# Intracellular Perfusion, IP1

Introduction

The system is designed for solution exchange inside small compartments like patch pipettes. The system comes with thin polyimide tubing to fit inside the pipette. Short pieces of Teflon and silicone tubing are provided to fit and seal the ends of polyimide tubing inside the pipette holder. Ferrule-type fitting is provided to fit polyimide tubing to soft tubing of flow control units, and to small volume delivery system SVDS1. The thin polyimide tubing should be filled with test solution (primed) before using the system. This can be done by connecting an empty plugged patch pipette to the pipette holder and applying a negative pressure using a syringe (similar to procedure used while forming a seal with a cell). Note: priming the tubing with solution should be done while soft tubing of flow control units is removed (not squeezed). This soft tubing can be inserted back into flow control units (while units are rotating) after priming is done.

A BNC pipette holder with thin metal port to feed polyimide tubing inside the pipette is provided for your convenience ready for use. You can also keep using your pipette holder, and feed the polyimide tubing through the port used for suction. T- and Y-connectors are provided to seal the tubing. The BNC connector fits standard patch clamp amplifier headstages.

Note: 1) Some headstages might have different from BNC connectors. In this case you might try feeding thin polyimide tubing through the port used for suction.

Operation

When all valves, but Valve 1, closed, applying slight suction will result to seal formation and subsequent excised patch or whole-cell generation. Opening Valve 2 - turning one flow control unit ON - will provide replacement of pipette solution near the tip for another solution from a small reservoir, which is connected to the pipette and Valve 2 through microbore polyimide tubing.
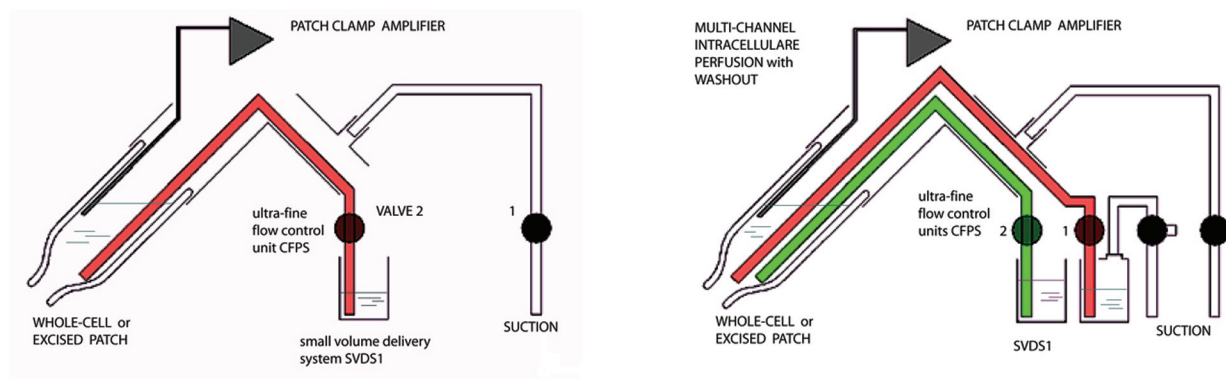
If washing out of this new pipette solution is required, or if the level of solution inside the pipette needs to be constant, the system can be used with two thin tubing inside the pipette. In this system two small reservoirs are pre-

filled with solutions, one with test solution (red) and another with control/wash pipette solution (green). Again, when all, but Valve 1, closed - applying negative pressure will result to seal formation and subsequent generation of whole-cell or excised patch. After flow unit 3 is ON, the test solution will replace previous solution near the pipette tip. After flow unit 4 is ON, the test solution will be replaced for the control/wash (or second) solution near the tip.

Note: the flow units can operate in reverse to withdraw solution from the pipette as well. This might help to wash out test solutions faster.

If both units 4 and 3 are ON, but one of them operates in reverse and withdraws solution from the pipette at the same flow rate, the level of solution in the pipette will remain unchanged. This can be easily achieved if double tubing provided is used inside the same flow control unit.

Procedure

Thin polyimide tubing should be inserted through pipette holder so that it reaches the end of the tip after a patch pipette is installed. The polyimide tubing is sealed with short pieces of silicone or Tygon tubing. Another end of polyimide tubing should be connected to a flow control unit, which is used to turn the solution flow ON/OFF.

The flow control units can be connected to 1 ft. long rod with x-block provided to position valves closer to your sample and pipettes.

Electrode Replacement

A silver-chloride electrode should be soldered to the pin of BNC connector. You can replace the silver electrode by unscrewing the pipette holder from the metal BNC part. After soldering the silver electrode, put some silicone grease around the junction to seal exposed silver wire.