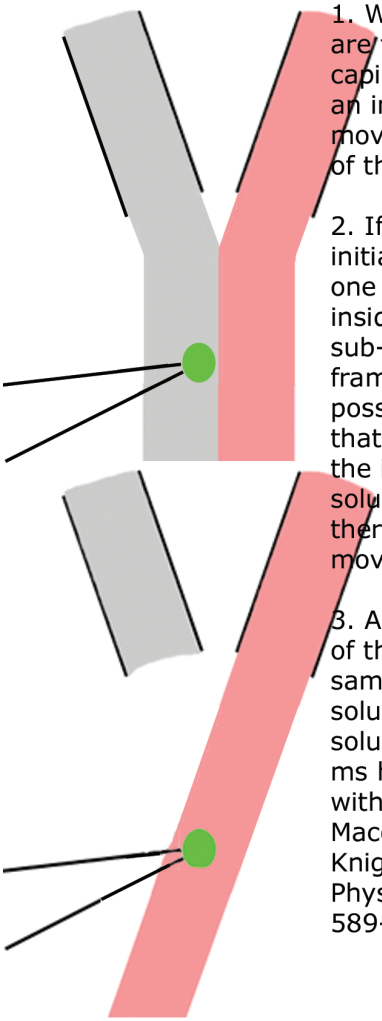
Fast Solution/Temperature Switch,UFPS1



1. When both solutions are flowing out of square capillaries, they form an interface, which can move if the speed of one of the solutions changes.

2. If the sample is initially positioned inside one solution, it can be inside another one within sub-milisecond time frame. This high speed is possible due to the facts, that the sample is small, the interface between the solutions is thin and that there is no mechanical movement involved.

3. After the flow of one of the solutions stops, the sample is inside another solution. Times of the solution exchange of 0.2 ms have been reported with this technique (D.J. Maconochie and D.E. Knight, European J. Physiology, 1989, 414: 589-596).

INTRODUCTION

This system is based on manipulation the interface between two solutions. Since there is no mechanical movement involved, sub-millisecond rates can be achieved. This also makes the system very gentle on the cells because the speed of the solutions surrounding the cells usually does not change.

Two solutions are constantly flowing from two square 1 x 1 mm tubing forming an interface, which moves if the flow of one of the solutions stops. Initially, the cell (or patch) is inside the first solution, which is usually the same as the bath (control) solution. After the flow of the first solution stops, the second solution takes its place. As a result the cell becomes immersed inside the second solution. To go back to the control solution, turn the second solution flow OFF (or turn the first solution ON again), and the cell becomes immersed into the bath (control) solution again. Since the size of the cell is small the solution interface moves across the cell surface very rapidly. Exchange rates of 0.2 ms have been achieved using this technique.

The system includes a two-capillary holder with tilting, pivot and one-axis movement controls, which allows you to adjust two square outflow tubing relative to each other. Slightly bent tubing allows you to use the system under water immersion objectives because the pipette holder can be position at a distance from the sample. The system includes 2-channel miniature fast perfusion system and polyethylene tubing that fits over the capillaries.

Can be used with gravity driven perfusion lines. Consider adding a coarse manipulator to mount the pipettes holder, and a small reservoirs pressurized system SVDS1.

Because of continuous solutions flow, which might result to contamination of the bath solution, it is desirable to washout the bath chamber constantly. Consider upgrading this system for additional valves for bath perfusion. Bath perfusion chambers are also available. Multiple solution lines can be also connected to the capillary through a manifold to allow switching multiple solutions in sequence between control solutions.

The Fast Temperature Switch system is based on ultra-fast perfusion system and utilizes two square profile heated capillaries. Initially, a sample is submerged in one flowing solution. Fast temperature switch happens after turning one (control) solution flow

off. As a result, the sample is submerged to the second solution at a different temperature.

Note: You might need additional heated bath chamber to keep your sample at control temperature during long experiments (this option is available as an upgrade).

More stable temperature control during switching is achieved if optional temperature controlled inline pre-heater is put before the capillary. This will increase the heating surface twice and will allow you to keep the temperature during fast solution flows. For even better temperature control use syringe heaters, which will heat the solutions before they enter the heated elements.

If jumps to temperatures below ambient are needed, the solutions should be cooled down first to 4°, for example. The fine temperature control is achieved by using the same heated capillaries and inline pre-heaters, which will elevate the temperature to the desired level.

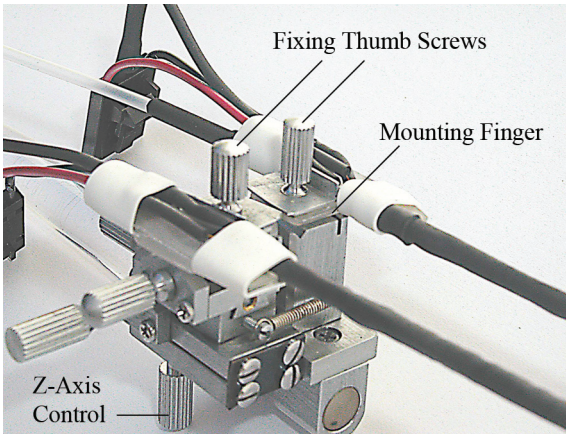
CAPILLARY POSITIONING

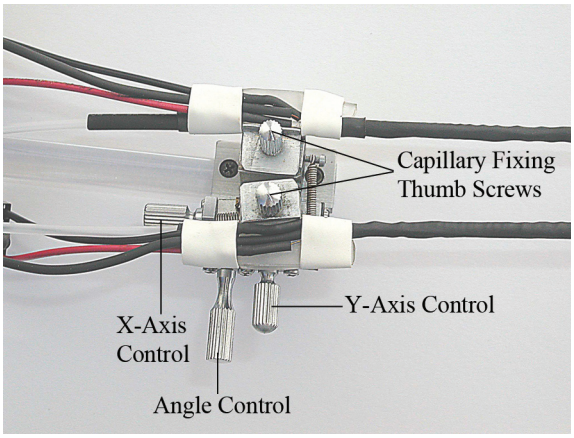
Both heated and non-heated capillary assemblies are fragile and easy to brake. Extreme care should be taken while handling the capillaries. Do not bend the capillaries or apply force. Handle the heated capillaries using plastic fingers, which are permanently attached to the capillaries. The non-heated capillaries can be formed at a required angle after heating the glass over a burner, and gently pushing the tip end of the capillary after the glass is soft enough.

Use polyethylene tubing attached to the capillary to connect to valves of the perfusion system. Fast 1.5 ms switch rate valves can be connected directly by putting tubing over the valve's barbs.

The polyethylene tubing is permanently attached to the heated glass capillary. The non-heated capillary fits inside polyethylene tubing. You need to put some grease (silicone, for example) over the capillary to make air-tight connection. If the default length is too long, the tubing can be cut to the required length.

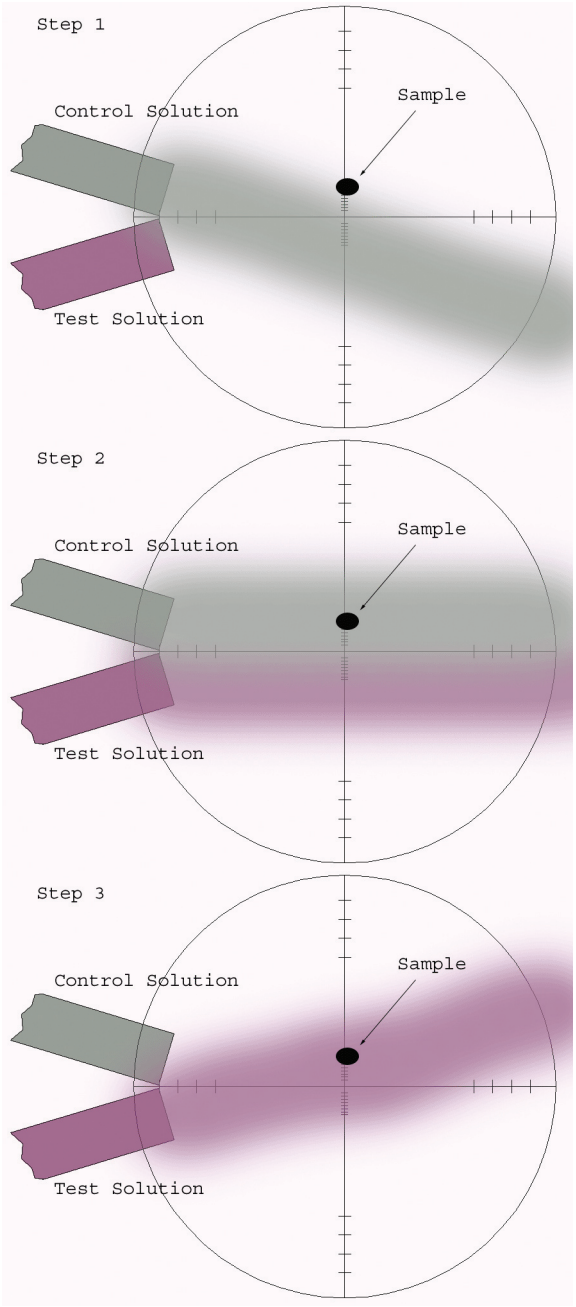
First, mount the capillaries on the holder using miniature thumb screws. Heated capillaries are attached to a plastic mounting finger with a mounting hole. It is used to attach the capillaries to the positioning manipulator with thumb screws. The manipulator has four additional control screws to move one of the capillary relative to another in X, Y, Z-directions and change the angle. After attaching the capillaries, bring the capillaries tips close to each other, so that two square outputs are in the same plane and touch each other. This is required to provide a parallel flow of both solution and to form a continuous interface between flowing





solutions. This is usually done only once before the experiment. The positioning relative to you sample is done using an optional regular 3-dimensional coarse/fine manipulator. Use a plastic rod of the two-capillary positioner to attach the capillary assembly to the optional manipulator.

After the capillaries are connected to the solutions, pre-filled with solutions (and connected to the temperature controller), position the capillaries relative to your sample.



Use a microscope eyepiece with a grid to position the capillaries (see schemas). The "X-axis" will indicate the border – interface - between two solutions flowing out simultaneously. If the length of tubing connecting the capillaries to the solutions and the level of solutions are the same, the flow rates of both solutions should be roughly the same. In this case, both solutions will flow parallel the "X-axis". The capillary tips do not have to be inside the visual field (after switching the magnification, for example) to provide adequate sample positioning. Markers on the grid can be used to position the sample after initial calibration. Using virtual projections from the walls of the capillary with Test Solution, position your sample within the projections.

For samples adherent to the bottom of the bath chamber, the capillaries should be position next to the bottom as well, so that the solutions flow along the bottom, or hit the bottom at the point of your sample.

For suspended samples (on a patch pipette, for example), use patch pipette manipulator to position the sample between virtual planes of walls of the capillary tip. The right level of the sample can be verified using the microscope's focus adjustment. If the capillaries are far from the sample, so that the solutions are flowing below the bottom walls of the capillaries, additional calibration is required to determine the required level of the sample.

The initial calibration of required level and relative sample positioning in X-Y plane, can be performed with either a live sample while recording a response to a test solution, or using colored solutions. Using manipulator's scales readings and the microscope eyepiece grid's markers, write down the required position of the sample and capillaries for consistent positioning during future experiments.

The schemas show the solution positioning during simple flow switching ON and OFF.

FAST SOLUTION/TEMPERATURE SWITCH

After sample positioning relative to the capillary tips, turn the CONTROL solution flow ON first. This ensures that the test solution will not hit the sample after turning the flow ON.

Turn the TEST solution flow ON. Your sample is still not inside the test solution, since it is protected by control solution flow.

Turn CONTROL solution flow OFF. At this point fast solution exchange happens, because it does not take long time for interface between two solutions to move across the sample. Now your sample is inside TEST solution. To wash out the sample, either turn the TEST solution OFF, or turn CONTROL solution ON.

Finer interface control can be achieved through flow rate adjustments using Pressure Controllers and SVDS1 system.

NOTE

Since the capillaries are long (10 cm) some spacers might be needed to align both capillaries in the same plane. A set of plastic spaces and longer thumb screws are provided to elevate one (or both capillaries). If longer screw is used, some spacers might be positioned under and above the capillary holder (see picture below.)