Frequently Asked Questions

1) In the interface style of chamber (in the pre-incubator) are the slices resting directly on the nylon mesh, or is there a small piece of lens paper or filter paper underneath them?
We put small pieces of lens papers on the mesh, on which slices are placed before transferring them into the setup chamber.

2) How exactly is done the transferring the slices from the pre-incubator to the chamber?
We grab the lens paper with a forceps, and bring them to the setup. Using a low level of solution in the chamber (we stop the flow for some moments), slices are faced done on the surface of the solution, thus the upper part of the slices in the interface chamber (in the pre-incubator) will touch the mesh, and the other surface of the slices will be faced towards the objective lens. By this way, you can remove the lens paper from the slice. If the slices are "swimming" on the surface of the solution, you may use either the lens paper or the forceps to push the slices gently below the surface of the solution. When it is done, you can put your "weight" on the slices to hold it in a place.

3) Does the slice need to be anchored to ensure adequate mechanical stability (and if so, how)?
Yes. We use a conventional technique, i.e. we have a U shape platinum wire that has 2-3 nylon fibers glued on it, which fibers keep the slice down, protecting from the movement.

4) Is it important to keep the air-exposed face of the slice facing up, when the slice is moved to the recording chamber?
No, we actually found the other surface is better.

5) Is the slice placed directly on the stainless steel mesh? Yes.

6) How are the inlets directed to the upper and lower parts of the chamber?
The steel mesh has a thicker part in front of the mesh. This thicker part holds the bended inlet that directs the flow below the slice. The upper inlet is glued to that inlet and directed above the mesh (see Fig. 1B in Hajos and Mody, 2009 or http://exasol.hu/index.php?page=dual). This is the current version. We are working on a type where two small tubings will be built in the chamber (one below and one above the mesh), so the connection could be made more easy from the in-line heater-head (http://www.super-tech.eu/Products.html).

7) The two volume parts in the chamber will be connected anyway by the uncovered parts of the mesh, so is it necessary to have two completely separate perfusion systems, or can a single solution line be split into two 'jets' with a Y-tube connection (either before the in-line solution heater or after the heater)?
I tried this solution, it does not work. Based on my experience, you need two separate perfusion systems.

8) Must the final outlets of the tubes be bent so that they are parallel to the bottom of the chamber?
No. I am using a single outlet that is adjustable, and has a larger diameter than the two inlets together to ensure the proper suction.

9) Is the bottom of the bottom chamber sealed with a glass coverslip? Yes.

10) Can the same chamber be used as an interface chamber by adjusting the outlet, and limiting the inlet to the bottom part of the chamber?
Yes. I tried.

11) For maintaining oscillations, did it require a total flow rate of 3-5 ml/minute, or is that per channel ? To use a peristaltic pump, it seems that the outlet would need to draw at twice the flow rate of each inlet. Alternatively, can the outflow be done with vacuum suction?
Vacuum should work. The flow rate of 3-5ml/min is a total volume under our conditions, but it depends on the type of tubing used (teflon, tygone is preferred). Yes, we are using for outlet a tubing with larger diameter. We have a peristaltic pump from Ismatec. We are using two tubings for inlets (1.2 mm i.d.), and one (or two) tubing for outlet(s) (2.3-2.6 mm i.d.) Plus the suction strength can be regulated with this pump by increasing (or decreasing) the pressure on the tubings.

12) Exactly what type of tubing do you use in your peristaltic pump?
We are using MS/CA casettes with pressure lever (IS 0649) in a 4-channel version of Reglo Digital type (http://www.ismatec.com/int_e/index.htm). The tubings are the 3-stop colour-coded style: for inlets two of red-red SC0404, and for outlet(s) one or two of purple-purple SC0316 or purple-white SC0319.

13) How practical is it to do patch clamp in the dual-superfusion slice chamber at the flow rates required to sustain oscillations?
We do not have any problem with patch using higher flow rates either. The slices can be (and should be) kept properly without any movements. The visibility of cell bodies are not compromised in the dual chamber (we have not try to patch on the dendrites yet). Obviously the condensor should be appropriately adjusted (it should be elevated ~ 2mm toward the cover slip to get similar picture as in the classical chamber).

WARNING: To get oscillations, you should use ultra-clean water for ACSF. Usually water from water purification systems used in the labs is not clean enough, unless it is extensively treated with UV. If the outlet pistol is not kept in alcohol (as many people do not do), then microorganisms could infect the outlet tubing plus the outer side of the column. We could not solve this problem properly, therefore we decided to buy water from a supplier, which guarantee the microorganism-free water. So, if you do not get any oscillations, buy a clean water from a supplier to make ACSF, it might sole the problem.

WARNING: According our experience, the metal mesh is wise to change in every 3-4 months.

If you have any further question, please feel free to ask: hajos@koki.hu