CryoSectioning – Insect Tissues

1. Fix tissue and embed in molds in 15% gelatin[1]. First half fill the mold with gelatin and put in the fridge to partially set. Then position your tissue in the gelatin, and maybe put it in the fridge again to fix it in place. Then pour on gelatin on top to cover, and again in the fridge to set. Don't let them dry out while they are setting.
2. Blocks can now be popped out of molds and stored at –20 until ready to section. The gelatin will take on a waxy texture when it freezes, suitable for sectioning. Make sure it is in a bag or something so it doesn't dry out in the freezer.
3. Set the block on top of the chuck and fix it there with a layer of gelatin. You can pipette a small quantity of gelatin around the base of the block to hold it in place more firmly. Adjust the angle of the chuck surface according to the way you want your samples sectioned.
4. Keep these at –20 until ready to section.
5. Just before sectioning (now at the microtome), spray the sample with CryoSpray to freeze instantly, and put immediately into the cryochamber to equilibrate to the temperature of the chamber (should be –20).
6. Adjust the thickness of the sections (minimum with gelatin-embedded tissue might be 5 microns; Sara Bevan usually does 20 microns for locust ganglia).
7. Start cutting!
8. Put sections, one by one with a tweezer, on to gelatin coated slides. Try to do this at the "entrance" to the cryochamber so that the sections don't warm up and curl too quickly, but rather stick to the slide.
9. Dry sections onto the slide onto a heat block.
10. Many sections can fit onto the same slide, so all sections of a given ganglion, embryo, whatever can be put onto the same slide. This makes it easier when scoring under the microscope.
11. Proceed with staining protocol.
12. Instead of staining right away, maybe you could store sections on slides in the fridge, but probably not for very long.

[1] Or you could use OCT compound, which is liquid at RT but sets into a get when you freeze it.