Array Tomography

Kristina D. Micheva and Stephen J Smith
Stanford University School of Medicine

1. Tissue fixation.

Note: *Below follows the protocol that we use for chemical fixation, but tissue prepared in a number of different ways can also be used – for example, fixed by perfusion. It should be dissected into small pieces of < 1 mm in at least one dimension. If you already have your tissue fixed, continue from section 2.*

Required materials and equipment:

Halothane (Sigma #B4388)
Paraformaldehyde (16%, EM grade; Electron Microscopy Sciences #15710)
PBS (Sigma #P3813)
Sucrose
Guillotine: for adult rats
or
Sharp scissors: for young rats and mice
Dissection instruments: handling forceps, small scissors, bone rongeur, forceps #5; small spatula; scalpel, glass pipette (tip broken off) with a bulb to transfer the tissue pieces.
Red biohazard bag
Petri dishes (35 mm)
Glass scintillation vials (20 ml)
Optional: PELCO 3451 laboratory microwave system with a ColdSpot set at 12°C; Ted Pella: speeds up sample preparation. In the protocols below two options are provided: **MW** - microwave processing or **BT** - bench top processing.

To prepare:

PBS: Dissolve 1 packet in 500 ml to prepare 0.02M PBS.

Fixative (prepare the same day, keep at room temperature): 4% paraformaldehyde, 2.5% sucrose in 0.01M PBS

For 4 ml:
1 ml PF (16%)
2 ml PBS (0.02M)
1 ml H2O
0.1 g sucrose

Procedure (for rodent brain)***:***

►Anesthetize the animal using halothane.

►Remove head using the guillotine for adult rats or sharp scissors for young rats and mice.

►Quickly remove the brain out of the skull. Dissect out the region of interest and cut into small pieces (< 1 mm in at least 1 dimension) in cold PBS.

►Quickly transfer to a scintillation vial with fixative solution (room temperature).

Note: For **MW**, u*se approximately 1ml of fixative per vial, or just enough to cover the tissue; excessive liquid volume will cause overheating in the microwave.*

MW: ►Microwave using a cycle of 1 min on – 1 min off – 1 min on at 100 – 150W. Note: *After this and each following cycle feel the glass vial to check for overheating. If solutions are getting too warm (>37°C), decrease the amount of liquid added.*

►Microwave using a cycle of 20 s on – 20 s off – 20 s on at 350-400W: repeat 3 times.

►Leave at RT for about 1 h.

BT: ► Fix @ room temperature for up to 3h or overnight at 4°C.