Optimizing SF-77 Performance

A brief discussion on solution flow and solution switching dynamics

Introduction

The SF-77 is capable of producing extremely fast solution changes at a point of interest, usually a single cell adhered to a coverslip or an excise patch on the tip of a microcapillary electrode. This apparatus is designed to select between multiple solutions, each emitting from its own outflow tube. Solutions appearing on the point of interest are selected by a lateral stepper that provides rapid movement of the tube assembly.

One necessary requirement for this system to operate effectively is that there be no mixing of solutions between the separate SF-77 outflow tubes, or between the outflow tubes and the surrounding bath. In addition, an understanding of how the bath solution flow affects switch times from the perspective of the cell can provide powerful insight on the how best to configure the SF-77 system.

This white paper discusses the underline dynamics for both solution flow and for solution switching.

Dynamics of differential solution flow

Flow velocity vs flow rate

It's useful to make note of the distinction between solution flow speed (or velocity) and solution flow rate.

We're most accustomed to thinking of perfusion in terms of flow rate, which is commonly referred to in terms of ml/min. However, the speed at which a solution flows (i.e. its velocity) has significant dependence on the size of the channel in which it's flowing. For example, a solution flowing at 2 ml/min in a large bath will move at a leisurely pace while the same solution flowing at the same flow rate in PE tubing will come out like a garden hose.

Our discussion here is framed in terms of flow velocity, not flow rate.

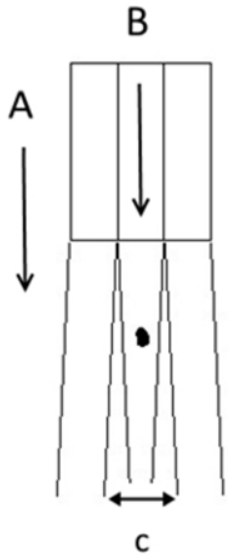
Linear flow

The principle guiding the expected behavior of solution flowing out from the SF-77 perfusion tubes is commonly termed a 'sewer pipe'. In this model, solution emitting from any single tube is speed

matched to the solution flowing in its nearest neighbors (e.g., other tubes and/or the surrounding bath). When the movement of these solutions are speed matched, the solution leaving a tube enters the surrounding environment with no velocity difference and turbulent mixing does not occur.

Lateral diffusion

In the sewer pipe model, mixing that does occur is driven by *lateral diffusion* stemming from solutions spreading out as they move downstream from the tubes. This is illustrated in the figure to the right.



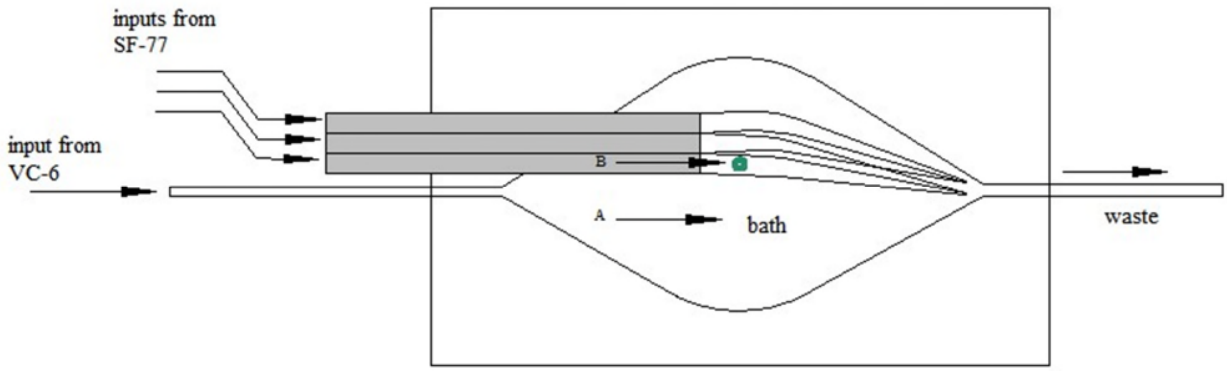
A is the flow velocity of the bath and *B* is the flow velocity of the solution leaving the tubes. Note that the length of the A and B arrows is the same, meaning that *flow velocity A = flow velocity B*.

This figure shows three solutions leaving three tubes. The width of one of these solutions (illustrated by C) is a representative example of lateral diffusion as it moves away from the tube output. Note that under normal conditions, solutions from adjoining tubes mix with their neighbors, but only after passing the cell (the black dot in the figure).

Configuring the system solution flows

Since the chamber bath and the SF-77 perfusion tubes have significantly different cross sectional areas (the bath is larger), it's not feasible to supply solutions to both the chamber and the bath from a single source.

In other words, a properly assembled SF-77 application uses two perfusion systems to achieve comparable solution velocities in both the bath and flow tubes. A larger perfusion system (often a VC-6 or VC-8 package, but can be a simple solution reservoir with a stopcock) is dedicated towards regulating the bath solution flow. A smaller perfusion system (usually a VC-6M or VC-8M) is dedicated towards regulating solution flow out of the SF-77 perfusion tubes. This concept is illustrated below.



Note that the flow rate in the bath and in the tubes has been adjusted so that the *flow velocity* (or speed) of both is the same (e.g., A and B arrows are the same length). Since the relative speeds are the same, the only mixing is lateral diffusion and the cell (green dot) sees only a single, pure solution.

Warner offers a composite system comprised of an SF-77 combined with a VC-6M for managing solution flow in the SF-77 tubes. This is called the VC-77SP. A separate valve control system (eg, VC-6 is needed for managing solution flow in the chamber bath.)

A technique for matching flow velocities

Unfortunately, there's no simple way to calculate flow velocity for the SF-77 perfusion tubes and an appropriate flow rate must be determined empirically. This is most easily done using a colored solution and a microscope.

As we will discuss in the next section, the bath flow velocity dominates the solution switching speed from the perspective of the cell. As such, it's most useful to first set the bath flow rate, then match the SF-77 outflow tubes to the bath flow.

For the sake of this setup method, let us assume the solutions to be simply H_2O, with or without coloring.

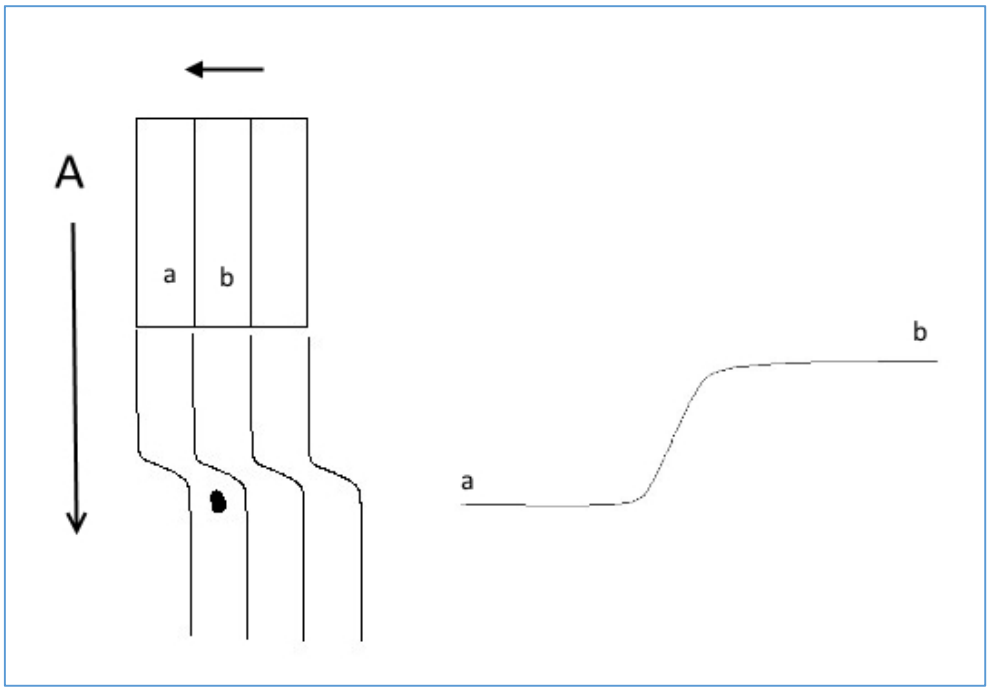
1. Begin by assembling a flow chamber without any cells or samples.
2. Load the bath solution reservoir with clear liquid.
3. Load the solution reservoirs that deliver to the three SF-77 perfusion tubes with clear solution.
4. Place a drop or two of blue food coloring (or equivalent) into the reservoir that delivers to the SF-77 perfusion tube immediately adjacent to the bath.
5. Begin perfusing the chamber bath at the desired flow rate.
6. Place the SF-77 perfusion tubes into the bath and begin flowing the blue solution. (Go as slow as possible.)
7. Under the microscope, observe the blue solution relative to the bath.
    a. If the bath is flowing significantly faster than the tube, then you'll see clear vortices entering into the blue dye.
    b. If the tube outflow is moving significantly faster than the bath, then you'll see blue vortices entering into the bath.
    c. Depending on the relative differences, these vortices can occur well downstream from the perfusion tubes. The greater the difference, the closer to the tubes they will appear.
8. In any case, adjust the perfusion tube flow rate by raising or lowering the reservoir rack up and down until the flow speeds are closely aligned.
9. Start the solution flow for the other tubes and check that the blue solution remains well behaved.
10. Record the height of both the bath solution reservoir and the tube solution reservoirs for future reference. Setting your working solutions to these heights will reproduce the driving

force for solution delivery and reproduce the desired flow speeds in your experiment. Useful for when you are unable to resolve the different solutions under the microscope.

Solution switching dynamics

Effect of bath flow velocity on solution changes observed at the cell

Now that we've properly analyzed, assembled, and configured the SF-77 perfusion dynamics, let us consider how the solution flow rates determine how the cell sees a solution change. Let us consider a nonlinear flow condition wherein we change from one solution to another by stepping the SF-77 tubes from side to side. This is shown the figure below:



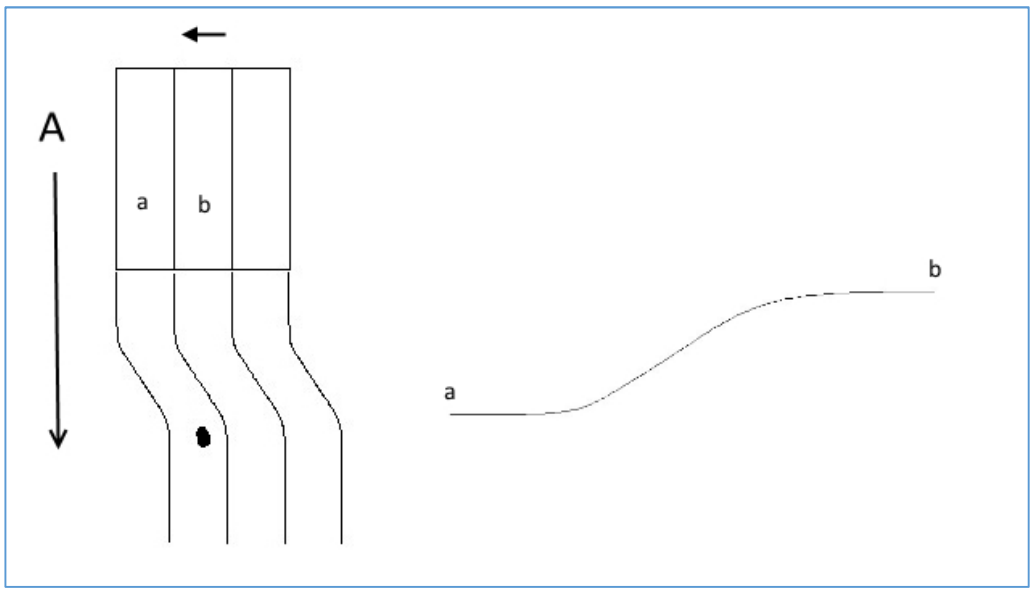
All solutions (including the bath, solution a, and solution b) are flowing at the bath speed A. You can see from the drawing that while the stepper has shifted to target the cell with solution b, this change is still en route and has not yet arrived at the cell. Since all solutions are flowing at the same speed, the boundary between solutions a and b (the a-b boundary) is also moving at the bath flow velocity A.

The physical geometry of this arrangement dictates that the cell will experience the solution change only when the a-b boundary sweeps across it. Since the a-b boundary is moving at the bath velocity, it is this speed that primarily determines the rate of solution change seen by the cell. This effect is illustrated in the line graph to the right in the figure on the previous page.

Effect of slow stepping speed on changes observed at the cell

Let us now consider how changing the stepping speed between channels a and b could modify how fast the solution changes at the cell.

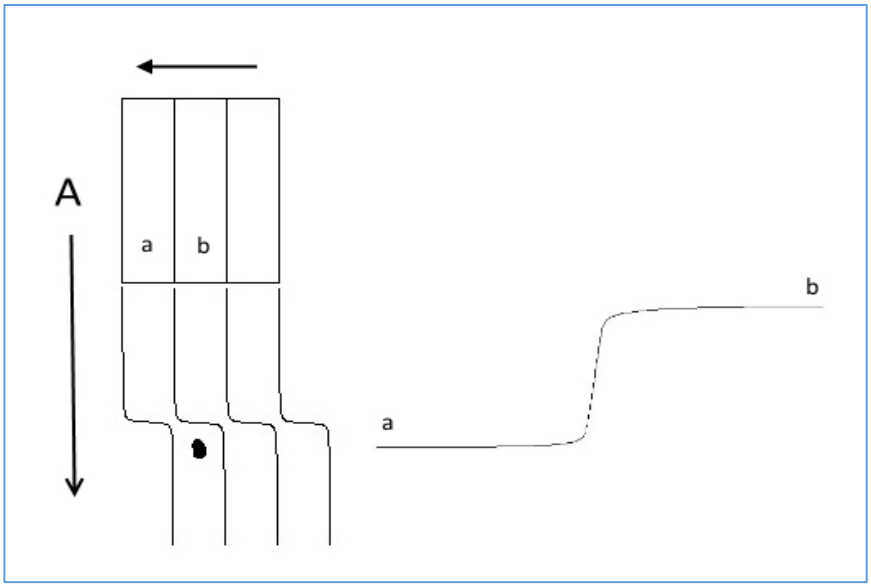
Take a look at the figure below:



In this example, we have deliberately chosen to step the perfusion tubes sideways at the same speed that the solution flows in the bath. In other words, both the sideways step velocity and overall bath velocity are at speed A. As you can see from the drawing, the a-b boundary reflects this arrangement in that the boundary angle is now 45°. In this case, it will take longer than in the previous example for the boundary to fully cross the cell. This is illustrated in the graph to the right.

Effect of fast stepping speed on changes at the cell

Now let us consider the opposite case when the sideways step speed greatly exceeds the bath flow speed. Take a look at the figure on the next page:



In this example, the sideways step speed is approximately ten times faster than the bath flow speed. As can be seen, the a-b boundary is now almost a horizontal line. (Note that a horizontal line represents the a-b boundary configuration that can sweep across the cell the fastest.) This is illustrated by the graph to the right in this figure.

Stepping speed conclusions

Generally speaking, the stepping geometries we've been discussing dictate that the crossing speed of the a-b boundary will follow the relationship:

$$A\cos\left(\frac{bath\ speed}{step\ speed}\right)$$ where A is also the bath speed

The SF-77 steps sideways at a uniform and rapid rate. This rate is 2.9 ms/step, which certainly exceeds any bath speed by a very wide margin. As such, the shape of the a-b boundary will normally appear as is shown in the figure above and the equation will converge to the bath flow velocity A.

In conclusion, since the sideways step speed of the tubes greatly exceeds the flow velocity of the bath, the rate of solution change at the cell is almost completely dependent on the flow velocity of the bath solution and not on the step speed.