Carbocyanine Labeling of Fixed Cortical Slices or Fixed Brain

[as used in Polleux *et al.* (1998) Science 282:1904.]

N.B. This technique works well for brain tissue younger than P7 in rodent i.e. before main phase of myelination which slows down or even prevent the diffusion of most carbocyanine.

We mainly used two different fluorescent carbocyanine dyes: DiI (bright - red) and DiA (a little less bright than DiI but green and can be combined with DiI using appropriate optics).

1. Fix your slices after various days in culture or your brain (perfused if possible) with fresh prewarmed 4 percent paraformaldehyde (in PBS). Store overnight or longer @ 4°C (not longer than a couple of weeks for better results).

2. There are then two main options to insert the dyes into your tissue. First you can shape your crystals using fine forceps or insect pins and try to insert small crystals into your structure of interest. The second way is more suitable for very fine injections and consists in coating tiny glass pieces with DiI or DiA. Start by diluting your dye in 25 percent DMSO and then brake small Pasteur pipette tips into very small fragments. Then put small drops of your dye solution onto the broken glass and let it dry (it can take a while so I usually do that under a ventilated hood). Then the small pieces of glass coated with DiI can be inserted easily into brain tissue. the drawback is that the total amount of dye is less than for a crystal so the amount of neuronal labeling is reduced but it's a more accurate way to target a structure.

3. Importantly try to remove as much small dye particules floating around on your sample as they contaminate very easily your labeling ! I usually use small pieces of Kimwipe dipped into 4% PFA to remove most of it. Then I dip the labeled sample into clean PFA before putting it into a 6 well plate half-filled with fresh 4% PFA. Seal it with parafilm and aluminium foil. Keep it @ 37°C for diffusion. Carbocyanine are lipophilic dyes which diffuse passively through phospholipid membranes, this process is temperature-dependant. As a rough estimate, for axonal tracing in embryonic brain, an "axonal distance" of 1 mm takes up about a 7-10 days for optimal diffusion. But this diffusion time varies a lot according to the type of tissue, the distance, the quality of fixation.

4. Embed your sample in 3 percent agar (in ddH2O) and cut it with a vibratome (80-100 microns thick sections). Mount with PBS and observe immediately ! For better visualization of the cytoarchitecture, you can counterstain the tissue with Hoechst 33258 (Molecular Probes) , a blue dye which labels cell nuclei (1:2000 in PBS for 15 minutes).

As a general advice you should visit the Molecular Probes website which provides a lot of valuable technical infos on this and other dyes.