liquid ion exchanger have not been fully determined. Ingestion or contact with the human body may be harmful. Exercise due care! Liquid ion exchangers should be stored in a cool place out of direct sunlight.

microelectrode holder	WPI	M3301EH	to hold ion-selective microelectrode prefabricate for silanization and filling the tip of ion-selective barrel with liquid ion exchanger; WPI sells two versions of this holder, clear M3301EH and black M3301EH. In our experience, the clear M3301EH appears to be sturdier than the black M3301EH.
micromanipulator	Narishige	MM-3	to position ion-selective microelectrode prefabricate during silanization and filling the tip of ion-selective barrel with liquid ion exchanger; can be substituted with any three-axis micromanipulator in good working condition
micropipette puller	Sutter Instruments	Model P-97	to pull double-barreled glass tubing; other pullers can be used as long as they can accommodate large diameter double-barreled glass tubing
microprobe thermometer	Physiotemp	Model BAT-12R	fine probe of this thermometer is placed close to recording site
needle	BD	Syringes and Needles # 305122 (25 gauge)	for silanization; BD PrecisionGlide needles 25 G x 5/8 in (0.5 mm x 16 mm)
objective 5X dry	Olympus	MPlan N	
objective 10X water immersion	Olympus	UMPlan FL N	10X objective is water immersion, numerical aperture is 0.3, working distance is 3.3 mm
plastic containers (with lids)	Fisher	#14-375-148	to store tetramethylammonium standard solutions and microelectrodes
platform and x-y translation stage for fixed-stage microscope	EXFO	Gibraltar Burleigh	platform holds slice chamber, micromanipulators and accessories, x-y translational stage moves microscope without compromising recording stability
porous minicup			for RTI measurements in a dilute agarose gel; homemade
reusable adhesive	Bostik	Blu-Tack	for securing microelectrodes to holding vessel and other uses; various suppliers, available from Amazon
robotic micromanipulator with precise x,y,z positioning	Sutter Instruments	MP-285	two micromanipulators are needed to hold separately ion-selective microelectrode and iontophoretic microelectrode. Also possible to glue micropipettes in a spaced array (see text).
signal conditioning unit with low-pass filter	Axon Instruments	CyberAmp 320 or 380	no longer available from the manufacturer but may be available from E-Bay; alternatives: e.g. FLA-01 Filter/Amplifier from Cygnus Technology. This is a single channel instrument with a minimum cutoff at 10 Hz using a multipole Bessel filter but the company may be willing to modify it for a lower cutoff frequency (2 Hz) if needed.
silver wire	A-M Systems	#7830	diameter 0.015", bare (no coating)

slice chamber	Harvard Apparatus	Warner Model RC-27L	this is submersion slice chamber; do not use interface slice chamber
stereomicroscope			for silanization and filling the tip of ion-selective barrel with liquid ion exchanger; horizontally mounted; various suppliers
syringe, 10 mL	BD	Syringes and Needles #309604	to backfill microelectrodes and for silanization; BD Luer-Lok tip
syringe filter 0.22 µm pore	Whatman	#6780-1302	to filter backfill solutions; available from Fisher
syringe needle, 28 gauge, 97mm	World Precision Instruments	MicroFil MF28G-5	to backfill microelectrodes
Teflon (=PTFE) tubing	Component Supply	STT-28 PTFE tube light wall (28 gauge)	for silanization of ion-selective barrel; fits on BD PrecisionGlide needles 25 G x 5/8 in. Note: Teflon is essential, PVC tubing would melt by hot wax.
temperature control system	Harvard Apparatus	Warner Models TC-344B and SH-27A	TC-344B is a dual automatic temperature controller, SH-27A is an in-line heater; controller and heater work with Warner slice chambers
tetramethylammonium (TMA) chloride	Sigma-Aldrich	T-3411	5 M solution; CAUTION: acute toxicity (oral, dermal, inhalation), carcinogenicity, hazardous to the aquatic environment, see Sigma-Aldrich Safety Information for full description
vibrating blade microtome	Leica	VT1000S	to cut brain slices
xylenes	Fisher	X5-1	for silanization; CAUTION: flammable, acute toxicity (oral, dermal, inhalation), skin corrosion, eye damage, carcinogenicity, see Fisher Safety Information for full description